Basic and Clinical Tumor Immunology

Cancer Treatment and Research

WILLIAM L. MCGUIRE, *series editor*

R. B. Livingston, ed., Lung Cancer 1. 1981. ISBN 90-247-2394-9.
G. B. Humphrey, L. P. Dehner, G. B. Grindey and R. T. Acton, eds., Pediatric Oncology 1. 1981. ISBN 90-247-2408-2.
J. J. DeCosse and P. Sherlock, eds., Gastrointestinal Cancer 1. 1981. ISBN 90-247-2461-9.
J. M. Bennett, ed., Lymphomas 1, including Hodgkin's Disease. 1981. ISBN 90-247-2479-1.
C. D. Bloomfield, ed., Adult Leukemias 1. 1982. ISBN 90-247-2478-3.
D. F. Paulson, ed., Genitourinary Cancer 1. 1982. ISBN 90-247-2480-5.
F. M. Muggia, ed., Cancer Chemotherapy 1. 1982. ISBN 90-247-2713-8.
G. B. Humphrey and G. B. Grindey, eds., Pancreatic Tumors in Children. 1982. ISBN 90-247-2702-2.
J. J. Costanzi, ed., Malignant Melanoma 1. 1983. ISBN 90 247-2706-5.
C. T. Griffiths and A. F. Fuller Jr., eds., Gynecologic Oncology. 1983. ISBN 0-89838-555-5.
F. A. Greco, ed., Biology and Management of Lung Cancer. 1983. ISBN 0-89838-554-7.
M. D. Walker, ed., Oncology of the Nervous System. 1983. ISBN 0-89838-567-9.
D. J. Higby, ed., Supportive Care in Cancer Therapy. 1983. ISBN 0-89838-569-5.

Basic and Clinical Tumor Immunology

edited by

RONALD B. HERBERMAN

National Cancer Institute
Frederick Cancer Research Facility
Frederick, Maryland, USA

1983 **MARTINUS NIJHOFF PUBLISHERS**
a member of the KLUWER ACADEMIC PUBLISHERS GROUP
BOSTON / THE HAGUE / DORDRECHT / LANCASTER

Distributors

for the United States and Canada: Kluwer Boston, Inc., 190 Old Derby Street, Hingham, MA 02043, USA
for all other countries: Kluwer Academic Publishers Group, Distribution Center, P.O. Box 322, 3300 AH Dordrecht, The Netherlands

Library of Congress Cataloging in Publication Data CIP

Main entry under title:

Basic and clinical tumor immunology.

(Cancer treatment and research ;)
Includes index.
Contents: The immunological surveillance hypothesis / Osias Stutman -- Role of macrophages in host resistance against tumors / William E. Fogler and Isaiah J. Fidler -- Specific antitumor immunity and its role in host resistance to tumors / Robert W. Baldwin -- [etc.]
1. Tumors--Immunological aspects--Addresses, essays, lectures. I. Herberman, Ronald B., 1940- . II. Series. [DNLM: 1. Neoplasms--Immunology. 2. Neoplasms--Therapy. W1 CA693 v. 14 / QZ 200 B311]
RC268.3.B37 1983 616.99'4079 83-8052

ISBN-13: 978-1-4613-3875-8 e-ISBN-13: 978-1-4613-3873-4
DOI: 10.1007/978-1-4613-3873-4

Copyright

© 1983 by Martinus Nijhoff Publishers, Boston.
Softcover reprint of the hardcover 1st edition 1983
All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, mechanical, photocopying, recording, or otherwise, without the prior written permission of the publishers,
Martinus Nijhoff Publishers, 190 Old Derby Street, Hingham, MA 02043, USA.

Contents

Foreword vii

Preface ix

List of contributors xi

1. The immunological surveillance hypothesis 1
   OSIAS STUTMAN

2. Role of macrophages in host resistance against tumors 83
   WILLIAM E. FOGLER and ISAIAH J. FIDLER

3. Specific antitumor immunity and its role in host resistance to tumors 107
   ROBERT W. BALDWIN

4. Cultured human antitumour T cells and their potential for therapy 129
   B.M. VOSE and A. HOWELL

5. Human tumor antigens: Detection and characterization with monoclonal antibodies 159
   KENNETH O. LLOYD

6. Immunocompetence in cancer patients 215
   LINDA M. KARAVODIN and SIDNEY H. GOLUB

7. Biological response modifiers: Current status and prospects as anti-cancer agents 257
   RICHARD V. SMALLEY, STEPHEN A. SHERWIN, CEDRIC W. LONG and ROBERT K. OLDHAM

8. Therapy of established tumors by adoptive transfer of T lymphocytes 301
   PHILIP D. GREENBERG, MARTIN A. CHEEVER and ALEXANDER FEFER

Index 337

Cancer Treatment and Research

Foreword

Where do you begin to look for a recent, authoritative article on the diagnosis or management of a particular malignancy? The few general oncology textbooks are generally out of date. Single papers in specialized journals are informative but seldom comprehensive; these are more often preliminary reports on a very limited number of patients. Certain general journals frequently publish good indepth reviews of cancer topics, and published symposium lectures are often the best overviews available. Unfortunately, these reviews and supplements appear sporadically, and the reader can never be sure when a topic of special interest will be covered.

Cancer Treatment and Research is a series of authoritative volumes which aim to meet this need. It is an attempt to establish a critical mass of oncology literature covering virtually all oncology topics, revised frequently to keep the coverage up to date, easily available on a single library shelf or by a single personal subscription.

We have approached the problem in the following fashion. First, by dividing the oncology literature into specific subdivisions such as lung cancer, genitourinary cancer, pediatric oncology, etc. Second, by asking eminent authorities in each of these areas to edit a volume on the specific topic on an annual or biannual basis. Each topic and tumor type is covered in a volume appearing frequently and predictably, discussing current diagnosis, staging, markers, all forms of treatment modalities, basic biology, and more.

In Cancer Treatment and Research, we have an outstanding group of editors, each having made a major commitment to bring to this new series the very best literature in his or her field. Martinus Nijhoff Publishers has made an equally major commitment to the rapid publication of high quality books, and world-wide distribution.

Where can you go to find quickly a recent authoritative article on any major oncology problem? We hope that Cancer Treatment and Research provides an answer.

WILLIAM L. MCGUIRE
Series Editor

Preface

Over the last several years, the field of tumor immunology has been undergoing considerable evolution. For a period of 10 to 15 years, since the demonstration of tumor-associated transplantation antigens on some rodent tumors, experimentation and discussions in tumor immunology centered around the distribution of such antigens in various types of tumors, particularly their possible expression in human tumors, and the role in resistance against tumor growth of immune T cells specifically directed against these tumor-associated antigens. The failure to demonstrate tumor-associated transplantation antigens on most spontaneous tumors of aged mice or rats, and the considerable difficulties in documenting the existence of such antigens on human tumors or the presence of immune T cells with reactivity against them, led to much pessimism and overall skepticism about the importance of tumor immunology. We are now in a period of reassessment, in which the earlier dogma is being critically questioned and novel or alternative effector mechanisms are being considered for their involvement in host resistance against tumor growth. There has also been a considerable shift from the predominant focus of tumor immunology studies on transplantable, local tumors to the biology and resistance mechanisms associated with tumor metastases. This is a particularly important and welcome redirection, since control of metastasis is the major clinical challenge that may be met by immunologic approaches.

The present volume is designed to reflect the changes in direction in tumor immunology and to provide some indications of the promising new approaches in this field of research, particularly in regard to the potential for therapy of cancer. As reflected by the title, a balance has been sought between the extensive basic information in experimental animal systems and the clinical applications of such information and insights.

The immune surveillance hypothesis of Burnet, Thomas and others has been a dominant driving force for much of the activity in this field for the

past 20 years. The continuing controversy about it has closely reflected the problems in the discipline itself. It is therefore quite appropriate to include an extensive analysis of the current status of this hypothesis by Stutman, who has been a major contributor to the experimental evaluation of the major predictions of the hypothesis.

As a reflection of the recent awareness of the heterogeneity of potentially important effector mechanisms, a chapter has been included on macrophages in resistance against tumors (Fogler and Fidler), as well as a chapter by Baldwin on the status of information on specific antitumor immunity. Even this last area has been considerably revitalized by the demonstrated ability to propagate antitumor T effector cells in culture in the presence of interleukin 2 and the rapid progress in this area is summarized by Vose and Howell.

The advent of monoclonal antibody technology has found almost immediate application in tumor immunology. The nature of tumor-associated antigens is being critically reexamined and dissected in much finer detail than was previously possible by the earlier technology. The chapter by Lloyd summarizes the recent progress in the characterization of human tumor-associated antigens by a variety of monoclonal antibodies.

One of the major practical applications of tumor immunology is the monitoring of the tumor-bearing host. There are emerging indications that specific or general alterations in immunologic function may be useful for the diagnosis of cancer, for the assessment of prognosis of tumor-bearing individuals, and for monitoring of therapy. In addition, documentation of immunologic deficits in tumor-bearing individuals would provide the rational basis for therapeutic attempts to overcome these deficiencies. The chapter on immunocompetence in cancer patients, from a group with much expertise and firsthand experience (Karavodin and Golub) provides a good overview of the status and potential in this area.

A final and major section of the volume is devoted to the potential applications of immunologic approaches to the therapy of cancer. In recognition of the likely importance of nonimmunologic host factors as well as immunologic effector mechanisms in defense against tumors, and the frequent findings that agents modulating immune reactivity also affect other host responses, biological response modification is a term which is rapidly replacing immunotherapy. Smalley and his colleagues summarize the current status of the National Cancer Institute's program on biological response modifiers and the prospects of this general area for therapy of cancer. One of the major, recent approaches in this area, the treatment of established tumors by adoptive transfer of immune T cells, is summarized by Greenberg et al.

This volume should provide the reader with extensive, recent information

on selected important advances in tumor immunology. The overwhelming number of publications in this area has been well assimilated and this overview should be of value to the non-immunologist with interest in oncology, as well as to tumor immunologists.

Ronald B. HERBERMAN, M.D.

List of Contributors

BALDWIN, Robert W., Cancer Research Campaign Laboratories, University of Nottingham, Nottingham NG7 2RD, U.K.

CHEEVER, Martin A., Division of Oncology, University of Washington School of Medicine and the Fred Hutchinson Cancer Research Center, Seattle, WA 98195, U.S.A.

FEFER, Alexander, Division of Oncology, University of Washington School of Medicine and the Fred Hutchinson Cancer Research Center, Seattle, WA 98195, U.S.A.

FIDLER, Isaiah J., Cancer Metastasis and Treatment Laboratory, NCI-Frederick Cancer Research Facility, Frederick, MD 21701, U.S.A.

FOGLER, William E., Cancer Metastasis and Treatment Laboratory, NCI-Frederick Cancer Research Facility, Frederick, MD 21701, U.S.A.

GOLUB, Sidney H., Department of Surgery, Division of Oncology, University of California at Los Angeles School of Medicine, Los Angeles, CA 90024, U.S.A.

GREENBERG, Philip D., Division of Oncology, University of Washington School of Medicine and the Fred Hutchinson Cancer Research Center, Seattle, WA 98195, U.S.A.

HOWELL, A., Department of Immunology, Paterson Laboratories and Cancer Research Campaign Department of Medical Oncology, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, U.K.

KARAVODIN, Linda M., Department of Surgery, Division of Oncology, University of California at Los Angeles School of Medicine, Los Angeles, CA 90024, U.S.A.

LLOYD, Kenneth O., Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, U.S.A.

LONG, Cedric W., The Biological Response Modifiers Program, Division of Cancer Treatment, National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD 21701, U.S.A.

OLDHAM, Robert K., The Biological Response Modifiers Program, Division of Cancer Treatment, National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD 21701, U.S.A.

SHERWIN, Stephen A., The Biological Response Modifiers Program, Division of Cancer Treatment, National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD 21701, U.S.A.

SMALLEY, Richard V., The Biological Response Modifiers Program, Division of Cancer Treatment, National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD 21701, U.S.A.

STUTMAN, Osias, Cellular Immunology Section, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, U.S.A.

VOSE, B. M., Department of Immunology, Paterson Laboratories and Cancer Research Campaign Department of Medical Oncology, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, U.K.

1. The Immunological Surveillance Hypothesis

OSIAS STUTMAN

1. INTRODUCTION

Three peculiar features have developed in recent years regarding the concept of immunological surveillance (IS). Firstly, the interest in the subject by *bona fide* immunologists who in the past only had marginal attachment to tumor immunology [1–7], in one case producing actual experimental work [2, 3] and in another [7] by involvement in natural killer (NK) cell research. Secondly, the possible 'rescue' of IS [8] as a viable hypothesis by the recent explosion of information on natural-cell-mediated cytotoxicity (N-CMC), especially on NK cells and similar effector mechanisms in animals and humans [9]. The third, and most important point concerning the actual role of the IS hypothesis in the analysis of clinical data, will be discussed at the end of this Introduction. Implied in the first point are also the views that NK cells may be possible mediators of IS [3, 6, 7], with very little else that can be considered as new insights into the problem. Especially since the possible role of NK cells in IS was proposed some time ago by several tumor immunologists [10–14] as well as by researchers in the NK area [12–14]. What was aptly termed as the 'rescue' of IS by NK cells [8] can still be considered simply as an adjustment of this persistent theory to the total debacle of the concept of IS as a general phenomenon that applies to all forms of malignancy and to the notion of being mediated by T cells, as originally proposed by Burnet [15]. Both concepts, as well as the sole prediction of the theory (i.e. the higher incidence of tumors in immunodeficiency states affecting T cells) proved wrong both at the experimental as well as at the conceptual level [16–29]. Furthermore, the possible role of N-CMC as some form of defense against cancer was proposed almost ten years ago [30], when discussing the problems posed by the detection of N-CMC against tumors in the normal controls, during the attempts to show specific anti-tumor cell-mediated cytotoxicity in cancer patients [31]. In addition,

R.B. Herberman (ed), Basic and Clinical Tumor Immunology. ISBN-13: 978-1-4613-3875-8
© 1983. Martinus Nijhoff Publishers, Boston.

'alternate pathways' mediated by non-T cells, were proposed initially to explain the resistance observed in some models of experimental oncogenesis in nude mice [21]. Also the normal or high levels of NK cells in nude mice were considered [13, 26, 32–36] as the possible explanation for the 'normal' incidence of spontaneous or induced tumors in such a T cell deficient model [18–29]. As a matter of fact, since the IS theory started to develop problems with its putative thymus-dependency (see [21]), a series of alternative devises mediated by macrophages [37–41], natural antibodies [42, 43], antibody-dependent-cell mediated cytotoxicity [44] or some other 'macrophage-like' or still undefined cell-mediated mechanisms [20, 45, 46], in addition to NK cells, were proposed as mediators of an IS-like function against tumor development. On the other hand, with some murine models, the T cells appear to play an important role, especially in the selection of progressor variants [47, 48]. Thus, it may well be that a variety of effector mechanisms, including NK cells, may act as surveillance devises in different experimental models [29, 43], depending on the type of oncogenic stimulus [12] and probably also the bias of the researcher. Most of the studies cited above in which some surveillance mechanism was proposed, used transplanted tumors (with exception of the nude mouse studies, see [20]; or some of the UV-light-induced models, see [47]) instead of actual tumor development, which may not be totally relevant to a true surveillance model, since it presupposes a site selection for transplantation (usually a subcutaneous site) and ignores possible regional differences in putative regulatory or IS mechanisms which may affect tumor development (i.e. tumors may actually develop on 'weak spots' of the defense system, see [29]). This point will be further discussed during our analysis of N-CMC as a possible IS mechanism. In summary, from the above statements, it is apparent that IS has the protean capacity to be mediated by a variety of alternate non-T mechanisms, depending on the temporary waves of research and on the experimental models used. It is also clear that, as proposed initially by Burnet [15], the only testable component of the hypothesis is the incidence and behavior of spontaneous and/or induced tumors in individuals with selected or combined deficiencies of the putative effector mechanism mediating IS [16–22, 25–29]. The prediction would be either augmentation of tumor incidence, acceleration of tumor appearance or more aggressive behavior of the tumor in the deficient hosts [15, 21, 25, 29]. From our own studies, as well as from the available literature, we argued that neither T cell deficiencies, nor macrophage deficiencies [21, 26–29], nor to some extent NK deficiencies [46, 49] seemed to affect tumor development in mice, while many of these deficiencies clearly affected local growth as well as metastatic spread of transplanted tumors [29, 49]. This point will be discussed further.

Finally, the third feature of the recent fate of IS has been the beginning of

studies on the mechanisms and properties of lymphoma development in immunodeficient patients [50]. As I indicated in a previous review [29], the persuasive power of the IS theory was so strong that the tumors appearing in the immunodepressed patients, even if they were predominantly of a rather unique type of lymphoma [50], were automatically considered to be the natural corollary of the immunodeficiency, as predicted by the IS theory, and only catalogues of their occurrence were built. In sum, as I indicated in a previous review, '... this paralyzing effect of the immunological surveillance theory in its dogmatic version on further research on these clinical materials is probably the single most damaging negative comment that may be made against the theory...' [29], a negative component which is certainly not balanced by the extensive hyperactivity that the theory has generated in other areas. Thus, I feel that it is extremely important that the lymphomas and other tumors in immunodepressed patients are finally being studied as unique clinical entities and interpreted by a variety of alternative hypotheses which may pertain more to lympho-hemopoietic differentiation and regulation, and other important issues of tumor progression [51, 52], rather than as the consequence of the putative absence of the IS mechanism preventing tumor development [53].

1.1 *Some definitions*

Before going into the analysis of some of the current trends in IS, especially those developments in the past three years, since my last review on IS was written [29]; it may be pertinent to present a basic definition of IS as proposed in its more organized form by Burnet [15]: '... when aberrant cells with proliferative potential arise in the body, they will carry antigenic determinants... When a significant amount of new antigens have developed, a thymus-dependent immunological response will be initiated which eventually eliminates the aberrant cells in... the same way as a homograft is destroyed...' [15]. The two axioms contained in this definition (i.e. the distinct antigens on tumor cells and the capacity of such antigens to provoke an immune response '... based on thymus-dependent immunocytes...' [15]), have been considered in 1971 as '... acceptable to all...' [54]. In summary, regardless of the proposed mechanism, IS was a mechanism to control incipient tumor development.

The main prediction of the theory, and indeed the only one that could be tested experimentally in both clinical and animal models, was that '... conditions associated with depression of the thymus-dependent system, whether genetic, induced by drugs or of other origin should increase likelihood of cancer...' [53]. More specifically, the prediction of the theory that could be tested in the clinic and the laboratory was that in genetic or induced immunodepressive conditions, a facilitation of spontaneous or induced tumor

development was expected. It was this lack of a significant increase in general tumor incidence (if the lymphomas in immunodepressed patients are excluded) in most clinical and experimental models [21, 25, 29], which constituted the stronger, and probably the sufficient argument at that time, that the IS theory in its original formulation was incorrect. The axioms (i.e. 'antigens' on the abnormal cells and some type of host response) can still be applied to any form of putative IS or plain 'surveillance'. In the sense that whether the proposition is that it is mediated by macrophages, NK or any other type of effector cell, belonging to the 'para-immunological' arsenal, in Woodruff's terminology [55]; the phenomenon implies the interaction of the abnormal cell expressing some surface structures that make it susceptible or resistant to the action of the host effector cells, with the putative effector cells, which probably recognize such surface changes in a 'specific' way, as is proposed for NK cells [36]. Thus, making it independent of the arguments concerning more conventional tumor associated transplantation antigens [12]. Concerning N-CMC, whether mediated by NK or other cell types, one comment seems relevant, which is, that for the first time there is an effector mechanism that may actually fulfill the properties required for IS, in the sense that it does not require time-consuming priming and thus can act rapidly; is independent of conventional tumor associated transplantation antigens and thus can handle non-antigenic tumors and finally, can handle small numbers of tumor cells *in situ* [29, 35, 49].

1.2 *Tumor-host interactions*

There are at least three critical levels of interaction which may take place between the tumor and the 'defense' mechanisms of the host: (1) The possible early recognition of the malignant change *in situ*, and some form of reaction against it, hopefully eliminating the abnormal cells (i.e. the IS view, actually preventing tumor development); (2) The complex immunological responses of the host to the developing tumor, once it has attained a certain critical mass *in situ*; with all the putative effector and regulatory circuits, involving different types of responses, some of which appear to benefit the host and some which may be beneficial for the tumor proper (i.e. the domain of tumor immunology proper, which is the *post factum* specific and non-specific response triggered by the developing tumor) and (3) The immunological or para-immunological mechanism, if any, which affect metastatic spread of the tumor, which also includes interactions between different components of the host's anti-tumor responses, as well as selection of tumor variants [56]. By definition, the appearance of 'tumor immunity' as in (2) above, means that 'surveillance' as in (1) above has failed. Similarly, the appearance of metastatic spread as in (3) above, means that in spite of the ongoing response as in (2) above, such response was ineffective in prevent-

ing the progression of the tumor to the clinically dangerous metastatic stage [49, 57]. In this review I will still use the abbreviation IS (immunological surveillance) for some of the alternate mechanisms to be discussed, such as N-CMC, which may not be immunological in the classical sense, such as responses mediated by T or B cells.

1.3 *Escapes from surveillance*

The detection of immunological or para-immunological host responses against the tumor in certain clinical and experimental situations, usually of the type 2 discussed previously, prompted the theories (and experimental studies) on the 'escape' mechanisms from surveillance or from the ongoing tumor immunity (as in 1 and 2 in the previous sub-section) and on the 'subversion' of the immune response of the host by the tumor itself (see [53, 58–65]). The mechanisms for escape/subversion of the IS or of the anti-tumor responses once the tumor is established (for a detailed discussion of 'concomitant immunity' see ref. [57]) have been variously attributed to different possible mechanisms, such as: (1) immunodepression by the oncogenic agents themselves ([15, 66]; see also [16, 17] and [21] for critique of this concept; this point will be discussed again in the section on N-CMC as a possible mediator of IS); (2) blocking factors produced by either the host and/or the tumor [58–61] and (3) more recently, by suppressor cells [62–64]. It was recently indicated [65] that '... the current tendency to attribute the absence of expected immunological reactions to suppressors is a new occupational hazard in the field of tumor immunology...'. By 'suppressors' is meant, suppressor cells [65]. I feel that such 'occupational hazard' has been quite endemic in the tumor immunology field, even before suppressor cells were discovered, and could be applied to any of the temporary waves of enthusiasm, which all of the proposed escape mechanisms have generated (suffice it to read the 'blocking factors' literature of the early seventies, see also [68]). I think however, that the commentary by George Klein cited above [65] contains one very critical point which relates to the peculiar psychology of studies in oncogenesis, including tumor immunology, and that is the 'absence of expected' part; since high expectations from the different branches of the immune system, whether conventional or not, have certainly dominated this area of research. Thus, when an expected result is not supported by the experimental data, an escape or subversion argument is proposed, to explain the negative correlation. I think that this approach stems from the neo-darwinian notion that the body, in its internal wisdom, *must* have some sort of defense mechanism, either against incipient cancer (as in IS) or against the established tumor (as in tumor immunology proper). This notion has been a most powerful driving force in cancer-oriented research (see [21, 25] and [29] for further discussion of this point), and certainly is also a driving force in the present studies of N-CMC. Whether as

an absolute theory applied to all forms of cancer [15, 54] or as a more restricted one related only to some forms of cancer [52, 53], such a defense mechanism against incipient cancer has been thought to be one of the main functions of the immune system. This belief has not been shaken either by the lack of experimental and clinical support of the IS theory nor by the concept that the evolutionary pressure for the development or maintenance of IS against tumors is probably negligible [1, 21, 25, 29]. As a matter of fact, it is most probable that what is measured as anti-tumor immune and para-immune responses, including N-CMC, may be just a functional by-product of the rather efficient immunological (and para-immunological) defense mechanisms against microbial, viral and parasitic invaders in mammals and other species [9]. To support this argument, suffice it to inspect the cause of death in the immunodepressed patients for organ transplantation, the patients with primary or acquired immunodeficiencies and even the recently described 'acquired immunodeficiency syndrome', where the uncontrolled infections by a variety of facultative organisms, and not malignancy, are the main mortality factor. Thus, at the time of the question, why do we have such an efficient system to reject surgical artifacts (i.e. the homograft reaction) exemplified by the premonition of IS [67]; such question could have been simply answered by the statement of Leslie Brent [68] that '... the homograft reaction may be regarded as the price paid for an efficient system of defense against bacterial and viral invasion...'. I have argued in the past, that if the same experiments in immunodepression and tumor development which failed to strongly support the IS theory, would have been done with microbial, viral or parasitic infections, a very strong case for IS against such invaders would have been constructed [21]. Even the peculiar endotoxin-induced 'tumor necrosis factor' in murine serum, which under certain circumstances produces rather spectacular tumor cures [69], has recently shown a marked specificity for lysing lethal murine malarial parasites [70]. One important point of the malarial study, is that '... other components of the immune response appear to act together with these soluble mediators...' to eliminate the parasites [70]. This last comment should alert us that probably there is no *single* mechanism which mediates these types of defensive responses (including the putative defenses against tumors). As will be seen in our discussion of N-CMC in this review, it may be quite probable that a similar strong case is emerging for this putative surveillance device.

1.4 *Scheme of this review*

In this review I will discuss two main aspects of IS: (1) A brief survey of the experimental and clinical studies on what can be called 'conventional' IS (Section 2), where the effects of procedures that depress immune func-

tions or other immunodeficiencies are used in tumor development studies and where the interpretation is directly related to the definition of IS as in Burnet [15]; these will be studies mostly performed during the past 3-4 years, since writing my last review on IS [29]. (2) In Section 3, I will discuss the studies on tumor development in nude mice, which will serve as an introduction to Section 4, which will discuss the possible role of N-CMC, especially NK cells as effectors of IS, or plain surveillance if preferred. As in the past, I will only discuss tumor induction experimental studies, and only marginally the experimental studies using transplanted tumors (see [21] for detailed reasons).

2. STUDIES ON CONVENTIONAL IS

Since the writing of my last review on IS, which was completed in September, 1979 and published in 1981 [29], a few experimental studies have appeared dealing with tumor development in different immunodepressed conditions, which will be discussed here. Almost all of these studies could be considered to have the original IS theory as conceptual background. The clinical studies will also be discussed under this heading, since in spite of some highly interesting divergences from the IS dogma, these studies can still be considered as a result of the IS conceptual framework.

2.1 *The experimental studies*

The actual number of animal studies on the effects of immunodepression on tumor development has been relatively low, when compared to the early seventies, at the peak of acceptance of IS. However, some studies deserve mentioning.

2.1.1 *Immunodepression and tumor development*

Several studies have used skin carcinogenesis in murine models. One study using mice and a combination of local skin application of low-penetrating beta-rays followed 10 days later by topical application of 4-nitroquinoline, showed that the procedure caused marked immunodepression of T cell functions; however, there were no significant differences in the degree of T-cell immunodepression between the groups of mice which developed malignant skin tumors versus either those that developed benign skin papillomas or those that did not develop any tumors [71]. The authors conclude that reduced T cell function was not a direct cause nor even a prerequisite for development of skin tumors in mice [71].

A second study, using UV-induced or benzo(a)pyrene skin carcinogenesis in mice subjected to a variety of 'immunodepressing' chronic treatments,

showed that although some of the treatments (such as cyclophosphamide, cortisone or anti-lymphocyte globulin for the UV studies, and only cortisone for the benzo(a)pyrene studies; with methotrexate having no effect on both systems) produced an acceleration of tumor development, with in some cases, a shift of predominant tumor type from fibrosarcomas to squamous cell carcinomas; such effects could not be considered a function of immunosuppression, since none of the treatments used were actually immunosuppressive in the animals [72]. These authors conclude that, either the treatments were producing some form of subtle imbalance of immune functions or that the reduction in latency periods was due to some non-immunological reason, ranging from direct oncogenic effect of some treatments to other mechanisms suggestive of 'cocarcinogenesis' [72]. In a different study, it was shown that acceleration of skin carcinogenesis after topical application of benzo(a)pyrene (BP) correlated with levels of anti-BP antibodies; with tumors appearing sooner in the animals with the highest levels of anti-BP antibodies [73]. The authors concluded that this was an example of antibody stimulation of carcinogenesis [73], as was observed in other in vivo models [74]. However, it was also stated that 'how antibody to BP enhances BP tumor induction is not known', and specificity of the effect was not controlled in the experiments [73].

A third study, on the effects of anti-thymocyte (ATS) or antilymphocyte (ALS) serum on two-stage skin carcinogenesis in mice, also showed some differential effects [75]: tumor incidence went from 48 (per 30 animals at risk) in the controls to 62 and 88 in the ALS and ATS-treated animals, when the treatment was given after initiation; with no effect of ALS or ATS on tumor development when the treatment was given after promotion. The authors conclude that ATS after tumor induction increased tumor incidence per mouse [75]. One puzzling observation in this study, which is not discussed, is that there was a disproportionate increase of regressing skin tumors (mostly papillomas, in all the groups) in the animals treated with ATS or ALS at initiation, when compared to the controls; in addition, there was no monitoring of the immunological effects of the treatment [75]; thus it is difficult to interpret these results as being truly immunological. This is important since in the same model of two-stage skin carcinogenesis, using 9,10-dimethylbenzo(a)anthracene as inducer and phorbol myristate acetate as promoter in the same type of Swiss mice, it was shown that age of initiation is an important factor; with a significantly lower tumor incidence when the initiation (and promotion) is given at 68 weeks of age, than when initiation is given at 8 or 48 weeks [76]. This last set of results, and somewhat similar lower incidence of fibrosarcomas induced by methylcholanthrene in a single stage carcinogenesis study with nude mice when the carcinogen is given to older mice [28], would suggest that other factors, related

to the effects of age on target cells for induction-promotion, may be operative. Concerning immunological factors, most mice at the ages tested in ref. [74] begin to show signs of age-dependent immunodeficiencies [28]. On the other hand, epithelial cells in culture derived from old murine donors are more susceptible to *in vitro* transformation after exposure to chemical carcinogens than cells from young donors [77]. An apparent increased resistance to *in vivo* carcinogenesis in older mice would certainly not have been predicted by the conventional form of IS as postulated by Burnet [15, 64]; for further discussion of the putative effects of aging on experimental carcinogenesis see ref. [21]) nor by some other defenders of IS in its more absolute forms [78]. In summary, it is apparent from the studies described above using skin carcinogenesis models, that a variety of 'pseudo-immunological' interpretations have been proposed to explain the results, but that it is difficult to make a strong case for truly immunological events affecting the observed results.

One additional experiment using a special model of skin carcinogenesis in mice, is that of Outzen [79], showing that local skin papilloma development on carcinogen-treated skin grafts showed a bi-phasic curve for incidence, when applied to immunodepressed syngeneic BALB/cByJ mice, which correlated inversely with the degree of partial immune competence as tested by skin allograft rejection: the higher tumor frequencies were observed in the animals with the intermediate levels of immunity. These results are considered to be supportive of the 'immunostimulation' theory of tumor development [80, 81]. This is an interesting observation which is not easy to evaluate, for the following reason: although some differences are observed between the different groups, when the results are scored as percent of papilloma bearing grafts per total number of mice grafted (i.e. the frequencies are 22% in the normal animals, 52% in thymectomized + 450R whole body irradiation and 73% in thymectomized + 750 R + bone marrow + 10^7 syngeneic spleen cells), such differences disappear in another experiment using thymectomized irradiated animals (dosages from 200 to 750 R + syngeneic marrow). Furthermore when the Tx + 750 R group of both experiments are compared, the incidence of papillomas per grafted animal is 39% in the first experiment versus 55% in the second, which is within the ranges of the differences observed in the first experiment between the different groups. Thus, it is not easy to decide what is actually being supported by these experiments. To some extent they seem more confirmatory of the interesting experiments by Andrews [82] using a somewhat similar model, showing that papilloma frequency was lower and papilloma regression more frequent in autografts of carcinogen-painted skin, when compared to the non-grafted carcinogen-treated skin. This was interpreted as indicating a failure of surveillance to detect *in situ* lesions, since the surveillance me-

chanisms required the complex inflammatory reaction associated with skin autografting to be effective [82].

The observation that anti-tumor immunity to a transplanted chemically-induced fibrosarcoma was increased, as evidenced by slower tumor growth and fewer spontaneous lung metastases in B-deprived mice [83], was interpreted at that time as probably related to the effects of the B-cell deprivation on conventional anti-tumor immunity, where the antibodies were thought to block cell-mediated responses [83]. B-cell deprivation was produced by life-long treatment with heterologous anti-mouse-mu-chain antibodies [83]. Later studies showed that anti-mu treatment either did not affect the NK-branch of N-CMC [84] or actually increased NK levels [85]. Thus, the transplanted tumor data was re-interpreted as being due to the increase in NK activity in the anti-mu-treated animals [85]. This point will be further discussed in subsequent sub-sections in this review. However, it is worth mentioning here that when a tumor development study was done in anti-mu-treated mice which were injected subcutaneously with 0.5 mg of 3-methylcholanthrene, tumor incidence was reduced in the anti-mu group: 38% in the anti-mu versus 72% in the controls injected with rabbit serum and 73% in the untreated animals 84 days after the carcinogen [86]. Unfortunately, this interesting study was terminated 94 days after carcinogen administration, and at that time it appears that the incidence differences were disappearing, suggesting that only a delay in tumor appearance may be produced by the anti-mu treatment. Furthermore, using a different mouse strain and including athymic nude mice, as well as different dosages of methylcholanthrene, we could not detect differences in tumor incidence nor latency periods in anti-mu-treated animals (our unpublished results).

Finally, one study showed that life-long immunodepression with anti-lymphocyte serum (ALS) and/or azathioprine, did not affect spontaneous tumor incidence in normal BALB/c mice [87]. In addition to this well known fact (see [21, 22, 25, 26, 29] for detailed reviews which contain the appropriate references), this study also showed that the treament could accelerate mortality and decrease latency periods when the animals were infected with a Friend-derived leukemia virus [87]. The authors conclude that '... immunosuppressive therapy was not innately oncogenic and the concept of immunological surveillance was not confirmed...' [87]. However, this statement published in 1980 contains little new information, and may be a good example of what Professor Merton called 'cryptomnesia' in science [88], which is a peculiar amnesia of known facts (in this particular case both by the authors as well as the editors of the journal where the paper was published). Anyway, the paper is confirmatory of one of the major arguments against the general validity of IS.

2.1.2 *'Immunopotentiation' and tumor development*

A somewhat reverse approach to the previously described one, is the study on the effects of 'immunopotentiators' on spontaneous or induced tumor development in experimental animals. These experiments stemmed from the use of some of the products as adjuvants in clinical protocols. The expected results were (again in accordance with the IS theory) reduction of tumor incidences in the treated animals. Unfortunately, some of the critiques that we made in the past to the immunodepressive studies, especially on the lack of concurrent immunological monitoring of the effects of the treatments (see [21, 25, 29]), also apply to most studies with 'immunopotentiators'.

Most of the studies that I consulted show what may be called negative or questionable results: (1) Corynebacterium parvum or BCG either enhanced tumor development or increased metastatic spread in a diethylhydrazine model of colonic tumors in rats [89, 90]; (2) BCG or vitamin A had no detectable effect on carcinogenesis induced by benzopyrene [91], dimethylhydrazine [92] or transplacental carcinogenesis with ethylnitrosourea [93] in extensive and detailed studies using rats, with large numbers of animals at risk and different protocols of drug administration; (3) Levamisole had no effect on tumor induction by dimethylnitrosamine in rats [94]; (4) pyran copolymer accelerated benzo(a)pyrene-induced skin carcinogenesis in mice [95]; (5) BCG administered after initiation (with dimethylbenzanthracene) and before promotion with croton oil of skin carcinogenesis in mice, although not affecting overall incidence of animals with tumors, reduced the mean number of papillomas per mouse [96]; (6) BCG administered 2 weeks before 0.5 mg of methylcholanthrene in Swiss mice reduced frequency of local tumors from approximately 80 to 50%, no effect was observed when BCG was given 9 weeks after carcinogen [97]; (7) Another study with C3H/He mice receiving the same dose of methylcholanthrene as above, showed quite different results: BCG or Wax D decreased latency periods (and with Wax D there was also a slight decrease in tumor frequency) when BCG was given 8 weeks after methylcholanthrene, with no effect or enhancement of tumor development when given concurrently or 4 weeks after the carcinogen; with Wax D, the protective effect was noted at 4 weeks after carcinogen, but not concurrently or after 8 weeks [98]; it is worth mentioning that in a previous study on urethane-induced lung carcinogenesis in mice of an unknown strain, BCG and cord factor reduced tumor incidence, while Wax D had no detectable effect [99]; (8) Perhaps explaining part of the discrepancies between examples number 6 and 7, another study showed that the effect of BCG (as cell-wall skeleton) on local tumor development after exposure to 0.5 mg of dimethylbenzanthracene in mice was strain dependent [100], with some strains being quite susceptible to the BCG

retarding effect, while some others (such as C3H/He) being quite resistant to such effect; unfortunately, the protocol of BCG administration given one day before carcinogen and subsequently at 1, 3, 5, 7, 9 and 11 weeks is not comparable to that used in examples 6 and 7; (9) In the first paper in which the effects of BCG were studied methodically for its effect on tumor development using the mammary tumor model in rats after dimethylbenz(a)anthracene administration [101], it was clear that timing was a very critical and totally empirical factor, and in this study, the only two detectable effects were: decrease tumor incidence when BCG was administered at the time of appearance of the first tumor, with no significant effect when BCG was given before or after the carcinogen at various timings [101].

The summary of these observations is that it is not easy to predict the results of the treatment, based on known immunological effects of the products tested. In addition, if one takes care of noting the dates of the different studies cited, it will be apparent that the more positive and enthusiastic papers are those published during the highest excitement on the possibilities of immunotherapy of human cancer with BCG and similar products. These waves of enthusiasm appear to be important factors in biasing experimental design and interpretation in cancer-oriented research (see [21] for further discussion of this problem).

Before ending this sub-section, I will discuss the effects of a variety of immunopotentiators on spontaneous tumor development in mice [102, 103]. Both these studies have extensive immunological monitoring of the effect of the treatment on a variety of immune functions as well as NK activities [102, 103]. In one of the studies, while BCG treatment actually reduced survival to 19.5 months mean, approximately 50% of the untreated animals or those receiving levamisole were still alive at 24 months of age, when the experiment was terminated [102]. Concerning tumor incidence, although the text indicated that it was lower in the levamisole-treated group, the figure showing that data seems to contradict the statement, since it indicates the detection at autopsy of 11 tumors in 15 untreated mice and 12 tumors in 9 of levamisole-treated animas [102]. It was not possible to determine the actual strain of the animals used in this experiment [102]. In a similar study using C57BL/6 mice treated with azimexon, retinoic acid or tuftsin, again involving extensive immunological monitoring; tumor incidence in 19-month old animals was 22% in the untreated controls, 10% in the azimexon group, 3% in the retinoic acid and no tumors in the tuftsin-group [103]. It should be stressed that the common feature of all three agents was to increase the age-dependent depressed tumoricidal capacity of peritoneal macrophages, while no detectable effect of any of the three regimens seemed to increase the age-related depressed NK activity in spleen [103]. However, both BCG and levamisole could also

increase the age-dependent depression of tumoricidal capacity in macrophages [102]. Again, the interpretation of these results is not easy.

2.1.3 *Suppressor cells and tumor development*

The title clearly indicates that I will not discuss any studies on suppressor cells appearing after tumor transplantation or affecting growth of transplanted tumors (see [60-64] and [104] for review of such studies). Many of such studies are not even relevant to biology, let alone tumor immunology, as I indicated in a previous review [104]. Who actually cares about the suppressor cells that appear in a mouse bearing a tumor which represents 10% or more of the animals' body weight? How would one translate such 'model' to a clinical situation?

However, I will discuss here, some studies which attempted to determine the role of suppressor cells in tumor development. As with many of the other trends, the 'occupational hazard' represented by the epidemic of suppressor cells in tumor immunology [65], which unfortunately mostly belong to the category of experiments discussed in the previous paragraph, also produced studies on tumor development which are difficult to interpret.

For example, the observation that a rather stern schedule of cyclophosphamide administered before 0.1 mg of 3-methylcholanthrene to BALB/c mice (2 mg per mouse 13 days before the carcinogen and at 10 day intervals thereafter for the duration of the experiment) delayed the appearance of local fibrosarcomas and reduced tumor incidence, was interpreted as cyclophosphamide 'interfering with either a suppressor cell mechanism or an immunostimulatory mechanism' [105]. However, the authors also indicate that the results are 'equally consistent with a non-immunological interpretation...' related, for example, to the direct effect of the drug on the developing tumors [105]. However, it is worth mentioning that the motivation for performing the experiment was the putative effect of cyclophosphamide on suppressor cells [105].

The UV-light-induced carciongenesis in rodents [47] is a good example of a tumor system which is strongly influenced by a complex set of host-mediated immunological responses. In this rather unique model, the sarcomas induced by irradiation are highly antigenic, usually do not grow when transpanted to normal animals and require immunosuppression or UV-irradiation of the host for successful growth [47]. The few progressor variants which grow in normal syngeneic hosts without need for further suppression, appeared to have lost the surface determinants recognized by T cells, and not by either NK cells or non-specific cytotoxic T cells [48]. In addition, an age-dependent decline of tumor-specific and idiotype-specific T cell-mediated immunity, correlates with transplantability of tumors which otherwise would have been rejected [106]. Thus, the case of a role for T cells

in this system is indeed strong [47]. In addition, suppressor T cells are also induced in this system, which interfere with the development of the specific T cell immunity [47, 63]. The common interpretation is that the suppressor cells are the permissible factors allowing the development and growth of the tumor in the original UV-irradiated host ([47], this is an excellent review containing all the pertinent references). The mechanism of suppressor cell induction by UV-light is probably through its action on antigen-presenting accessory cells [47, 63]. However, only one study addresses the possible role of all such immunological concomitants described above using transplantation methods [107]. In these experiments, mice were lethally irradiated and repopulated with lympho-hemopoietic cells from normal or UV-irradiated syngeneic donors (the UV-treated animals having the putative suppressor cell component), these animals were subsequently grafted with large skin flaps of UV-irradiated light, and local tumor development followed; the animals receiving the cells from the UV-treated donors had increased probability of tumor development [107]. In a different type of experiment, partially purified T cells from normal or UV-treated donors were injected intravenously on 4 occasions, before and after UV-irradiation of the hosts, and tumor development monitored; the study showed an augmentation of tumors and acceleration of tumor appearance in the animals receiving the UV-treated T cells [107]. These two experiments combined, make a rather strong case for the possible role of suppressor cells (T) regulating tumor development in this model; although one may consider the first type of experiment to be a variation of the experiment by Andrews [82] discussed previously, in the sense that the skin grafting procedure may actually trigger the immunological recognition or lack of it in the hosts; or the remarkable effect in the second experiment of the injected cells into normal hosts, which contradicts most information on cell transfers, which usually require 'space' for proliferation [108]; or by the fact that such cells behaved that way in the midst of the UV-irradiated environment of the host and retained their qualities (especially the normal T cells, which actually reduced tumor incidence when compared to the uninjected controls, see [107]). However, in spite of these criticisms, these experiments make a strong case for a new interpretation of one of the 'old' arguments in the discussions of IS, i.e. the immunodepressive effect of the carcinogenic agent proper [15-17, 21, 25, 53, 54, 66]. This model would suggest that in the case of UV-light, the carcinogen would also act as an inducer of a suppressor cell population, probably T, which would enable the highly antigenic tumor to get established in its original host.

Finally, I will briefly discuss one example from our studies with the mammary tumors which appear spontaneously in mice infected with the murine mammary tumor virus [109]. In the tumor-prone virus-infected ani-

mals, it has been shown that the tumors appear in these animals, in spite of a variety of specific and non-specific host responses to viral components ([109] contains the appropriate references for this point). Such a situation is accompanied by the development of specific suppressor T cells, which prevent the interaction of helper T cells with the precursors of cytotoxic T cells, producing as a consequence, a rather inefficient cytotoxic T cell response, when compared to that obtained in mice not infected with the virus [109]. In these studies, the correlation between the *in vitro* with the *in vivo* responses was not easy, and even the putative role of the suppressor cells affecting tumor development could be questioned [109]. The best example of these problems, is the effect of adult thymectomy [109]. Adult thymectomy produced a total disappearance of the suppressor T cells in spleen, allowing an excellent *in vitro* cytotoxic T cell response against the tumor [109], however it did not affect the overall tumor incidence nor time for tumor appearance in these animals, which were technically devoid of suppressor cells [109]. If the *in vivo* studies on tumor development mentioned above, showing that adult thymectomy did not affect mammary tumor incidence, would not have been performed, it certainly would have been tempting to decide that the observed effect of thymectomy on the suppressor cells measured *in vitro*, could finally explain the puzzling observation made in 1964 on early thymectomy actually decreasing mammary tumor incidence in the virus-infected mice [110; a phenomenon that at some time, during the peak of interest in blocking factors, was attributed to thymectomy inducing a decline in blocking factor production [111]. However, we found that only thymectomy at 6 days of age, but not at 30 or later (as done in the suppressor T cell experiments) had any effect on reducing overall tumor incidence [109]. Thus, I was forced to indicate that in many cases one could not avoid the impression '... of forcing the *in vitro* data to fit the *in vivo* results...' [109], based on the expectancy of the investigator. As I have indicated in another text [46], imagine the discussion that could have accompanied the description of the effects of thymectomy on suppressor T cells measured *in vitro*, if the experiments on the effect of adult thymectomy on tumor development would not have been performed!

However, and in spite of the proliferation of studies on suppressor cells in tumor bearing hosts, the actual studies on the possible regulation of the putative surveillance mechanisms by suppressor cells has bearly been studied in tumor development models.

2.1.4 *Selection, clonality, carcinogen dose and other problems*

The ideas of some form of selection of tumor variants *in vivo* has been quite dominant in attempting to explain escapes from IS [53, 112]. The initial thoughts were related to selection of variants by the host defense

mechanisms, which were resistant to such mechanisms [53, 112]. These views have been recently revived in relation to tumor progression [2, 3, 6, 113]. In addition it is also apparent that tumor heterogeneity [56], with predominance of certain variants, especially in metastasis, is an intrinsic component of tumor behavior [56], which may or may not be influenced by host responses.

One of the arguments used against surveillance [4], is that most human tumors appear to be monoclonal in origin [114]. Thus, the argument against IS states that it does not exist because it permits the development of a tumor from a single progenitor cell [4]. In experimental tumors produced by methylcholanthrene in mice, using the comparable enzymatic markers for assessment of clonality, it was found that at high dosages of methylcholanthrene (MCA), 0.2 and 2 mg/mouse, the tumors were polyclonal [115], while at lower dosages (5 μg/mouse) the tumors were monoclonal [116]. In another study, using either 0.5 mg of MCA injected subcutaneously (as in [115] and [116]) or implantation of plastic discs impregnated with 0.1 mg of MCA, it was found that of 34 tumors studied, 13 were clearly monoclonal, with the rest being pleoclonal and showing no clear correlation with carcinogen dose [117]. However, the important finding in this study is that the clonal composition could change markedly during tissue culture or *in vivo* transplantation [117]. The authors conclude that the number of clones present at any given time depend on the number of cells originally transformed and the growth rate of each clone; that the number of cells transformed will depend on nature and dose of the carcinogen and that the growth rate of each clone may be affected by its inherent properties, the host reaction or interactions between different clones of transformed cells [117]. In addition, if growth rates remain constant, the clones with the greatest growth rate will outgrow the others [117], which we termed clonal dominance while discussing the monoclonality of tumors [29]; however this does not preclude the persistence of small clones of slower growing cells nor the possibility of sudden changes in growth rate [117]. In summary, this last paper shows the complexities of the interactions between different cell populations within the tumor as well as with host components [117], which also fit with the biological data on heterogeneous behavior of tumors [56]. The question of clonality versus polyclonality (or 'pleoclonality' to use the terminology of ref. [117]) may be contingent on a variety of complex interactions between different tumor cell populations within the tumor as well as with stromal and other host components, which shows marked temporal variability.

Although I will not discuss here the question of conventional antigenicity of tumors, as measured by transplantation and similar techniques, which in the past presented an obstacle for the IS theory, when tumors with no

detectable antigenicity were described [12, 21, 25, 29, 118, 119]; I will discuss the relationship of carcinogen dose and antigenicity, which bears some relationship to the conceptual framework of IS.

In our previous studies we showed that the antigenicity of MCA-induced tumors in severely immunodepressed animals was comparable, with approximately 30% of the tumors belonging to either the high antigenic or the non-antigenic variety, in two different mouse strains at a single carcinogen dose of 0.2 mg/mouse in oil, injected subcutaneously [16]. Subsequently we expanded the studies and tested a large number of tumors (110 tumors) induced by 0.01, 0.02, 0.03, 0.05, 0.2 and 2.0 mg/mouse of MCA in C3Hf/Umc mice [21], showing that, again, the proportion of tumors with high or with undetectable antigenicity was comparable for all the carcinogen dosages. In a concurrent study, using (BALB/c×DBA/2)F_1 mice, receiving paraffin pellets impregnated with different amounts of MCA, it was shown that the tumors appearing in animals receiving 5% MCA had a tendency to display high antigenicity (3 or perhaps 4 out of 8 tumors being highly antigenic), while those animals receiving 0.05% MCA showed tumors with low or undetectable antigenicity [120]. The author concluded that if indeed 'spontaneous' tumors may result from low levels of carcinogen, such tumors will not be affected by IS, since they will have a tendency of having '... little or no immunogenicity as demonstrated by excision-challenge type assays...' [120]. However, it is not easy to compare the actual amounts of MCA in refs. [16] and [21] with that in ref. [120], since the 'percent' value is not specified; in addition, there is a confusion on techniques, since ref. [120] indicates that the paraffin pellets were prepared as in the reference by Bartlett cited at the beginning of this discussion [118], which however, uses MCA in melted paraffin which impregnates plastic discs, with each disc containing approximately 4 µg of MCA [118]. Thus, it is not clear whether 'pellets' or 'discs' were used in the Prehn study [120]. This difference may be of importance, since Prehn indicates that '... the total amount of MCA in the pellets is not a significant variable in this method of oncogenesis, since only the chemical near the surface of the pellet is functional...' [120]; however, a recent study has shown that MCA in benzene applied to plastic discs, is rapidly metabolized and removed from the discs in 7 days and distributed widely via the blood stream; provided that an appropriate local macrophage reaction takes place around the disc, with the macrophage being the candidate for providing the enzymatic degradation of the MCA [121]. It is worth mentioning here that in the study of Bartlett with the plastic discs containing approximately 4 µg of MCA [118], the proportion of highly antigenic and non-antigenic tumors was comparable to our own studies [16, 21]; with the only correlation being a tendency of the highly antigenic tumors to have short latency periods, and for the tumors arising from 'spontaneous' trans-

Table 1. Antigenicity or immunogenicity of fibrosarcomas induced in C3Hf/UMC mice by different dosages of 3-methylcholanthrene (MCA).

MCA dose mg[a]	Number of tumors tested	Mean antigenicity ratio±SE and range[b]	Antigenicity[c]	
			None	High
0.01	16	5.48±1.33 (1.0-20.2)	4	7
0.02	20	6.28±1.37 (1.0-24.1)	6	9
0.03	20	5.31±1.12 (1.0-20.1)	5	8
0.05	21	5.57±1.37 (1.0-26.0)	5	8
0.20	20	5.54±1.36 (1.0-24.3)	6	8
2.00	22	5.56±1.29 (1.0-26.3)	6	8

[a] The indicated amount of 3-methylcholanthrene in mg, in 0.1 ml of corn oil was injected subcutaneously into 8-10 week old mice as described in ref. [16]. Latency period for the tumors used ranged from 170-250 days.

[b] The antigenicity ratio using normal and immune syngeneic hosts was determined as in ref. [118], using tumor cell suspensions for immunization, as in refs. [118] and [122].

[c] Number of tumors with undetectable antigenicity, i.e. with an antigenicity ratio of 1.0; "high" antigenicity was arbitrarily defined as having a ratio of 5.0 or more.

formation of fibroblasts enclosed in cell-impermeable diffusion chambers to be non-antigenic [118]. Furthermore, a study of the techniques for detection of antigenicity of the tumors, comparing, among others, the technique used in our study [16, 21] with that used in Prehn [120] and Bartlett [118], showed no significant differences in sensitivity of the techniques [122]. Thus, with the proviso that the actual amounts of MCA in the Prehn study [120] are undetermined, although the 'high' and 'low' differ by a hundred-fold, and that different mouse strains were tested, it is difficult to explain this discrepancy. Furthermore, if the tumors from ref. [21] are retested using the 'immunogenicity ratio' as in Prehn [120], which is similar to the procedure defined by Bartlett [118], where a ratio of 1.0 menas no antigenicity, the lack of difference in antigenicity between the different MCA dosages is still apparent (see Table 1). For comparison, in the Prehn study [120], the mean ratio for the 5% MCA dose was 8.8 (the individual values for the 8 tumors being 25.3, 18.7, 9.3, 4.0, 3.2, 1.7, 1.3, 1.1) and for the 0.05% being 1.5 (with the individual values being 2.3, 1.9, 1.9, 1.7, 1.4, 1.1, 1.0). It is also possible that some intrinsic variability may exist in the paraffin-disc-MCA procedure, since in a different experiment the low dose of MCA required to detect differences in susceptibility to oncogenesis within a inbred strain, was 0.01% MCA, while 0.05%, which was the low dose discussed previously, had no effect [123].

In relation to strain differences, a study using the same Bartlett paraffin-embedded MCA plastic discs [118] containing again 5 and 0.05% MCA, 10

mouse strains and F_1 hybrids showed a reversed rank order of susceptibility to tumor development depending on the MCA dose, i.e. C3H/HeJ being more susceptible to the 5% MCA than to the 0.05% dose; with the reverse being apparent for C57BL/6 [124]. The authors hypothesized that this was due to the effects of the concentration of MCA on antigenicity of the tumors, discussed previously [124]. However, a recent study using a similar protocol, but with the difference that MCA at 625 μg and 5 μg/mouse in tricaprylin injected subcutaneously were used, could not show any differences in tumor development between C3H/HeJ and C57BL/6J mice [125]; confirming other studies with these two strains [126]. It is also possible that the 'rejection' of the MCA discs, which was observed in one study [124] as well as the rejection of the tumor-free animals from the analysis [124], may have affected the results, as was proposed by Bernfeld and Homburger [125]. Thus, it is not easy to decide if the effects of carcinogen dose on antigenicity and susceptibility to tumor induction are indeed documented enough to incorporate such concepts into any discussions of conventional or unconventional IS. As will be discussed in Section 4, the alternate hypotheses of IS do not rely on recognition of the conventional tumor-associated transplantation antigens discussed in this sub-section. An alternative interpretation, which appears to be documented by facts [121, 127] may be that the plastic disc procedure of MCA carcinogenesis, with its peculiar dependence on a macrophage-mediated radiosensitive host component required for the metabolism of the MCA in the plastic disc [121], may be a unique model from which generalizations may not be warranted. It is also possible that most of the experimental models of chemical carcinogenesis, especially those using injection of a single and usually rather large dose of the chemical, may not be representative of the putative environmental attack by such products, although they are certainly highly relevant in determining metabolic pathways as well as other host responses to the chemicals.

The issue of immunodepression by the chemical and our criticisms to the concept (see [16, 17, 21, 25, 28, 29]) will not be repeated here, but will be discussed again in the context of N-CMC in Section 4. Suffice it to close this paragraph by indicating that one still finds, in the relatively recent literature, studies expressing some surprise that the chemical carcinogen tested at low but still carciongenic dosages, has no detectable effects on the conventional immune responses of the host [128].

Finally, breaking my own rule of not discussing tumor transplantation models, I will discuss briefly the 'sneaking through' phenomenon, initially observed by Old et al. [129]. This phenomenon consists in the preferential take of tumors from small size or cell number inocula, in comparison to the large size inocula, with rejection of the middle size inocula. A variety of interpretations following the trends of the times have been used to explain

this phenomenon, ranging from discrepancy between growth and recognition [53, 112, 130] to low-zone tolerance [131] probably mediated by suppressor T cells [132, 133]. However, it was considered as a strong example against IS, since it appeared that the conventional immune system was incapable of detecting small inocula of tumor cells [29, 112, 130]; and comparisons were made between similar situations in immunity to bacteria and other parasites, including concomitant immunity [21, 25, 29, 49, 57, 134, 135]. The reason for discussing sneaking through here is that some studies have used the Meth A chemically-induced fibrosarcoma [129, 133] as a prime example of an antigenic tumor with sneaking-through capabilities, which is also one of the prototype tumors that we used for the definition of the natural cytotoxic (NC) branch of N-CMC [136]. However, it is tempting, based on the extreme sensitivity of Meth A to NC cell killing *in vitro*, to interpret some of the data in ref. [133], showing that sneaking through of Meth A is not detected in T deprived or nude mice, as a consequence of a T cell interference with NC or N-CMC effector cells in general, as shown for NK cells under certain circumstances [137], rather than to conventional antigen-specific suppressor cells operative in a low-zone tolerance situation [133]. This point will be further discussed in Section 4.

2.2 *The clinical evidence*

It is apparent that as the clinical studies on the association of immundeficiency and certain forms of malignancy become more incisive and complex (see [50] for a good example), my own comments on the subject become more shallow and cursory, compared to those in previous reviews [21, 25, 29].

In general, it is agreed that once the clinical material was analyzed following proper age-related incidence for the different tumors, defining the 'expected' frequency of the tumors; the great preponderance remains for the general category of non-Hodgkin lymphomas (NHL), and vanished for most of the other forms of malignancy, with the exception of some forms of skin cancer. This statement applies to the group of patients undergoing renal transplantation [138, 139]; the cardiac transplantation group [140, 141]; the patients receiving immunodepressive drugs for other causes besides transplantation [139, 142], although the risk for NHL or skin is much lower than for the transplanted group [139, 143] or for the patients with immunodeficiency syndromes [143–145]; the group of patients with the severe immunodeficiency syndromes of different types, especially the severe combined deficiencies [144, 145], especially if the pediatric populations are analyzed [144, 145], this group has been collectively known as 'naturally occurring immunodeficiencies' and represent a very heterogeneous group of patients, regarding age of onset, severity of immunodeficiency, possible gen-

etic abnormalities, etc. [145], and the group of uremic patients in chronic renal dialysis without transplantation [146]. In this last case, the initial anecdotal literature, as was the case in most of the other examples, gave the impression of actual increase in the more common types of malignancies [147–149], which was not corroborated in other series with larger number of patients and better estimation of tumor risks [146, 150]. For further information on these tumor frequencies, as well as some other categories of increased lymphoid malignancies such as the group of patients with autoimmune disorders, see the excellent review in ref. [143], as well as the factual data in references [151] and [152] (in [152] concerning malignancies in 1,348 organ allograft recipients) and the analysis of the naturally occurring immunodeficiencies and 733 malignancies in transplant recipients in [145].

Before discussing some aspects of the lymphoid tumors and NHL, a brief comment on the skin tumors appearing in the transplant group. Firstly, the preponderance is of squamous cell carcinomas of the skin and lip, with basal cell carcinomas being less frequent, which is the reverse situation from the normal population, and showing some variations in incidence in relation to sunshine exposure [5, 138, 139, 152]. Secondly, the frequency of melanomas was not increase or affected, even in series from regions where melanoma is frequent [139, 152]. Thus, one may conclude that a very peculiar type of skin cancer, which in most cases is not life threatening for the patients, although it may have a more aggressive behavior than in the normal population [152], is the predominant one in the patients undergoing kidney transplantation. Whether this is indicative of certain skin cancer being controlled or affected by some form of immune response of the host is still a mostly hypothetical question. An alternative view, since some increased risk for similar types of skin cancers was observed in patients receiving immunodepressive drugs for causes other than transplantation [139], may be that some of the drugs used for immunodepression may be related to this type of skin cancer, either as inducer or promoter of the squamous cell carcinomas.

The most important advances in the studies on the association of malignancy with immunodeficiency, have been, firstly, the beginning of the analysis on the pathogenesis of the lymphoid tumors [50, 153–165] and secondly, the possible grouping of immunodeficient patients into different clinical entities in which defective immunoregulation, as proposed some time ago [51], may be the permissive factor allowing the uncontrolled lymphoid proliferation [145], perhaps permitting a better understanding of lymphoma-leukemia development. Thus, it seems that the negative conceptual influence exerted by the IS hypothesis over the studies of this unique clinical material, discussed in Section 1 of this review and in reference [29], has finally been overcome. And indeed, with increasing numbers and better analysis, some interesting correlations are emerging. For example, in a study

of 35 cases of lymphoreticular disorders in primary immunodeficiencies of different types, it was found that 60% were NHL (non-Hogkin lymphomas) of which 38 were B-immunoblastic sarcomas; and 23% were Hodgkin's disease, predominantly of the lymphocytic depletion type, which is quite rare in young patients [153]. Three of the lesions, of the 35, could not be categorized as benign or malignant [153], a problem that we stressed some time ago [21, 25, 29] concerning the accuracy of the registries of lymphomas in immunodepressed patients. Furthermore, while the predominant type of disorder in the Wiskott-Aldrich syndrome was the B-immunoblastic sarcoma, in the ataxia-telangiectasia group there was a predominance of either Hodgkin's (of the lymphocytic depletion type) or NHL of the type usually associated with the 14q translocation [153]. In addition, all of the 7 lymphocytic leukemias studied in the ataxia group proved to be of T lineage [145]. On the other hand, in the heart transplant patients, all of the tumors were of the NHL type, with predominance of HLA-DR positive-immunoglobulin and T-cell markernegative cells [159]. The presence of Epstein-Barr virus (EBV) has been documented in the lymphoproliferative disorders associated with the x-linked lymphoproliferative disorder [155, 156], as well as in some of the lymphomas in renal transplantation patients [154, 157]; where it is postulated that the EBV transformed B cells in association with the impaired host defense responses, may be the mechanism for the development of such tumors [154–157]. Further analysis of these tumors showed marked B cell abnormalities and polymorphism [153, 158], and the term 'polymorphic B-cell lymphomas' or 'polymorphic diffuse B-cell hyperplasias' has been proposed for such type of lesions [158]. On the other hand, some probable monoclonal B-lymphomas have also been detected during the evolution of these tumors [154, 157], possibly as a result of some type of clonal dominance as discussed in Section 2.1.4 in the experimental models. Diffuse polyclonal B-cell lymphomas have also been observed in primary infections with EBV [160] or in some cases evolving directly from infectious mononucleosis [161]. In addition, donor type of immunoblastic B lymphomas, in one case actually bearing EBV genome, have been observed in patients with leukemia after bone marrow transplantation [162, 163]. Thus, a strong case for EBV as an important oncogenic factor in patients with immunodeficiencies [154] as well as for conditions of unregulated growth of lymphoid cells, especially B cells [51], is emerging from the analysis of this clinical material. Finally, while NHL are common in the untreated cases of severe combined immune deficiency (SCID) in children [144, 145], these have not been observed in 35 patients with successful bone marrow engraftment [164]. However, such fatal B lymphomas frequently appear in SCID treated with thymus grafts derived from allogeneic donors [164–165], in one case also associated with EBV [166].

Thus, the question concerning these lympho-proliferative disorders is, whether they are associated to actual deregulation of normal controls for differentiation, related to the underlying disease or triggered by the immunodepressive therapy which has the lymphoid system as target; or whether, as proposed by some [155], the main permissive factor for the proliferation of the abnormal lymphoid cells is the underlying natural or induced immunological deficiency, which otherwise would have controlled such abnormality, especially if triggered by factors such as EBV [155]. Thus, this second alternative would still be in line with some form of IS. Interpretations somewhat combining both views have also been proposed [52]. I will discuss in some detail the possible role of N-CMC, especially NK cells, both as regulators of lymphoma proliferation and as mediators of some homeostatic control of normal lympho-hemopoiesis in Section 4.1. It is possible that a model for development of B-lymphomas via de-regulation of the immune system may be represented by the lymphomas appearing in anti-mu-treated nude mice [46] to be discussed in Section 3.

The Kaposi sarcoma issue deserves mentioning here, since increased frequency has been reported in immunodepressed patients receiving immunosuppressive drugs for organ transplantation [152, 167] or for other reasons [167, 168] as well as for its association with other malignancies [169]. Furthermore, a possible association with cytomegalovirus has been suggested [170], thus, the possibility of another malignancy with putative viral triggering and with accompanying immunodepression allowing progression, is comparable to the NHL previously discussed. In addition, Kaposi sarcoma has also been observed in the recently described 'acquired immunodeficiency syndrome' (AIDS) seen in homosexual men and other patients [171–176]. I will not speculate too much about this unusual entity, since there is enough speculation in the scientific [177, 178] as well as the non-scientific publications [179, 180]. However, it is worth pointing out that in the severe immunodeficiency which accompanies AIDS, whatever its pathogenesis may be, the main cause of death still is the rapid and uncontrollable infections by a variety of opportunistic organisms [172 176], and to a much lesser extent the Kaposi sarcoma or other malignancies [171–175]. This bring us back to some of the arguments about IS and its role discussed in several parts of Section 1 of this review. To further stress this point, a recent survey of primary immunodeficiencies in children, showed that while NHL were observed in 2 instances out of 201, mortality was 18.4% (32 children) of which 74% (28 cases) were due to different types of infections or administration of live vaccines, especially in the patients with combined deficiencies [181]. Thus, at the risk of being repetitious, while there is a strong case for IS as very important in defense against a variety of infections (and as a corollary, a substantial and deathly risk for uncontrolled infections

in immunodepressed patients), the case for IS against malignancy seems to be restricted to a very narrow spectrum of tumors, in which lymphoid malignancies predominate.

Finally, some conditions in which increased chronic antigenic stimulation as well as immunological dysfunction appear associated, also have been described as associated with increased NHL, such as gluten entheropathy, Sjogren's syndrome, schistosomiasis, autoimmune thyroiditis, and also to some extent rheumatoid arthritis or BCG vaccination (see [143] for further discussion and appropriate references; see also [151]). However, such correlation does not apply to patients with leprosy, where no differences between observed and expected NHL as well as Hodgkin's or leukemia were observed in a careful study [182]. Thus, although it is possible that alteration of the immunological balance may favor NHL development, it is also clear that neither immunodepression nor chronic antigenic stimulation *per se,* may be the sole factor.

3. TUMOR DEVELOPMENT IN NUDE MICE

Although I have extensively discussed this subject in past reviews on IS [21, 25, 29] as well as in experimental and review papers on tumor development in nude mice [18–20, 22, 26–28, 46], it still is a topic of relevance in relation to IS, whether in its orthodox form mediated by T cells [15] or in the newer forms as possibly mediated by N-CMC and other effector cells, to be discussed in Section 4. As a matter of fact these studies represent almost a connecting point between the two areas: on the one hand, they served to cast serious doubts about the experimental support of IS as mediated by T cells [21, 25, 29]; on the other hand, the demonstration of normal or increased levels of N-CMC effector cells in nude mice, prompted interpretations that the 'normal' tumor incidence (i.e. comparable to that of the euthymic controls) could be due to N-CMC [5, 7, 13, 26, 29, 32–36]. However, the data on tumors in nude mice has been handled rather loosely, and two examples will illustrate this situation. Firstly, it has been described that germ-free nude mice partially inbred in the BALB/c background have a high incidence f lymphomas [23]; which however, although within that particular experiment the nudes showed more lymphomas than the normal controls, the actual incidence was well within the variation range of lymphoma incidences in BALB/c mice, including germ-free animals (see [25, 26, 183] for discussion of this point, with the appropriate references supporting the argument). However, statements such as '... a high incidence of lymphoreticular tumors has been observed in congenitally immunodefective germ-free nude mice...' [151], citing ref. [23], are quite common in present day literature.

An even worse example is found in ref. [184], where it is stated, that, 'The low incidence of spontaneous tumors in congenitally immunodeficient nude mice...', again citing ref. [23] as the source. This statement is doubly incorrect since ref. [23] shows possible, although questionable (see previous paragraph), increase of spontaneous lymphomas and normal (i.e. comparable to controls) incidence of chemically-induced fibrosarcomas, which confirmed our earlier findings [18]. Secondly, statements such as '... nude mice lacking T cells do not develop spontaneous tumors...' [4], citing ref. [24], are also inaccurate, as will be seen in this Section (see also [19, 22, 26, 27], for further discussion of this point).

3.1 *Spontaneous tumors and lymphomas*

Before discussing our own results, I will briefly analyze the available literature on spontaneous tumors in untreated nude mice, especially the literature published since 1979, the date of my last review on IS [29] or on nude mouse tumors [26].

The reports of no detectable tumors in rather large numbers (in one case 11,000 mice) of nude mice in either BALB/c or NIH Swiss background [24, 185, 186] as part of the survey of a production colony, in which most of the animals were observed for very short times, usually less than 4 months [24, 185] or where only a small number of animals were actually autopsied [186], are certainly non-informative, and have been criticized in the past in some detail [25, 26]. Similarly, the studies on tumor incidence in nude mice which are used for other experimental purposes [24], especially the grafting of human tumors [187–190] should be considered with some care, since in the case of the lymphomas, such tumors may actually be induced by the putative antigenic stimulation [188, 189]. This point will be further discussed in the next paragraphs. And certainly, the frequencies of tumors developing in such animals will probably not '... throw some light on this confusing picture...', as recently proposed [190]. In addition, most of those studies do not have actual controls and thus, except for stating frequencies for the group of experimental nudes in question, no comparisons with nu/+ or other euthymic controls are possible [187, 190].

It is only through careful studies on tumor incidence in which the animals are kept untreated, in which the observation period includes the whole lifespan of the animals at risk, whether in germ-free or other conditions, and where a complete autopsy of the animals is included, that could be considered to be truly indicative of the actual status of spontaneous tumor development in nude or in any other animal model [26].

One careful study of tumor incidence in athymic germ-free nude mice partially inbred (four backcrosses) in the C3Hf/He background, is unfortu-

nately flawed by the lack of appropriate controls [191]. In this study, the nude mice were compared with the C3Hf/He inbred strain and not with the appropriate heterozygous littermates; thus, although worthless for the comparison between tumor incidences in athymic nude versus euthymic controls, it still shows that nude mice, certainly develop tumors [191]. For example, with groups of approximately 50-60 animals at risk which were observed for their whole natural lifespan, overall tumor incidence was 72% for the nude females and 65% for the nude males (with 93% and 67% incidence in the normal C3H mice), which included both benign and malignant tumors, and where lylphoreticular tumors were observed in approximately 35% (versus 8% in the normal C3Hf/He) and 55 or 33% of other benign and malignant tumors in females and males, respectively [191]. In the normal C3H/F/He, there was a high incidence (28%) of fibrosarcomas in females, and an unusual incidence of hepatomas with 4% in females and 28% in males [191]. In the data compiled by Murphy for C3Hf/He, the incidence of fibrosarcomas is 3% in males or females; the incidence of lymphomas is 3% for both sexes and the incidence of hematomas is 25% in females and 17% in males [192]. In a study comparing frequency of common naturally occurring tumors from six different laboratories in (C57BL/6N × C3H/HeN)F_1 mice it was found that while the incidence for lung-liver and leukemia-lymphoma was relatively homogeneous within laboratories, significant inter-laboratory heterogeneity was observed, with the causes for such variability being unknown [193]. A good example of intra-laboratory variability is described in ref. [190]. In two separate studies in which nude mice in a Swiss outbred background were set aside for spontaneous tumor incidence, with 50 animals per group and sex, and nu/+ controls, which were studied for their whole life span; some marked variations in tumor incidence were observed [190]. The overall tumor incidence was 2% in nude males and 8% in nude females in the first experiment, versus 4% and 10% respectively in the second; while the incidence in the nu/+ was 2% and 4% respectively for males and females in the first experiment, versus 18 and 24% respectively in the second experiment, with practically no differences in the mean life-span of the nu/+ animals between both experiments [190]. With the lymphomas, the incidence was 8% in the nude females in the first experiment, with no lymphomas in either the nude males or the nu/+ of both sexes; while in the second experiment lymphoma incidence was 6% in nu/nu females and 4% in nu/nu males or nu/+ of both sexes [190]. Finally, with pulmonary adenomas, the first experiment did not show any such tumor in any group, while the second experiment showed no such tumors in nu/nu males and 2% in nu/nu females versus 12 and 15% respectively in nu/+ males and females [190]. I certainly hope that this last set of numbers is not quoted as evidence for the immuno-stimulation the-

ory [80, 81] and are just considered as comparable to the variability observed in tumor incidence in the control literature using outbred Swiss mice [194] and in the somewhat shorter observation period due to shorter-lifespan of the nu/nu versus nu/+ [190]. As a matter of fact, in ref. [23] where it is proposed that germ-free nude mice partially inbred in the BALC/c background had increased lymphoma incidence, one of the problems, in addition to those described previously, is that the nu/+ group, was actually observed for a shorter period of time than the nu/nu group, which may also effect the lymphoma incidence within that particular experiment [23].

Finally, another report described a high incidence of lymphomas in nude mice in the NIH(S) background [195]. This paper cited both reference [23] and [191], indicating that their results supported those previous observations. In spite of the fact that these animals were not used for experimental purposes (although they appear to be retired breeders), that they were observed for their lifespan and that careful autopsies were performed, this study has one serious problem for analysis of incidence, namely that the nu/nu group (38 animals) is much larger than the heterozygous coated littermate controls (15 animals); however, 16 lymphomas (42%) were observed in the nude group, versus a single lymphoma (7%) in the control group [195]. On important feature (besides an interesting comparison between the Lukes-Collins classification of human lymphomas with the Dunn classification of murine lymphomas) is that while 9 lymphomas were observed in 20 animals (45%) kept in non-sterile isolators, the incidence was 4 of 12 (33%) plus two animals developing non-lymphoid tumors in the group kept in sterile isolators; with 3 lymphomas in 6 animals kept in laminar flow units [195]. We will discuss again the possible induction of lymphomas in nude mice in Section 3.2. This study [195], when compared to the studies by Giovanella's group cited in ref. [190], may be an example of inter-laboratory variation [193], since both groups used nude mice in the NIH(S) Swiss background, with marked differences in lymphoma incidence both in the nudes as well as in the heterozygous controls.

Our own experience, summarized in ref. [26], showed no major discrepancies from expected results when the spontaneous tumor incidence was studied in otherwise unmolested nude mice (which were not even used for breeding purposes), partially inbred in the CBA/H background (mostly in the 5th backcross) observed for 18 months, or with nude mice inbred in the BALB/c background (8 backcrosses and 3 to 5 intercrosses) observed for 24 months, these animals could be kept for their whole lifespans, which in general were comparable to the normal animals in the laminar flow housing conditions described in ref. [28]. The CBA study included 80 nudes, 63 nu/+ and 121 CBA/H [26]. The BALB/c study included 189 BALB/c, 189

heterozygous +/+ and nu/+ (derived from heterozygous matings), 85 nu/+ (derived from matings and 80 nudes derived from homozygous matings [26]. In this study with the BALB/c animals, the most common tumors were lung adenomas which appeared in 12 to 15% of all groups, followed by a variety of other solid tumors which ranged from 3 to 5%; lymphomas were uncommon and observed in 1% of the animals, with no differences between nude and controls [26]. However, based on lymphoma incidence in BALB/c mice [196–198], it was apparent that the observation period in the study cited above was too short for a true estimate. On the other hand, in the CBA/H study, while again, the most frequent tumor detected was the lung adenoma (approximately 10% in all groups), lymphoma incidence was 6% in CBA/H, 3% in nu/+ and 11% in nu/nu [26]. Thus, we attempted to determine a crude lymphoma estimate in the partially inbred CBA/H nudes and the inbred BALB/c nudes, observed for longer periods of time, i.e. 33-47 months for the CBA and 30-32 months for the BALB [26]. Most of these animals were left unmolested in groups of 40-60, although some data on retired breeders are also included, and the studies were performed during the period from 1971-1976. The animals were housed in laminar flow units as described above (and in ref. [28] in detail). This study also showed no major difference in lymphoma incidence between the different groups [26]. For example, while lymphoma incidence was 8.9% in BALB/c mice (58 lymphomas out of 650 animals at risk, with no clear difference between males and females), the incidence of lymphomas in nu/+ and +/+ littermates from heterozygous matings was also 8.9 (35 of 390), while the incidence in nu/+ animals was 7.2% (13 of 180) and in nu/nu from heterozygous matings was 9.7% (35 of 360) and in nu/nu from homozygous matings was 8.4% (16 of 190 at risk, again with no differences between males and females); in summary, no major differences in lymphoma incidence between the original inbred strain and the inbred nudes and controls [26]; results in accord with the usual lymphoma incidence observed in other studies using BALB/c sublines [196–198].

Finally, the effect of the viable yellow gene, which produces an overall increase in spontaneous or induced tumor incidence of the types prevalent in the inbred background in which the gene is inserted [199], was studied in hybrids of the partially inbred (6th-7th backcross generation) nudes in the CBA/H background and C57BL/6J or C57BL/JA^{vy} mice [27]. Unfortunately, at that time we did not have nudes in the C57BL/6 background, thus the nu/nu A^{vy} were obtained by mating CBA/H nu/nu males with nu/+A^{vy} mice, A^{vy} being a dominant gene. Thus the nu/nu A^{vy} are in reality an F_1 backcrossed to the nu/nu parent; while most of the controls in the experiment are either F_1 hybrids or in the case of the plain nu/nu, partially inbred CBA/H mice [27]. With the proviso in mind that comparison between

groups is not warranted, we did observe the viable yellow effect increasing, especially lung adenoma formation in all groups and hepatoma formation in males, with only marginal effects on lymphomas or other tumors, and no clear differences between the nudes and the other controls [27].

In summary, with the exception of some viral induced malignancies such as polyoma [20, 26], it is apparent from our own studies that the incidence of spontaneous tumors in nude mice is usually comparable to that in the euthymic controls, and follows the patterns of the late-appearing spontaneous tumors developing in the original inbred strain in which the nude gene has been inserted [26].

This statement is also supported by the studies on spontaneous tumor development in nude mice (in BALB/c background?) which have been immunologically reconstituted with T cells, as described by the Lozzio group [190]. During an observation period of 14 to 29 months, 7 tumors were observed in these reconstituted nudes, from a total of 680 mice, of which 6 were lymphomas [190]. A detailed and careful study of nude mice in the C57BL/6 and BALB/c backgrounds (presumably inbred) reconstituted with thymus grafts was performed by Eaton et al. [200]. In the case of the C57BL/6 group, 108 nu/nu and 122 +/+ were observed for their whole life span, which ranged from 52-53 months in both groups; the overall tumor incidence was comparable with 59 and 51% respectively in nu/nu and +/+, with the only difference being the appearance of some vascular tumors, mainly hemangioendotheliomas (8 tumors) in the nu/nu group [200]. Concerning lymphoreticular tumors, the incidence was 18% in the nu/nu and 42% in the +/+; in the last case, these figures were quite similar to those observed by us in C57BL/6J mice, where we found 32% lymphomas in males and 42% in females [201], while the incidence of epithelial tumors was 54% in nudes and 11% in +/+ [200]. The study with BALB/c mice, as clearly indicated by the authors, had problems with differences in survival and group size at risk, since the nude mice had a mean survival of 56 months; however, even with such a proviso, and with a lower overall tumor incidence in the nudes (32% versus 73% in the normals), the incidence of lymphoreticular tumors in the nudes was 20% versus 74% in the normals [200]. The differences in survival in the BALB/c study were interpreted as due to incomplete restoration by the thymus grafts in the nude group [200]. However, it is quite apparent that the nude trait *per se* does not increase the risk for lymphoma development, and if anything, it seems to reduce it; while it may effect vascular tumor development in one inbred strain [200]. It is interesting to read the discussion of this paper at the Workshop, on page 365 of ref. [200], since it still contains the same ill-founded argumentation about increased incidence of lymphomas in the untreated nudes, discussed in the previous paragraphs of this sub-section.

3.2 *Lymphoma 'induction' in nude mice*

During the observation of 1141 non-inbred Swiss mice that received human tumor transplants, 24 tumors were found, of which 22 were found in the fraction of 324 mice which survived for 5 months or more, giving an overall tumor incidence of 2.1 to 6.8% respectively if calculated for the whole population at risk or the group surviving 5 months or more [187]. These studies were obviously published as a reaction to the belief that nude mice did not develop spontaneous tumors [185]. Of the 24 tumors observed, 18 were lymphoreticular in origin, and would have been classified as lymphomas, and 6 were pulmonary adenomas ([187], the same data also in [190]). The overall tumor frequency and tumor types found was comparable to that observed in normal Swiss mice in a variety of backgrounds, observed for similar periods of time [194], since the mean age for animals developing tumors in this experiment was 9.1 months [187, 190]; although lower than that described for ICR:Ha Swiss mice observed for their whole lifespan, where lymphoma-leukemia has a 20% frequency [202]. These animals were grafted with a large variety of different human tumors, and in spite of such tumor heterogeneity, there was a tendency for animals grafted with colon and urogenital tract tumors, to develop more spontaneous tumors than expected [187, 190]. In summary, although there is no data on tumor incidence in non-xenografted nudes, this study shows that the nude mice receiving human tumor grafts to develop tumors and that lymphomas do not seem to be over-represented [187, 190].

A different picture is described in the studies by Baird et al. [189], where they indicate that 19 of 30 nude mice in the BALB/c background receiving human tumor xenografts, developed lymphomas, giving a frequency of 63%, during a 6 month observation period. During this period, 5 control nudes did not develop lymphomas, which certainly does not seem to be an appropriate control [189]. They also show that in the same period, 9 of 16 (56%) nudes infected with pinworms developed lymphomas [189]; they had previously described the production of a transplatable complex lymphoma containing null, B and 'occasional T cells' in a nude mouse infected with pinworms, which the authors concluded was a result of chronic antigenic stimulation, and that could represent a model for human disease [188]. In a subsequent study, it was shown that some, but not all, of human tumors grafted into nudes could induce the expression of endogenous murine leukemia virus in nude mice in the BALB/c background [203]. Of 5 lymphomas produced in the experiments in ref. [189], all showed a pleomorphic histology and contained null, B and T cells; and all expressed surface Ig (which was usually lost after *in vitro* passage), and all expressed murine leukemia virus glycoproteins and proteins. Thus, the possibility of these tumors being the result of lymphoproliferation triggered by chronic antigen-

ic stimulation (by the pinworms or the xenograft) and the possible activation of endogenous murine leukemia virus, as well as other forms of 'deregulation' was considered in interpreting their pathogenesis [188, 189].

The ideas of lymphoma-leukemia resulting from chronic antigenic stimulation which eventually breaks normal regulation of the lymphoid system, was proposed some time ago (204], see also [51]), and experimental work supporting these views are abundant (for examples see [205-207], see also Section 2.2).

Our own studies [46] show that a high incidence of lymphomas can be induced in nude mice after life-long immunosuppression with anti-mu antibodies, which induces a B cell maturational arrest. During studies on polyoma oncogenesis in nude mice, we observed that the animals, although highly susceptible at ages in which the euthymic controls are resistant, developed a partial resistance to tumor induction at approximately 4 months of age, which was not mediated by T cells [20]. Such resistance could be transferred with spleen cells from 4 month old nudes to newborn nudes or normals, which are highly susceptible to polyoma tumor induction; and our cell separation procedures suggested a complex mechanism in which, however, B cells appeared involved [20]. Thus, we studied the effect of life-long anti-mu treatment since birth on polyoma oncogenesis [46], and indeed, a partial abrogation of the age-dependent resistance was observed. However, and pertinent to this discussion, 33% of the animals developed lymphomas with mainly splenic involvement, which is indeed a rare event in polyoma oncogenesis, which induces tumors in a variety of organs, but almost negligible tumors of the lymphoid system [20, 208]. Thus, we made a more detailed study on the effect of anti-mu on lymphoma development in nude mice [46]. Table 2 summarizes our studies in nudes partially inbred in the CBA/H background and nudes inbred in the BALB/c background. It is quite apparent that nude CBA/H mice treated with anti-mu have a marked increase in lymphoma development (42%), which is also observed in BALB/c mice (28%), when compared to controls. It should be noted that this is a short term experiment, terminated at 13 months, which as was discussed in sub-section 3.1, is relatively short for lymphoma studies. The first lymphoma in the anti-mu treated mice began to appear at 140 days, which is quite early for this type of tumor. Six of the 18 lymphomas in the anti-mu treated CBA-nudes, studied so far, proved to be surface Ig positive, and also all expressed murine leukemia virus antigens, as was observed by Beatie et al. (1980). Our interpretation of these results was that from the interaction of the maturational B cells arrest induced by anti-mu, the possible selective mitogenic effect of anti-mu, the lack of mature T cells (regulatory?), plus some form of additional triggering, perhaps via antigenic stimulation (our mice were not germ-free, but are certainly pinworm free) or

Table 2. Lymphoma incidence in anti-mu-treated nude mice and controls.

Mice	Lymphoma incidence (number lymphoma/number mice and %)		
	Untreated	Goat IgG	Goat anti-mu
CBA/H	2/54 (4)	2/60 (3)	3/58 (5)
nu/+ & +/+	1/49 (2)	3/43 (7)	6/52 (14)
nu/nu (CBA)	1/56 (2)	6/51 (12)	18/43 (42)
BALB/c	2/40 (5)	1/40 (2)	3/40 (7)
nu/+ & +/+	2/44 (4)	3/40 (7)	3/41 (7)
nu/nu (BALB)	3/40 (7)	5/52 (12)	12/43 (28)

The anti-mu or goat-IgG control animals were injected intraperitoneally since birth with 0.5 mg of IgG or anti-mu IgG, daily for the first 6 days and continuing 3 times/week for the duration of the experiment (see ref. [46]). All animals were sacrificied when obviously sick or at 400 days of age. The first lymphomas in the anti-mu-treated nudes began to appear at approximately 160 days of age. For further details on tumor type and nude animals see ref. [46].

related to endogenous murine leukemia virus, may start some abnormal B cell proliferation leading to lymphoma formation [46]. A similar observation has been made in BALB/c nudes treated with anti-mu or with endotoxin (M.E. Gerswin, personal communication). It is obviously too early to determine if these lymphomas may represent models for the lymphomas appearing in immunodepressed patients (see sub-section 2.2), but at least some testable possibilities are worht studying, such as the role of T cell subsets in the regulation of these lymphomas. However, it is important to note that these lymphomas in anti-mu treated nude mice do appear in the presence of an intact [84] or perhaps enhanced [85, 86] NK system, which will be discussed again in sub-section 4.2.

3.3 *Other studies on tumor development in nude mice*

I will not repeat here, our published studies on tumor induction by carcinogens in nude mice [18, 19, 22, 26, 28], which basically showed no major differences between nudes and controls; but rather discuss a few observations which were published since our last reviews on the subject [26, 28].

In a model of transplacental carcinogenesis with urethan, given at 17 or 19 days of gestation, the incidence of primary lung tumors in the progeny of heterozygous BALB/c nu/+ matings, 4 months after exposure, was comparable to the nude and normal offspring, with a higher incidence in both cases when urethan was given on day 19 [209]. However, in a rather complex system of scoring by 4 different pathologists, the authors concluded that the tumors had more atypical cells and showed more invasiveness in the nude than in the controls; however, with respect to invasiveness, there were some

wide differences in the scores of three pathologists, i.e. while one scored all of 10 tumors in nudes and all of 16 tumors in controls showing invasiveness, another scored 3/10 and 3/16 for nudes and controls, while the third scores 8/10 and 3/16 for nudes and controls respectively [209]. Thus, it is not easy to agree with such conclusion, especially since the microphotographs accompanying the paper, while showing that a nude lung adenoma had more nuclear heterogeneity than a control tumor, certainly did not suggest differences in invasiveness [209].

A series of papers have dealt recently, with skin carcinogenesis in nude mice [210–214]. In one study, repeated painting of the skin with dimethylbenzanthracene produced a papilloma incidence, 5 months after beginning of the experiment, of 3 of 18 for the nudes and of 18 of 23 mice at risk for the nu/+, which also showed more than one tumor per mouse [210]. In addition, the frequency of papillomas in nu/+ skin transplanted to nude mice was also low, 3 of 15 [210]. Unfortunately, the lack of a control group of nu/+ skin grafts in nu/+, makes the evaluation of the result difficult. Anyway, the question of skin carcinogenesis in nude mice includes as a major variable the peculiar properties of the nude skin, and cannot be used as an argument for or against host immune intervention in tumor development. Two more studies used skin grafting techniques in nude mice to analyze skin carcinogenesis, unfortunately with quite different results [211, 212]. In the first one [211], the model used the technique developed by Lappe [215] who showed that when skin was treated with a subcarcinogenic dose of methylcholanthrene as initiator, applied by contact of a carcinogen-impregnated disc, and 5 days later transplanted to syngeneic hosts, transplantation seemed to act as promoter for papilloma formation. Following such technique, probably using normal BALB/c skin in all the experiments (it is not stated in ref. [211]), the carcinogen treated skin was grafted to nude mice partially inbred in the BALB/c background or in one case to syngeneic normal BALB/c mice [211]. The incidence of grafts with papillomas was 3/15 (20%) in the nudes and 10/15 (55%) in the BALB/c mice. In addition, a group of grafted nudes that also received 10^5 normal BALB/c spleen cells had an intermediate value of 7/15 [211]. A second experiment used the same type of grafts in nude mice which in addition had been irradiated with 300 R and the results were 5/18 (28%) of grafts with tumors; in addition, when the same irradiated nudes received either 10^2 or 10^7 BALB/c spleen cells, the tumor incidences were 7/18 and 3/18, i.e. 39 and 17% respectively [211]. These results were interpreted as suggesting a role for immunostimulation of tumor development, based on the differences observed between the nude mice restored with different numbers of spleen cells [211]. However, a major contradiction in these results is observed, since while the skin grafted into the normal BALB/c animals had the highest

papilloma incidence; the lowest incidence, even lower than the untreated nudes, was observed in the group receiving the highest spleen cell dose, i.e. 10^7 [211]. A subsequent and more detailed paper using similar procedures, unfortunately did not contain data using nude mice [79]. It is not easy to produce at present an explanation of these modest differences in papilloma incidence. However, it is worth mentioning here that technical differences may be important in this type of experiment using skin grafting. For example, in the Andrews, experiments [82], the tumors were induced by 2 mm in diameter paraffin pellets containing approximately 0.15 mg of methylcholanthrene and full-thickness skin grafts containing the pellet were transplanted; under these conditions tumor development (probably fibrosarcomas) was reduced in the grafted animals. On the otherhand, when skin carcinogenesis was initiated by contact with plastic discs embedded with paraffin containing methylcholanthrene and the treated skin grafted to normal or immunodepressed animals, grafting acted as a promoter for skin papilloma development, and the proportion and behavior of the tumors was directly proportional to the immunocompetence of the host [215].

A second study, used a variety of skin types grafted mostly into nude mice, which were subsequently treated by a two-stage carcionogenesis regimen using diemthylbenzanthracene and a phorbol acetate [212]. This study used skin from SENCAR mice, which are highly sensitive to this type of two-stage skin carcinogenesis, as well as BALB/c and BALB/c-nude skin [212]. The results show that papilloma development in SENCAR skin seemed to be unaffected by the immune status of the host; and more importantly that the papilloma incidence in intact nudes, nude grafts or the adjacent skin in the grafted nudes, were quite comparable and that such incidence, albeit much lower than in the SENCAR mouse, was quite comparable to the incidence in BALB/c skin, which belongs to the strains that are poorly sensitive to this type of carcinogenesis [212]. Both BALB/c and BALB/c-nudes showed a frequency of approximately 0.25 papillomas per square cm per mouse; while SENCAR skin showed values of 1.50 or more papillomas [212]. One interesting, albeit related point, is that the skin grafting procedure, even with SENCAR skin on SENCAR hosts, seems to reduce papilloma incidence and increase latency periods [212], supporting to some extent the Andrews' experiments discussed previously [82]. However, this paper shows quite clearly that in a two-stage model of skin carcinogenesis, BALB/c as well as BALB/c nudes show the same degree of 'resistance' to skin papilloma development. Finally, another study on two-stage carcinogenesis in nude mice also presents some confusing results, especially a marked discrepancy between the title of the paper and its contents [213]. In this study the nude mice are partially inbred in the C3Hf/He background, with the nude trait being derived from a non-inbred BALB/c nude stock,

and used a dimethylbenzanthracene-phorbol acetate scheme, similar to that in ref. [212], with the exception that the dose of phorbol used was higher and that croton oil was used as promotor in one of the experiments [213]. In the first experiment, in which the carcinogenesis protocol was changed and which the authors called '... rather complex experimental conditions...', 22 of 37 (59%) nudes and 21 of 22 littermates developed papillomas with an average latency of 208 and 145 days respectively, thus the title of the paper: 'Resistance of germ-free athymic nude mice to two-stage skin carcinogenesis' [213]. In a second experiment, they used nude mice which were either untreated, received a thymus graft or a sham-thymus graft; papilloma incidence in this study was 19 of 21 (90%) in the untreated nudes, 17 of 20 (85%) in the nudes with a thymus graft and 20 of 20 in the nudes with a sham-graft; with average latency periods of 74 to 77 days; the only slight difference being that papilloma multiplicity was higher in the unreconstituted nude mice [213]. Thus, the nudes in the second experiments developed more tumors and at shorter latency periods than the controls in the first experiment. In spite of these results, the title of the paper, and to some extent the tone of the discussion were not modified [213].

Finally, a recent study using transplacental carcinogenesis with ethylnitrosourea (ENU) in BALB/c nudes, showed that 45% of the nudes versus 5% of the normal littermates developed benign papillomas after exposure *in utero* to 50 mg/kg of ENU [214]. Furthermore, 17% of adult nudes injected intraperitoneally with 100 mg/kg of ENU developed skin papillomas, versus no tumors in similarly treated normal littermates [214]. As it was indicated by the authors, this susceptibility of the nude mice to skin carcinogenesis by systemic ENU may be explained by other reasons besides the lack of conventional IS [214]. In our own studies on transplacental carcinogenesis with ENU in nude mice partially inbred in the CBA/H background [26], we did not detect any skin tumors in nudes or controls, using a similar dose of ENU as in Anderson et al. [214]. However, we treated the pregnant mothers on day 16-17 of gestation, while the Anderson et al. experiments treated the mothers on day 15 of gestation [214]; these differences in ENU administration as well as the mouse strain differences, may explain the discrepancy. In summary, it is apparent from the analysis of the previous studies, that the case for actual resistance of nude mice to topically induced skin carcinogenesis is not very strong, and in some cases can be attributed to problems in experimental design or even to problems in application of the carcinogen to the skin (as suggested by Holland and Perkins, [213]). The variations described are difficult to interpret either within a classical IS framework or even within the revised versions of IS to be discussed in Section 4. On the otherhand, it seems that nudes are more susceptible to systematically-induced skin carcinogenesis than their normal littermates [214]. This is

Table 3. Effect of diethylstilbestrol on interstitial testicular tumor development in nude mice.

Experimental group [a]	Tumor incidence [b]	Latency period [c]
nu/+ and +/+ (BALB/c)	20/25 (80%)	245±9.0
nu/nu (BALB/c)	14/25 (56%)	248±7.6
nu/+ and +/+ (CBA/H)	2/30 (7%)	510-525
nu/nu (CBA/H)	2/30 (7%)	517-525
nu/+ and +/+ (F_1)	9/31 (29%)	360±9.2
nu/nu (F_1)	7/30 (23%)	376±6.6
BALB/c	21/29 (72%)	220±5.9
CBA/H	1/30 (3%)	528

[a] All males were implanted subcutaneously at 2 months of age with cholesterol pellets containing 20% diethylstilbestrol (as described in ref. [216]) and observed for 20 months. Groups of 20 controls implanted with simple cholesterol pellets did not show any testicular tumors in either the BALB/c or CBA/H system. F_1: (BALB+CBA)F_1.

[b] Tumor incidence expressed as animals with tumors per total studied (percent tumor in parentheses).

[c] Latency period as mean days for tumor appearance ±S.E. of the mean.

indeed, one of the few instances in which nude mice showed increased susceptibility to chemically-induced carcinogenesis (see [26, 28] for further comments). Whether this example belongs to the category of 'restricted' IS, applying only to certain tumor types, as we suggested some time ago [21], or whether other mechanisms unrelated to IS may be operative, deserve further analysis.

Finally, before ending this fastidious analysis of the available data on carcinogenesis in nude mice, I will discuss one more model, that of interstitial testicular tumors induced by diethylstilbestrol (DES), as described by Andervont et al. [216]. The reason is two-fold, firstly because it shows some additional properties of tumorigenesis in nude mice including possible genetic influences related to strain; and secondly because the depressive effects of DES on NK cells and its subsequent effect on chemically induced carcinogenesis [217], appears as one of the few clear examples where low NK activity correlates well with increased risk for tumor development, which will be discussed in sub-section 4.2. Our studies with nude-BALB/c mice were briefly described in ref. [26], and our completed study is presented in Table 3. Male nude mice inbred in the BALB/c and CBA/H background (in the latter case, 9 backcrosses and 3 intercrosses) or the nude F_1 hybrids of such strains, as well as the heterozygous controls, were implanted subcutaneously at 1 month of age with cholesterol pellets containing 20% DES, as described in ref. [216], and observed for testicular tumor development for 20 months. Two points are worth mentioning. Firstly, tumors in the

BALB/c groups had the shortest latency period for development and appeared at higher frequency than than in the CBA/H groups, which are practically refractory to this oncogenic treatment, as was observed by Andervont et al. [215], with the F_1 hybrids between the two strains showing intermediate values; secondly, that in spite of the difference in tumor incidence between the BALB/c, CBA/H and F_1 groups, DES treatment depressed NK activity in a control group of similarly treated animals to practically undetectable levels (data not shown) as was described by Kalland [217]. Thus, one may conclude that, the tumors appeared at their low or high frequency in spite of the absence of conventional T-cell-mediated IS, as well as in spite of the low levels of N-CMC activity. Thus, one may conclude that the strain differences and possible genetic control of this type of oncogenesis are not related to immunological or para-immunological host responses. I will discuss this subject again in sub-section 4.2.

3.4 *Conclusions*

I hope that it becomes apparent from this brief analysis of the available studies on induced and spontaneous tumor development in nude mice, that the predominant conclusion that can be made is the one made by us some time ago: that the nude mice express the spontaneous or induced spectrum or tumors characteristic of the inbred strain in which the nude trait has been inserted [18-22, 25-29], and that only in some selected models of virally-induced tumors (such as polyoma oncogenesis or Moloney sarcoma virus) do the nude mice show an increased susceptibility (see ref. [26] for detailed review and appropriate references). However, as we indicated in this text, even in the polyoma model with its marked T-cell dependency, the nude mice can develop 'alternate pathways' of resistance to oncogenesis, mediated by complex non-T mechanisms [20].

The possible role of N-CMC in this generally 'normal' susceptibility to tumor development observed in the nude mice will be discussed in Section 4. Finally, I hope that at least temporarily, and based on the available studies discussed here, we may put to rest two misconceptions concerning tumors in nude mice; firstly that the 'absence' of tumors in these animals, which we strongly criticized in the past [21, 25, 26, 29] is due to inappropriate experimental design; and secondly, that the 'higher' incidence of lymphomas in the nude mouse is questionable, especially with the possibility that chronic antigenic stimulation and other immunological imbalances may actually induce such type of tumors in the nude animals, which may explain the few instances in which an apparent increase of lymphomas was observed within an experiment (see sub-section 3.2). We also criticized this concept rather strongly in the past [21, 22, 25, 26, 29, 183]. However, it seems that in neither case we have been persuasive enough, based on the

unusual persistence of such misconceptions in the literature. I think that a similar case of poorly designed or analyzed experiments is emerging for the case of skin carcinogenesis in nude mice, and I hope that from this review, a sceptical eye is cast on the reports of 'resistance' to skin carcinogenesis in nude mice.

Finally, two points are worth mentioning, concerning future directions. Firstly, a small field of 'unique' tumors is emerging, which appear only in nude mice under certain conditions such as: uterine tumors in polyoma infected nude mice [20], vascular tumors in one strain of nudes [200], skin papillomas after systemic carcinogens [214] and 'B lymphomas' in anti-mu-treated nudes [46]. Whether these represent special effects of the nude gene, or actual 'weak spots' of the antitumor defense mechanisms, whatever they may be, as we proposed some time ago [29], deserves more study. Secondly, and this will be discussed in more detail in Section 4, additional immunodepression of the nude mouse, especially by procedures that affect N-CMC, seems warranted; however, other more conventional immunodepressive regimens (such as anti-lymphocyte serum, cyclophosphamide, anti-mu, etc.) have not seemed to influence tumor development (see ref. [46] for further discussion).

4. DOES N-CMC MEDIATE ANTI-TUMOR SURVEILLANCE?

Due to space limitations, and also a sense of avoiding too many repetitions from reviews past, this section will concentrate on the proposition described in its title: whether anti-tumor surveillance (or IS for short, as defined in sub-section 1.1) could be mediated by natural cell-mediated cytotoxicity (N-CMC), of which NK cells are one of the best studied prototypes.

As with our past analysis of IS, I will concentrate on the emerging studies on spontaneous or induced tumor development in animals with impaired NK activity (to be discussed in sub-section 4.2) and only marginally citing studies on the activity of N-CMC on transplanted tumors, which at present, make the bulk of the evidence for the possible *in vivo* role of N-CMC as an anti-tumor mechanism [5–9, 32–35]. As a matter of fact, it is probably in the stage 3 (i.e. metastatic spread) and to some extent in stage 2 (i.e. local tumor development), rather than at stage 1 (i.e. IS of *in situ* lesions) that the N-CMC system seems to be most effective (for details of the 3 stages of tumor-host interactions, see sub-section 1.2). A brief listing of some prototype studies with transplanted tumors and metastatic spread will also be discussed at the end of sub-section 4.1.

For the same reasons cited at the beginning of this section, I will not

discuss in detail the general properties of N-CMC in animals and man, nor the possible role of other effector mechanisms such as macrophages, since these subjects will be covered by other authors in this volume. In sub-section 4.1, I will briefly discuss some unique properties of the N-CMC system, which make it mechanistically suitable to function as a possible antitumor IS mechanism, based in part on our discussions of the subject in references [29, 35, 49, 218] and [219]. An unavoidable sense of *déjà vu* will permeate these pages, since most of the present discussions on the possible role of NK and other N-CMC effector mechanisms (whether 'non-adaptive' as in ref. [39] or 'para-immunological' as in ref. [55]) use much of the same argumentation utilized during the climax of popularity of the IS hypothesis when it was T cell dependent (see ref. [21] for extensive review). In addition, the present day enthusiasm about NK cells and their possible role in IS, has generated those same poorly planned experiments using beige mice, as those we criticized in the past using neonatally thymectomized animals [21]; probably as part of the wave of beige mouse studies predicted by Mitchison in 1980 [5]. Similarly, our past criticism [16, 17, 21] to some of the presistent although questionable correlations, such as the argument on the depressive effect of the carcinogen itself on the putative defense mechanisms, are still valid, whether IS is mediated by T or NK cells (see sub-section 4.2). I think that it is somewhat premature to build up a very strong case for or against an IS-like mechanism mediated by N-CMC effector cells which actually controls or affects *in situ* tumor development (see sub-section 4.2). As a matter of fact, the phenomenon of 'cryptomnesia' in science, so well described by Merton [88] – which in essence is the rediscovery of known facts as if they were new findings, in its 'positive' formulation – has acquired a 'negative' aspect in the IS studies, which is the forgetting or overlooking of already known facts which do not agree with the bias towards expected results (for some examples within the conventional IS studies see our refs. [21, 25, 29]; for some examples on the *in vivo* role of N-CMC see our refs. [46, 49, 218, 219]. However, it is worth ending this brief introduction with the comment that, whatever the source of the expectancy bias, all of these efforts and studies are an integral part of our overall effort for a better understanding of the complexities of the tumor-host interaction and its possible application to clinical situations favoring the host [49]. As indicated by Woodruff, polemical arguments are '... no substitutes for rigorous testing and for the generation of new hypotheses...' (page 147, in [55]). Thus, as was proposed some time ago by Popper, it is '... our way of choosing between theories...' in relation to a problem in question, '... which makes science rational...' [220].

4.1 *Some general properties of N-CMC*

Due to an unexpected bloating of the references cited so far in this paper, I will mainly use, in this sub-section a procedure that I have strongly criticized in the past, that of citing reviews instead of the original papers on the subject [21, 29]. However, I am almost obliged to do so, since this review has a page limit which, although generous, is certainly finite. Furthermore, I want to use additional original references to support the points in sub-sections 4.2 and 4.3.

N-CMC has the following general properties: (1) pre-existence at high levels in normal hosts, with no apparent need for priming [32–36, 137]; (2) 'specificity' for certain tumor targets which appear as 'susceptible' to N-CMC-mediated killing with no clear evidence for memory in the immunological sense [32–36, 137]; (3) no major histocompatibility restriction between effectors and targets for effective killing [32–36, 219]; (4) mediation by an interrelated but heterogeneous population of effector cells of still undefined lineage, although different from 'conventional' T, B, monocyte-macrophages [32–36, 219]; (5) in mice, N-CMC is mediated by several subpopulations of which NK and NC (natural cytotoxic) are the most prominent, which differ in surface markers, genetic control, target preference (although there is some overlap) and overall regulation [32–36, 219, 221–224];(6) complex genetic influences (different from those controlling T, B or macrophage functions) affecting levels of activity, target preference for lysis, interferon effects, etc. [32–36, 219, 221]; and (7) the levels of N-CMC are influenced by the interferons [32–36] as well as other regulatory products such as interleukin 2 [225] and interleukin 3 [226], which affect overall levels of activity as well as the properties of the effector cells [32–36]. These are a quite unique set of properties which set N-CMC as a distinct category of effector system, different from the rules and properties that govern the more conventional cytotoxic effector mechanisms; although it may well be that the final event of actual target lysis may be similar for most effector cell types [36].

How do the above properties of N-CMC apply to anti-tumor IS? Firstly, and quite importantly, once it has developed during early ontogeny, the N-CMC system can act directly without the need of time-consuming priming (as is the case for T cells) or of time-consuming production of macrophage-activating factors. In the past, one of our arguments against conventional immune responses mediating IS addressed this time-consuming initial step required for recognition and clonal expansion (or functional activation, in the case of macrophages) of the effector population capable of dealing with the *in situ* tumor [25, 29, 49, 57]. Secondly, and based mostly on *in vivo* studies with transplanted tumors, it is clear that N-CMC effector cells can deal quite well with a small number of tumor cells *in situ* [32–36, 184,

217, 227–238]; and as a matter of fact both for NK [227–231] and NC [232] cells, it is clear that increasing the number of tumor cells eventually overwhelms the capacity of the host N-CMC system to deal with the tumor at the local site. In the past, and drawing especially from the data with infectious agents [134], one of our arguments against IS being mediated by conventional T cells was precisely the point that such system needs a certain antigenic mass to be activated, and that sub-threshold levels of antigen are ineffective or tolerogenic; thus, the T cell system, by definition, would not recognize small numbers of abnormal cells [25, 29, 49, 57]. In summary, it would be blind to the emerging tumor *in situ,* and only be able to detect the tumor, assuming its 'antigenicity', when it reached a certain size [25, 29, 49, 57]. Thirdly, the argument of 'tumor antigenicity' in its conventional sense, is irrelevant for N-CMC, since the system is independent of such determinants [32–36]. However, targets and even variants within targets which are resistant to N-CMC do exist, and whatever the nature of the 'antigens' or surface determinants being recognized by N-CMC effector cells, the possibility of 'resistant' targets could be one of the explanations for escape of N-CMC [35, 36, 233, 236]. The argument of conventional antigenicity (usually measured as tumor associated transplantation antigens) was noted almost from the beginning of the IS boom [54], and generated considerable argumentation [12, 29, 40, 52, 53, 119]. The question of tumor clonality [114], which was considered as an argument against any form of IS, since it implied that the system allowed the escape of a single parent cell [4], was already discussed in sub-section 2.1.4. Macrophage-mediated IS would also be apparently independent of conventional tumor-associated antigens [40]. As I indicated in a previous review [219], the question of 'escape from surveillance' discussed in so many ways in the past (see [53] for a listing of almost all the possibilities as applied to conventional IS, which certainly apply to non-conventional IS mediated by any mechanism), is certainly a crucial one, besides the actual properties and mechanisms of the surveillance system [29, 219]. Indeed, the sneaking through phenomenon (also discussed in sub-section 2.1.4) was also observed with small doses of lymphoma cells [131], which later were shown to be susceptible to NK killing [137]. With the exception of some studies on changes in susceptibility to N-CMC killing after *in vivo* passage [32, 232, 239], or some studies on the N-CMC sensitivity of *in vitro* transformed cells [2, 3, 113, 232], the analysis of this important question has bearly begun. Finally, an important area of ignorance concerning the *in vivo* functions of N-CMC is that of regional differences or of delivery systems to the appropriate sites of the circulating N-CMC effector cells. I proposed some time ago that such factors could create '... weak spots where incipient tumors may thrive...' [219], which is somewhat a variation of the immuno-

logically privileged sites argument, discussed in the past in relation to conventional IS [21, 25, 29, 183]. Regional differences for tumor growth have been described in mice, although the actual mechanisms for such differences remain unknown [240].

One of the more spectacular *in vivo* correlations of NK cell function, has been the observation by Warren and Dennert [184], where a cloned cell line with NK activity administered to NK-deficient mice (beige, etc.) could restore the capacity of the hosts to reject allogeneic bone marrow transplants, resist challenge with transplanted tumor cells and show resistance to development of radiation-induced leukemia. This observation, as well as the remarkable recent accumulation of relevant information on NK cells and other N-CMC effector mechanisms (see ref. [9] for an impressive compendium of this new information in the field), has prompted a recent commentary that NK cells had come to the rescue of immune surveillance, which however, was wisely tempered by the addition of a question mark [8]. Similarly, there is a rather convincing case for an important role of NK cells in the clearing of blood-borne metastasis of lymphomas and solid tumors, usually measured as either clearance of intravenously-injected radiolabelled tumor cells from the lung, or the counting of lung-colony formation after spontaneous or artificial metastasis, either in beige mice or mice with other forms of NK deficiencies [237, 241-249]. This last point relates directly to stage 3 of the tumor-host interaction discussed in sub-section 1.2; although of the highest importance due to its clinical implications, is certainly beyond the scope of the present review. Similarly, a variety of important issues on N-CMC effector cells, such as heterogeneity, cell lineage, regulation and other properties, including their possible role in regulation of normal hemopoiesis or of initial defense responses to a variety of bacterial, viral or parasitic agents (see again [9], as well as [36, 137, 219, 250, 251]) are beyond the scope of the present review.

However, a few additional points will be discussed before closing this sub-section. Firstly, the initial ideas, derived mainly from murine studies, that NK cells were primarily involved with responses to lymphomas [32-34, 36] and that NC cells had a preference for non-lymphoid solid tumors [35, 136], is certainly incorrect, since solid tumors, for example, can be lysed both by NK and NC cells [252]. Thus, in spite of some of the differences between the two effector populations, they even share a certain degree of target preference. This point will be discussed again when commenting on the tumor induction studies in sub-section 4.2. Secondly, the idea of an heterogeneous, albeit related, system of effector cells acting in concert [35, 219] has also received strong experimental support in murine systems [222-224, 253]; although comparable studies in other species, including man, have barely begun, and are presently concentrated mostly on

NK-like cells, recognizable by their peculiar morphology as 'large granular lymphocytes' [254]. Although LGL have been detected in mice [255], it is apparent from the studies by Roder and collaborators [36, 256] on the ultrastructure of the NK cells that form conjugates with the targets, that most of the cells do not have the morphology of LGL. Whether the mouse represents a unique model; whether the comparisons are inadequate due to regional differences, since the murine studies use spleen cells while the human studies use peripheral blood lymphocytes or whether limited numbers of targets have been tested in the studies in other species, certainly needs further clarification. As will be seen in the next paragraphs, it is quite possible that the spleen may not be the total, nor the best, representative of murine NK activity [257].

Finally, an important point which needs mentioning, is the age-related decline of NK activity in spleen, observed in mice at approximately 10-12 weeks of age [32-34, 137]. It is obvious that if such is really the case, that after 3 months of age, which is quite far away from the age at which spontaneous tumors begin to appear in mice [192], NK cells disappear; the argument for a role of NK cells in antitumor IS becomes almost non-existent. However, this phenomenon is not general for all N-CMC effector cells in mice [136], it is not detected in peripheral blood, even at 16 months of age ([257], our unpublished observations), is readily augmentable in spleen [258], and such a decline is not observed in rats [259] nor humans [32, 250]. Thus, as mentioned previously, the spleen may not be the overall representative of the NK activity in the murine host. However, some correlations between spleen NK activity and behavior of transplanted tumors have been described, showing increased tumor growth in the 'older' animals (usually at approximately 4 to 6 months of age) with the putatively NK defect [33, 34, 36, 231, 260, 261], which are not easy to explain. For example, subcutaneous growth of YAC cells injected into 4 week or 6 week old mice, showed lower frequency of takes than when injected into 6 or 9 month old hosts [260]. Lung clearance of injected tumor cells was impaired in 13-20 week old mice [231], at a time when blood NK activity is high [257]. Thus, it is not easy to understand how clearance of intravenously injected tumor cells by the lung would correlate with spleen rather than with blood NK activity. On the other hand, clearance of labelled cells from other sites, such as footpads, seems to have other mechanisms and properties [262]. The same paradox concerning what has been termed 'senescence' of NK activity [36] is exemplified by the experiments of Haller et al. [261], where 1 year old mice with low NK activity in spleen, could be restored to normal by lethal irradiation followed by injection of young bone marrow; however old bone marrow (1 year old) still produced good restoration of spleen NK activity in both young or old irradiated hosts [261], with the

differences favoring the young marrow being significant but very modest [36, 261]. These experiments were done using CBA mice, which were shown to have rather constant high levels of NK activity in blood, even at more than 470 days of age [257]. To conclude, this comment, whatever the mechanism of the age-related low NK activity in spleen [137] (which certainly is not observed with other N-CMC effector cells such as NC [136]), is not a generalized phenomenon, since 'normal' levels of NK effectors in blood can be detected in 'aged' mice, and well preserved levels of NK-precursors can be found in the 'aged' bone marrow [261]. As was indicated previously, such putative declines have not been observed when measured in spleen or other tissues in rats [32, 259] nor in human blood lymphocytes [32, 263].

Another important proviso for questioning the value of transplanted tumors in relation to IS studies, is the observation that the tumor cell inoculum itself, regardless of whether it contains passenger viruses, can actually induce a boosting of NK activity mediated mostly by interferon [260, 264]; which combined with our past criticisms on the use of transplanted tumors [21], explains why we concentrate exclusively on the discussion of spontaneous or induced tumor development in experimental models of IS.

4.2 *Tumor development in N-CMC deficient animals*

In this sub-section I will discuss two types of experimental evidence: firstly, the correlation between known levels of NK activity in spleen and spontaneous or induced tumor development in a particular mouse strain and secondly, tumor induction after exposure to carcinogens in animals with spontaneous or induced low NK activity, which include beige mice. I will also discuss some experiments on carcinogen-induced tumors in animals with low or normal NK activity, in which the effect of the carcinogen proper on NK activity was studied. An occasional experiment with rats will also be discussed. As can be seen from the paucity of actual studies (4 studies cited in ref. [7]; one study, as well as 5 other examples of related experimental and clinical observations in ref. [250]), this is a field that has just begun to be explored, and most of the conclusions have to be considered provisional. I will discuss mainly correlations with NK, since strain distribution of activity in mice is well established for such system, while it is still being defined for other N-CMC effectors [219], and because most of the studies on tumor induction use either beige mice or mice with induced deficiencies of the NK compartment.

4.2.1 *High NK-activity mouse strains*

How about murine strains with high NK activity? This has to be consi-

dered only as a suggestive correlation, but as seen in Table 4, the CBA sublines, which have been considered as prototype high NK reactors [33, 34, 36] have a low incidence of late appearing lymphomas and a moderately high, but variable, incidence of other tumors (mostly mammary tumors in females and hepatomas in males, see also sub-section 3.1 for discussion of lymphomas in CBA nudes, and refs. [21, 26, 266 and 273]). The only mouse strain described as having NK cell hyper-reactivity, of a level higher than that usually seen in other high NK strains or in nude mice, is the SM/J strain [265], and is clearly a strain with low spontaneous tumor incidence [266], although being one of the strains with an intermediate lifespan, comparable to strains with low NK activity, such as A/J [266]. Overall tumor incidence of varied types has been reported as 7% in females and 0% in male SM/J mice, observed during their whole lifespan [266]. It is obvious that these findings need confirmation and further analysis and should be considered presently, only as suggestive correlations. Especially since as will be discussed in the next paragraphs, there is not a good correlation between NK levels in spleen and spontaneous tumor development (including leukemia-lymphoma) in most murine strains [46, 49]. In addition, SM/J mice have other immunological hyper-reactivities which may also be affecting tumor development [265]. On the otherhand, some mutations like yellow and viable yellow (see ref. [27] and sub-section 3.1) which augment the tumor incidence characteristic of the inbred strain in which the gene has been inserted [199], have no effect on NK activity ([267] and our unpublished data) nor on NC activity (our unpublished data).

4.2.2 *NK activity and spontaneous tumors in rats and mice*

In a careful study in which NK activity in 7 different inbred rat strains was analyzed, no correlation with NK levels and spontaneous tumor incidence was observed [268]. For example, high tumor incidences have been reported in low-NK strains such as BN as well as in high-NK strains such as F344 and BD.X ([268, 269], see also [193] for further discussion on tumors in F344). Similarly, while both AKR and SJL mice (see Table 4, which develop a high incidence of spontaneous leukemias and lymphomas respectively) are low-NK strains; C58, which is also a strain selected for high leukemia incidence [192] can be included in the intermediate to high NK category ([34], our unpublished data). In a study of a leukemia-prone wild mouse population ([270]; see [271 for further details on tumor-prone sub-populations of wild mice, which in general have intermediate to low tumor incidences, see ref. [192]), some interesting information on NK cells was obtained: (1) the wild mice from this restricted area in California possess a wide range of NK reactivity against YAC target cells, with 32 mice (50% of all tested) falling in the low-NK range, comparable to A/J mice; (2) spleen cells from those

Table 4. Spontaneous tumor development in different mouse strains and sublines: Lack of correlation with NK activity in spleen.

<table>
<tr><th rowspan="3">Strain[a]</th><th rowspan="3">NK activity[b]</th><th colspan="4">Percent Tumors[c]</th><th rowspan="3">Reference[d]</th></tr>
<tr><th colspan="2">All tumors</th><th colspan="2">Lymphoid tumors</th></tr>
<tr><th>Male</th><th>Female</th><th>Male</th><th>Female</th></tr>
<tr><td>CBA/J (LS)</td><td>H</td><td>29</td><td>54</td><td>6</td><td>5</td><td>273</td></tr>
<tr><td>CBA/J (LS)</td><td>H</td><td>72</td><td>87</td><td>8</td><td>12</td><td>266</td></tr>
<tr><td>CBA/H (LS)</td><td>H</td><td>26</td><td>33</td><td>4</td><td>4</td><td>21, 201</td></tr>
<tr><td>CBA/H (18 M)</td><td>H</td><td>10</td><td>16</td><td>5</td><td>6</td><td>26</td></tr>
<tr><td>CBA/H (47 M)</td><td>H</td><td>?</td><td>?</td><td>5</td><td>6</td><td>26</td></tr>
<tr><td>CBA/H nu/nu (18 M)</td><td>H</td><td>24</td><td>22</td><td>12</td><td>10</td><td>26</td></tr>
<tr><td>CBA/H nu/nu (47 M)</td><td>H</td><td>?</td><td>?</td><td>5</td><td>5</td><td>26</td></tr>
<tr><td>BALB/c (24 M)</td><td>I-H</td><td colspan="2">20</td><td colspan="2">1</td><td>26</td></tr>
<tr><td>BALB/c nu/+ (24 M)</td><td>I-H</td><td colspan="2">19</td><td colspan="2">1</td><td>26</td></tr>
<tr><td>BALB/c nu/nu (24 M)</td><td>H</td><td colspan="2">20</td><td colspan="2">1</td><td>26</td></tr>
<tr><td>BALB/c (32 M)</td><td>I-H</td><td colspan="2">?</td><td colspan="2">9</td><td>26</td></tr>
<tr><td>BALB/c nu/+ (32 M)</td><td>I-H</td><td colspan="2">?</td><td colspan="2">9</td><td>26</td></tr>
<tr><td>BALB/c nu/nu (32 M)</td><td>H</td><td colspan="2">?</td><td colspan="2">10</td><td>26</td></tr>
<tr><td>DBA/2 (LS)</td><td>I-H</td><td>15</td><td>49</td><td>10</td><td>12</td><td>273</td></tr>
<tr><td>DBA/2 (LS)</td><td>I-H</td><td>18</td><td>37</td><td>?</td><td>?</td><td>266</td></tr>
<tr><td>C57BL/6J (LS)</td><td>I-H</td><td>37</td><td>46</td><td>32</td><td>46</td><td>201</td></tr>
<tr><td>C57BL/6J (LS)</td><td>I-H</td><td>7</td><td>14</td><td>?</td><td>?</td><td>266</td></tr>
<tr><td>C57BL/6J</td><td>I-H</td><td colspan="2">51</td><td colspan="2">42</td><td>200</td></tr>
<tr><td>C57BL/6J nu/nu*</td><td>I-H?</td><td colspan="2">59</td><td colspan="2">18</td><td>200</td></tr>
<tr><td>C57BL/10J (LS)</td><td>I-H</td><td>33</td><td>31</td><td>31</td><td>29</td><td>273</td></tr>
<tr><td>RF/J (LS)</td><td>I-H</td><td>49</td><td>43</td><td>49</td><td>43</td><td>266</td></tr>
<tr><td>RF/J (LS)</td><td>I-H</td><td>?</td><td>?</td><td>?</td><td>30</td><td>274</td></tr>
<tr><td>LP/J (LS)</td><td>I-L</td><td>26</td><td>20</td><td>1</td><td>9</td><td>273</td></tr>
<tr><td>LP/J (LS)</td><td>I-L</td><td>19</td><td>55</td><td>?</td><td>?</td><td>266</td></tr>
<tr><td>129/J (LS)</td><td>L</td><td>7</td><td>21</td><td>2</td><td>7</td><td>273</td></tr>
<tr><td>129/J (LS)</td><td>L</td><td>12</td><td>20</td><td>?</td><td>?</td><td>266</td></tr>
<tr><td>129/J (LS)</td><td>L</td><td>?</td><td>?</td><td>5</td><td>13</td><td>46, 49</td></tr>
<tr><td>PL/J (LS)</td><td>L</td><td>19</td><td>71</td><td>19</td><td>50</td><td>266</td></tr>
<tr><td>PL/J (LS/)</td><td>L</td><td>?</td><td>?</td><td>?</td><td>45</td><td>274</td></tr>
<tr><td>PL/J (LS)</td><td>L</td><td>?</td><td>?</td><td>6</td><td>32</td><td>46, 49, X</td></tr>
<tr><td>I/Umc (LS)</td><td>L</td><td>12</td><td>21</td><td>2</td><td>8</td><td>46, 49, X</td></tr>
<tr><td>A/J (LS)</td><td>L</td><td>13</td><td>30</td><td>?</td><td>?</td><td>266</td></tr>
<tr><td>A/J (LS)</td><td>L</td><td>65</td><td>95</td><td>3</td><td>5</td><td>46, 49, X</td></tr>
<tr><td>A/WySn (LS)</td><td>L</td><td>?</td><td>?</td><td>13</td><td>10</td><td>192</td></tr>
</table>

[a] Different strains and sublines, observed for tumor incidence for the indicated period of time (in parentheses, length of observation in months; LS: observed for whole life span). (*) these animals were grafted with a syngeneic tumors, see ref. [200] for more details.

[b] NK activity in spleen, against YAC targets, based from data in references [32–36, 221].

[c] Percent tumors in animals at risk, columns in the middle indicate overall tumor incidence without indication of sex. The question marks (?) in the table indicate that the information is not available.

[d] References cited in bibliography; (X) indicates our unpublished results. For additional consultation on lymphoma incidence in mice, see pages 169–173 in: *Inbred and Genetically Defined Strains of Laboratory Animals. Part 1. Mouse and Rat.* Altman PL, Katz DD (eds), Bethesda, MD, Federation of American Societies for Experimental Biology, 1979.

same animals showed no killing whatsoever against allogeneic lymphoma-leukemia or other tumor target lines derived from other mice trapped in the same area; conversely, CBA/J and C3H/HeJ mice, but not A/J, could kill quite well, those same lymphoma-leukemia cell lines; (3) using NIH 3T3 lines infected with wild amphotropic or ecotropic MuLV, no detectable NK activity of the wild mice against such determinants could be detected and (4) no indication of an overall defect of NK responses could be related to the expression of MuLV in this sub-population of wild mice with high incidence of leukemia [270]. In this detailed study, it is apparent that NK activity in spleen could not correlate well with the leukemia-proneness of this wild mouse population. Furthermore, the situation of this wild mice study, reacting against YAC but not against allogeneic cell lines derived from the same sub-population is reminiscent, although not supportive, of some of the problems detected in human studies [272].

The correlation between known NK activity in spleen and incidence of spontaneous or induced leukemia-lymphoma (or other tumors) in mice is presenting a complex picture, in which almost all the possibilities can be observed [46, 49]; or compare any well-detailed study on tumor incidence in inbred strains, such as those cited in ref. [192] or the studies in refs. [266, 273, 274], with their known levels of NK activity. There is also the additional problem of some inter-laboratory variation of spontaneous tumor incidence in the same mouse strains, even when using appropriate number of animals and lifespan observation periods of otherwise unmolested animals (compare [21, 46, 49, 192, 201, 266, 273–275]). However, as indicated previously, with the exception of AKR and SJL, which are both NK-low strains and which have a high incidence of spontaneous leukemia-lymphoma [192], tumor incidences, including leukemia-lymphoma does not seem to correlate well with overall levels of NK activity [46, 49]. Table 4 shows a good example of this situation, where a variety of low NK strains such as 129, LP, A, RF, I and PL do not show a very high risk for leukemia-lymphoma development, while strains such as C57BL/6 and C57BL/10, which have moderately high levels of NK activity, develop a much higher frequency of lymphomas. The case of PL/J deserves further comment. PL/J mice belong to a category of non-augmentable low NK strains, with normal NC activity ([276]; our unpublished data) which shows a variable leukemia incidence, which was moderately high in studies performed in 1966 [266] and much lower (our unpublished data) or very late appearing leukemia incidence in more recent studies [274]. In summary, Table 4 shows that several low-NK strains have a lower or comparable incidence of overall tumors or leukemia-lymphoma than the prototype NK-high CBA strain. I will not belabor this point any further, but it seems that overall spontaneous tumor incidence including leukemia-lymphoma for a particular inbred

mouse strain, does not correlate with the known levels of NK activity of that particular strain at optimal conditions of *in vitro* NK testing (usually measured against YAC.1 targets, using spleen cells within a limited age-range of donors). However, the overall strain pattern of NK reactivity, does not show variability in tissue distribution, and the pattern is preserved when NK [34, 36, 267] or NC [232, 267] activity is measured in other organs, including blood [267]. Whether the complex genetic control affecting NK [221] and NC cells [219] is part of the complex genetic control of tumor susceptibility or resistance, needs further detailed study.

4.2.3 *Tumor development in induced NK deficiencies*

Two studies have used procedures that reduce NK activity *in vivo* [217, 235] to determine the effects of such treatment on tumor development after exposure to methylcholanthrene (MCA). In one study, an antibody against the asialo-GM 1 marker, which is present on NK as well as other cells [235, 277, 278], and suppresses NK activity after *in vivo* administration was used [235]. This study used BALB/c nude mice injected subcutaneously with 600 μg of MCA [235]. The results, presented only in chart form, show marginal differences which are difficult to evaluate: 14/19 anti-asialo GM 1 treated animals developed local tumors versus 10/19 in the controls, 15 weeks after MCA treatment; although the differences between the two groups were seen almost at all time points [235]. The second study used a procedure which induces profound and apparently life-long depression of NK activity, seen after the administration of diethylstilbestrol (DES) during the first week of life [217]. The DES and control female mice of the outbred NMRI strain were treated at 8-10 weeks with a subcutaneous injection of MCA at doses of 10, 20, 50 or 100 μg/mouse. The experiment was terminated at 36 weeks, and the approximate tumor incidences (based on the charts) and with groups ranging from 20 to 44 mice, showed some suggestive differences: while tumor incidences were higher in the DES-group at 10 and 20 μg/MCA dosages, such differences were not observed at higher MCA dosages [217]. Unfortunately, tumor incidences in these animals, especially in the DES-treated group, do not follow a true dose response in relation to MCA, since the highest tumor incidence (approximately 50%) was observed at the 10 and 50 μg/mouse dosages; while the control mice showed a kind of plateau curve of approximately 20–30% tumors at all dosages, with the lower tumor incidence at 20 μg/MCA [217]. Thus, in spite of being suggestive of a role of NK in controlling tumor development, these results show enough intrinsic variation to warrant some caution in the interpretation. As mentioned earlier, both anti-asialo GM 1 and DES affected the growth of transplanted tumors [217, 235]. As was discussed in sub-section 3.3, the characteristic strain-dependent incidences of interstitial testicu-

lar tumors induced by DES [216] were not affected in normal or nude BALB/c (high tumor incidence) and CBA/H (low tumor incidence) after exposure to DES, which produced a marked decrease in NK activity, with intermediate values for the normal or nude F_1 hybrids of both strains. Thus, in spite of the low NK activity, the testicular tumor pattern characteristic for each strain of normal or nude mice was preserved.

4.2.4 *Depression of NK activity by carcinogens*

Urethan lung carcinogenesis was also studied [279–281]. The main contention of these studies is that the capacity of the carcinogen to depress NK cell activity may be '... an important factor in its carcinogenic activity...' [279]. Unfortunately, this contention is not supported by the experimental facts. While it seems to apply to the situation with A/J mice, it does not seem to be a general phenomenon applicable to other mouse strains [281, 282]. In A/J mice administration of 1 mg/g body weight of urethan injected into 6–8 week old animals, usually in repeated injections, produced a marked decrease in NK activity in spleen (this depression was biphasic, with depression at day 1 after urethan, normalization by day 4 and a more profound depression by days 7–8 which persisted 14–18 days, see ref. [279], decreased the lung clearance of injected tumor cells [280, 281] and produced high numbers of adenomas per mouse, 3 months after urethan [279–281]. A concurrent study showed that the same treatment in C57BL/6 mice did not produce depression of NK activity and did not induce lung tumors [280]. These results were considered as highly supportive of the view that the depressive effect of urethane on NK activity was a factor in lung carcinogenesis [280]. A more detailed study is described in ref. [281]. Here, the same dosage of urethan is given to A/J, CBA/J and C57BL/6 mice, and while the results in A and C57BL/6 are similar to those reported already, the situation with CBA/J shows that the generalization is impossible. In the case of CBA/J mice, the urethan treatment had no clear effect on NK activity (except for a mild decrease by day 1) and no effect on lung clearance of injected labelled tumor cells, while 67% of the animals developed lung tumors within the 3 month observation period [281]. Furthermore, the same dosage of urethane given to normal C57NL/6 and C57BL/6 bg/bg mice showed no differences in lung carcinogenesis [281]. This last finding was interpreted as a result of the lung-target cells in C57BL/6 mice (and bg/bg) being '... inherently resistant...' to carcinogenesis by urethan. However, it is worth mentioning here that C57BL/6 mice and sublines, are not resistant to leukemogenesis by urethan [282, 283] and that the A mice and sublines are the prototype strains for high spontaneous incidence and high susceptibility to carcinogen-induced lung adenoma formation, with usually 53% lung tumors at 12 months of age that rises to 90%

by 18 months ([284]; see also [192]). In addition, a certain degree of over-interpretation also permeates some experiments in this study: for example, the effect of cyclophosphamide (CY) on lung tumor development after urethan in A/J mice is indicated in the text as a 54% increase, and interpreted as indicative that such an increase is due to the further suppression of NK activity produced by CY [281]. However, the actual data shows 11.4 nodules per lung in the animals treated with urethan and 17.6 nodules in those treated with urethan plus CY, while in another experiment the incidence of lung nodules is 13 in animals receiving only urethan; suggesting that intrinsic variation between experiments also has to be considered in this type of study, as we have indicated in the past in relation to other studies on lung tumor development using immunodepressed animals of different types [21]. Ref. [285] contains a good example of marked variability induced by different factors on lung carcinogenesis by urethan in A/J mice. In addition, another study in which BALB/c mice were injected with 10 mg/mouse of urethan, also in repeated injections, showed a marked increase in NK activity in spleen, beginning 2 weeks after urethan and persisting for 8 weeks after the carcinogen [286, 287]. Thus, it is obvious that the urethan effect observed in A/J mice may not be a general phenomenon, and be perhaps related to other mechanisms.

In the past, we criticized the notion of immunodepression by the carcinogen being a required permissive factor for its carcinogenic effect [15, 54, 66], using two types of arguments [16, 17, 21, 25]: firstly, that the carcinogen doses which produced detectable effects on the immune responses (mostly T, B at that time) were usually 100 to 1000 times the actual carcinogenic dosages [16, 21] and secondly, that by manipulating the actual carcinogen dose or route of administration, a complete dissociation of its carcinogenic action from its immunodepressive action could be obtained, in which tumors developed in the absence of any detectable immunodepressive effect by the carcinogen [16, 17, 28]. It seems that similar approaches should be used in analyzing the effects of carcinogens on N-CMC effector cells, before a major generalization is made. I should add, that the urethan lung-carcinogenesis experiments have been cited already in reviews as supportive of a possible role of NK cells in anti-tumor surveillance (see refs. [7, 251, 280] for examples). Before closing this subject, I will discuss two additional sets of experimental data: one dealing with the effects of dimethylbenz(a)anthracene on NK activity and tumorigenesis [286, 287, 288] and the other with the effects of leukemogenic dosages of radiation on NK activity [289, 290]. In the first set of studies, BALB/c mice were treated with dimethylbenzanthracene (DMBA) orally at a dose of 1 mg per feeding, in 6 weekly administrations, in mice that also had or had not received a pituitary syngeneic graft [286, 287] and NK activity was found

depressed in the spleens of carcinogen-treated tumor-bearing animals. The interpretation was that the tumor-bearing state in this system (but not in a lung-carcinogenesis model induced by urethan, see previous paragraphs) depressed NK and cytostatic activity in the spleen, via a putative suppressor cell mechanism [286, 287]. In a second study, using the same mice and DMBA schedule of administration [288], it was found that although the carcinogenic treatment depressed NK activity in spleen, the low levels of NK activity did not correlate with tumor risk, since comparably low levels were observed in animals with and without mammary tumors [287]. In addition, chronic administration of poly I:C, although it augmented the lowered NK activities, did not seem to affect tumor development in this model [288]. The authors indicate that the correlation between decrease in NK activity and tumor development in their model is still unknown [288].

The leukemogenic split-dose radiation (175–179 R whole body, weekly for 4 weeks) in C57BL/6 mice has been shown to have a marked depressive effect on spleen NK activity, which was observed by 12 weeks post-irradiation and that could not be fully restored by poly I:C treatment [289]. Similar results were obtained in another study [290], which also showed that lung clearance of YAC labelled cells was also impaired, and that bone marrow cells from normal C57BL/6 mice, but not from C57BL/6 bg/bg mice, could restore the deficiency of NK in spleen and the lung clearance [290]. However, the levels of NK activity in spleen (expressed as percent lysis at 100:1 ratios, while in 281 is expressed as lytic units per spleen, making comparisons difficult) is quite minimal, ranging from 1 to 8%, which makes this study difficult to evaluate [289]. The authors indicate that this is probably related, even in the non-irradiated normal mice, to the age-related decline of NK activity in spleen, since the animals were 10–12 week old when tested [290]. For further discussion of this point and the lack of age-related decline of NK cells in blood, see previous paragraphs. The demonstration that radiation-induced-leukemogenesis could be prevented by administration of cloned cell line with NK activity [184] is probably the strongest argument for a possible correlation between NK activity and leukemogenesis in this complex experimental model [291].

Before closing the discussion of these models, I would like to return to the urethan-C57BL/6 problem discussed previously [281]. The older literature contains some interesting results, which could be reinterpreted in the view of the possible role of N-CMC in tumor development. For example Berembium et al. [282] noted that injection of adult bone marrow, failed to inhibit urethan leukemogenesis in newborn C57BL/6 mice. In a subsequent study, they showed that urethan leukemogenesis was fairly potent (35% incidence) when treatment was begun soon after birth, but weaker when urethan was

begun at 45 days of age, with only 6% incidence [292]. These results may suggest a certain role for NK cells due to their age-related development, but also shows that adult C57BL/6J mice whose NK compartment is not affected by urethan [281], are still susceptible to leukemia development. They also showed that leukemogenesis could be inhibited by thymectomy, but that in contrast to radiation-induced leukemogenesis, such inhibition would not be reversed by thymus-grafting [282]. Furthermore, they also showed that in a two stage-carcinogenesis model of a single dose of irradiation (150 to 400R) followed by a multiple injection dose of urethan at 1 mg/g body weight dosages, leukemia incidence would be increased in C57BL/6 mice, and that the younger group (28–32 day old) was more responsive than the older (37–44 day old) animals [293]. Finally, in a subsequent study, they showed that when newborn C57BL/6 mice were used, the animals did not prove to be more susceptible to radiation-induced leukemia than the older animals and that while urethan injected within the first day after birth failed to induce leukemia, two weekly injections started at 4 days of age, were leukemogenic [294]. However, leukemia incidence declined when urethan was begun at 14 or 21 days of age; with latency periods being 24 weeks for the early injections versus 44 weeks for the later urethan administrations [294]. It is apparent that these studies have a reminiscent flair following the ontogeny of NK development in mice [32–34, 137], and may support some of the views on urethan or radiation being operative in depressing NK activity and allowing the leukemogenic process to develop, as proposed for the urethan-lung model [281], or the split-dose radiation model [289, 290]. However, the failure of adult bone marrow administration to reverse this process [282], would argue against a direct role of NK cells in this model. In addition, in spite of the data with urethan and lung carcinogenesis quoted in ref. [281], C57BL/6 mice are quite susceptible to urethan carcinogenesis and develop a variety of tumors, including thymic leukemias when treated at 10 days of age [283]. It is worth mentioning here that the synergistic effect of urethan and a single low dose of irradiation has been known since 1958, and the studies included C57BL mice, from which the C57BL/6 and other sublines have been derived [295]. In summary, it is clear that further analysis of these complex biological models of induced leukemogenesis may produce interesting correlations with our present knowledge of N-CMC, especially NK-mediated responses in mice. The only aim of this tirade was to caution the first value interpretations of these possible correlations.

4.2.5 *Studies with beige (bg/bg) mice*

A few studies on spontaneous or induced tumor development in beige (bg/bg) and control mice have been recently reported [238, 243, 297–299].

In spite of the studies with transplanted tumors showing differences in behavior between normal versus low-NK beige mice ([296]; see also previous paragraphs and [36, 241–243, 296]) and in spite of the beige mice being heralded as a model for NK surveillance [5, 6, 36, 300] which may '... provide a critical test of the hypothesis that NK cells are a first line of defense against neoplasia...' [300], the studies on tumor development in beige have produced only marginal results. This absence of a spectacular confirmation of NK-mediated IS by the beige mouse, could be interpreted in several ways: (1) The NK deficiency in beige mice is not as complete as originally thought in 1979 [301] and can be detected and augmented [276, 302–304]; (2) The beige mice have an intact NC [276, 305] and promonocyte NK-like [306] compartment, which may act as IS against the induced tumors; with or without an additional macrophage defect [306, 307]; (3) The possible, albeit questionable, increase of lymphoma incidence in the untreated beige mice may be part of the complex influences of the beige gene and independent of the NK deficiency [297, 299]. Thus, the beige model was not such a good model to start with, and the disappointment voiced already by some [7, 238] may be due to the unwarranted initial high hopes [300]; (4) The lack of difference between beige and controls in tumor induction studies [238, 243, 298, 299] could be due to the 'equalizing' effect of the carcinogen-induced depression of NK activity in the normal controls (see previous paragraphs). Thus, it is possible that the variety of murine mutations, like beige, which, among other effects, influence NK activity may not be ideal models [267]. And in spite of the complex genetics of N-CMC effector cells [221, 308], it is quite probable that the 'critical test' for the role of NK or N-CMC effectors in anti-tumor IS will require the actual production of high versus low N-CMC activity congenic mouse strains.

Before discussing the more recent data on tumor development in beige mice, two older studies are worth mentioning [309, 310]. During genetic studies of the SN/Le strain which carries the beige (and satin) gene and a variety of crosses with C57BL/6J [309] it was observed that the homozygous beige animals had an increased susceptibility to spontaneous neumonitis and that 23 animals out of 558 (4%) under study at 7 to 17 months of age, involving a variety of crosses and having better correlation with homozygous satin than with homozygous beige, showed moderate to marked generalized lymphoadenopathy, '... suggestive of lymphoma...', which in 3 cases was confirmed as reticulum cell neoplasms, again with a better correlation with satin than with beige [309]. Due to Dr. Murphy's expertise in murine pathology, this finding should be considered as a cautionary note in the evaluation of beige mouse studies, since not all of the lymhoid tissue enlargements are related to actual lymphoma development [309]. A second

study, using beige animals in the C57BL/6J background (as all of the tumor studies to be discussed here), showed that lifespan was only slightly diminished when compared to the C57BL/6J controls: 23.4 months in beige versus 27.9 months in controls [310]. Unfortunately, this study does not contain information on tumor development nor cause of death [310]. However, it contrasts with the lifespan data discussed in ref. [299].

Two recent studies on spontaneous tumor incidence in beige and controls [297, 299] suggest an increase in lymphomas, however, both reports can be considered as preliminary, and the definitive publication of the studies will clarify this issue. In one letter to Nature, as a result of the publication of ref. [301] and the enthusiasm about beige mice, it was observed that 11 of 40 (27%) of beige mice in the $(C3H \times 101)F_1$ background developed '... a variety of disseminated lymphomas...' at 15-18 months of age [297]. It is also indicated that neither of the parent strains nor the F_1 hybrid kept at Harwell '... is noted for natural lymphoma...' [297]. The other study [299] indicates that in a total '... of over 200 C57BL/6 bg/bg and bg/+ mice...' studied for spontaneous tumor development, only malignant lymphomas have been observed in both groups, with a frequency of tumors in dead mice of 46% (7 of 11) in bg/bg and 27% (3 of 11) in bg/+; with no indication of age of appearance, except that '... the age profiles were the same for each group...' and that the beige mice had a shorter lifespan [299]. At first glance, in spite of the paucity of detailed information, one may conclude that the cause of death in beige mice may be related to a higher incidence of lymphomas. However, it is clear if compared to the data on normal C57BL/6J presented in Table 4, that the lymphoma incidence even in the beige is within that seen in the C57BL/6J strain.

The tumor induction studies using beige mice show mostly negative results. As already mentioned, there were no differences between beige and control C57BL/6 mice in lung tumorigenesis produced by urethan, with both groups showing resistance [281]. One study again using beige in the C57BL/6 background showed that local tumor incidence after subcutaneous injection of 100 μg of methylcholanthrene, showed no differences in tumor frequency, latency period for appearance, nor time of death due to tumor growth [243]. This is really a micro-study, since the number of animals at risk was 8 in each group; due to this fact it should be considered only as suggestive evidence, and indeed, this paper is a good example of the questionable experimental design of studies produced at the peak of enthusiasm, mentioned at the beginning of this section. A second and well-designed experiment studied tumor development in 64 bg/bg and 83 bg/+ littermates in the C57BL/6 background, which received 1 mg of DMBA intragastrically in 5 weekly dosages (as in refs. [286-288]) and tumor incidence monitored at 165 or 500 days after the first DMBA feeding [298]. The findings showed

that the beige and controls had a similar incidence of most tumors, including lymphomas, and only showed a slight increase in non-epithelial tumors arising in cutaneous or subcutaneous sites in beige mice when studied at 165 days and an increase in non-thymic lymphomas (7 of 27, 26% in bg/bg versus 1 of 33, 3% in bg/+) when studied at 500 days [298]. However, none of the differences were statistically significant [298]. In spite of that, the paper indicates that the beige mutation may cause '... a certain defect in a mechanism that may prevent or delay the onset of non-thymic lymphomas and of epithelial and non-epithelial cutaneous tumors...' in the DMBA treated animals [298]. Although indicating that the results are less spectacular than predicted from the studies with transplanted tumors, the dampening of the differences between groups may have been related to the depressive effect of the DMBA on NK activity [298]. In a subsequent paper from the same group, it is indicated that the lack of increase in tumor development in the beige may be due to the fact that '... clearly, the *bg* mutation does not provide an ideal model (since) the NK-defect it causes is neither absolute, nor selective...' [238]. This same study showed that at 200 days, tumor incidence after DMBA was similar in beige and controls (13 of 64 bg/bg and 15/83 bg/+, probably the same animals as in ref. [298]). Furthermore, they also showed no differences between groups of 20 beige and controls injected early in life with Moloney leukemia virus (M-MuLV): 7 bg/bg and 6 bg/+ developed leukemia within 165 days after infection with no differences in latency period; an additional group of beige and controls injected with M-MuLV at 4–6 weeks of age, did not develop any leukemias [238]. They also showed no differences in local tumor development, rate of growth or regression in a small group of beige and control animals injected with Moloney Sarcoma virus [238]. Thus, the procedure of denying the validity of the beige model is applied here for the interpretation of the negative tumor-development results.

Finally, another study showed no major differences between beige and controls receiving either a murine leukemia virus (9 of 13 beige and 13 of 16 bg/+ developed lymphomas); subcutaneous injection of three different (although undefined) dosages of benzo(a)pyrene (50 of 65 bg/bg and 85 of 124 bg/+ developed local tumors), or with split-dose irradiation (160R, weekly × 4), where lymphoma-leukemia incidence was 6/13 in bg/bg and 7/19 in bg/+ at 200 days [299]. In spite of this array of negative results, it is indicated that '... according to the evidence presented to date, it is quite possible that NK cells play a protective role in surveillance against the development of *some* primary tumors...' ([299], italics added). A rather florid, but interesting, scheme for a step-wise mechanism of IS in which NK cells would be the first step, is presented by Roder and collaborators in two reviews [36, 300]. Both were probably written at a time in which mostly the

Table 5. Lack of correlation between spontaneous levels of NK activity and leukemia development after exposure to methylcholanthrene.

Strain [a]	NK activity [b]	Ah [c]	Number of mice	Percent leukemia [d] (latency periods in days)	Percent skin tumors (latency periods in days)
AKR/J	Low	d	46	100 (149)	0
RF/J	Int-H	d	45	95 (135)	0
SJL/J	Low	d	40	85 (186)	20 (195)
DBA/2	Int-H	d	40	67 (239)	0
C58/J	Int-L	b	40	50 (188)	10 (250)
PL/J	Low	b	50	42 (344)	5 (375)
129/J	Low	d	48	29 (241)	10 (265)
$(I \times RF)F_1$	Low	d	32	12 (198)	16 (374)
A/J	Low	b	45	7 (550)	51 (358)
I/Umc	Low	d	49	6 (375)	6 (350)
CBA/H	High	b	53	6 (195)	92 (250)
BALB/C	Int-H	b	49	4 (323)	77 (290)

[a] Female mice treated at 10–12 weeks of age with 5 daily skin paintings with 3-methylcholanthrene in benzene, applied to a skin area in the back of approximately 2 sq. cm, using the procedure described in ref. [274]. In most of the experiments animals were observed for tumor development (leukemia and/or local skin tumors) for 400–450 days.

[b] NK activity tested in spleen cells against YAC.1 targets in 4 hr ^{51}Cr release assays. Int-H or Int-L means that the NK values are intermediate-high or low, respectively. For further details on NK activity in many of these strains see refs. [32–36].

[c] Aryl-hydrocarbon-hydroxylase (Ahh) levels of activity controlled by the Ah gene [126]; the b allele is the inducible or responsive; the d allele is the non-responsive. For details on strain distribution of Ahh levels of activity see p. 81 in: *Inbred and Genetically defined strains of laboratory animals, Part 1, Mouse and Rat.* Altman PL, Katz DD (eds), Bethesda, Md, Federation of American Societies for Experimental Biology, 1979.

[d] Tumor incidence expressed as percent of animals with tumor per total animals at risk at 400–450 days after treatment; mean latency periods in days, in parentheses. Strains are ranked by leukemia incidence.

tumor transplantation studies in beige mice were available, however in one of the reviews [300] it is indicated, as unpublished data, that beige mice showed a higher incidence of tumors after exposure to benzo(a)pyrene, although later it was found that the overall differences between beige and controls in this tumor model showed no statistical significance [299].

4.2.6 *Lymphoma-leukemia development in low-NK strains*

In a study still in progress, briefly reported in refs. [46] and [49], we indicated that spontaneous leukemia-lymphoma incidence (see Table 4 and subsection 4.2.2) did not correlate well with known levels of NK activity in

those strains. In addition, the incidence of T cell leukemias (and skin tumors) induced by skin painting with methylcholanthrene, using the model developed by Duran-Reynals and collaborators [274], also did not correlate well with the levels of NK activity, especially in a variety of NK-low strains [46, 49]. Table 5 presents some of this data in more detail. The procedure used female mice, whose flanks and back were shaved, and which were painted daily for 5 days with a solution of 1 g methylcholanthrene (MCA) in 100 ml of benzene, using a brush dipped once in the solution and painting an area in the back of approximately 2 cm^2; the treatment was begun at 10–12 weeks of age. It is obvious from the results in Table 5 that extreme caution should be used in interpreting these results, since examples to please almost any bias can be found, depending on the strain tested. For example, with the NK-low strains such as 129, PL, A, I and $(I \times RF)F_1$ the leukemia incidence was 29, 42, 7, 6 and 12% respectively, and in most of those strains the leukemias appeared much later than in other strains such as AKR, SJL (both NK-low) or C58 and DBA/2 (both with intermediate-high NK levels). In the case of PL, for example, at 250 days after treatment, leukemia was usually 10% or less. As was discussed previously, the PL/J have a more profound NK defect than the beige, which is not augmentable by either long-term assay nor interferon-inducers ([276]; our unpublished data; see also sub-section 4.2), and however, are relatively resistant to early induction of leukemia by MCA-painting and have an intermediate incidence of spontaneous leukemia-lymphoma which appears late in life (see Table 4 and 5). On the other hand, the incidence of skin tumors, followed a somewhat inverse pattern from leukemia, as was previously described [274, with all the permutations again being expressed by the different strains. It is interesting to note that the leukemia/skin tumor pattern in A/J and CBA/H (or CBA/J, see [274]) are quite comparable in spite of A being one of the prototype low-NK strains and CBA being the prototype high-NK strain [32, 34, 36]. It is also clear that if only one or two strains had been used in this study, depending on chance or some educated guess in selecting them, a rather compelling case for an important role of NK cells in controlling leukemogenesis, with no relevance for skin carcinogenesis would have emerged. For example imagine if the study would have included only AKR, SJL and CBA! I might add that in this particular model of leukemia development in the NK-low strains such as 129, PL, A, I and $(I \times RF)F_1$ mice, the putative depressive effect of the carcinogen on NK activity becomes almost irrelevant; since even assuming that the carcinogenic schedule depresses NK activity further (and our preliminary testings suggest that it does), some strains remain quite resistant to leukemia development in spite of their low NK levels. As was indicated for C3H and RF crosses [274] leukemia development does not correlate with expression of the responsive

allele at the Ah locus which regulates MCA metabolism [216], while skin tumor development usually does [274]. The Ah phenotype of the strains used is also indicated in Table 5. Similarly, as discussed in sub-section 3.2, the lymphomas induced by anti-mu treatment in nude mice in the CBA/H and BALB/c background, develop in the presence of an intact high activity NK compartment, especially in the CBA/H nudes (see Table 2 and ref. [46]). Thus, it seems that alternative interpretations besides the possible role of NK cells (or perhaps other N-CMC effectors), seem necessary to explain these results. In the case of the MCA model of leukemia development, it involves complex genetic influences [274, 275, 311]. It is also apparent that a narrow model, or the use of a limited number of mouse strains may provide some results which may seem to support the desired argument, but which may prove to be isolated cases and not a truly general phenomenon.

4.2.7 *Conclusions*

One has to conclude from the experimental data discussed in this subsection 4.2, that in spite of the arguments discussed in Section 4 and 4.1 concerning the general properties of the N-CMC systems, and in spite of some persuasive discussions in the published literature [5–8, 29, 32–36, 55, 219, 250, 251, 280, 300]; the experimental data on tumor development in conditions of impairment of NK function, do not support the idea of a major role for the NK branch of the N-CMC system as a surveillance device preventing tumor development in mice and rats, regardless of tumor type. It is obvious that most of these are still incipient studies, which in the case of the beige mice, seem more disappointing due to the high initial expectancy concerning that model [36, 300]. It is also clear that further studies, well planned, and with sufficient numbers of animals involved, will certainly help clarify the possible role of N-CMC mechanisms in anti-tumor surveillance, or at other stages of the complex tumor-host interaction.

4.3 *The clinical evidence*

I will start this sub-section with two provisos. Firstly, that the definition of 'normal' levels of NK activity in peripheral blood in healthy normals is not an easy task and high-low individual reactive patterns can be quite persistent and different [312]; and that a variety of extraneous factors such as alcohol or aspirin consumption, smoking habits (see [312] for appropriate references) as well as intrinsic variations of the assays and modes of handling the experimental data and expressing the cytotoxicity results can affect the overall values, making comparisons difficult [312]. Thus, the indication of abnormal values for NK activity in blood of patients with different diseases including cancer, requires careful analysis and control [312]. Secondly,

as indicated before, space limitations will make of this sub-section just a cursory excusion over some of the available data, especially in patients with the immunodeficiencies discussed in sub-section 2.2. I will just use a few examples to point towards some interesting questions, rather than evaluate in detail all of the available clinical literature with its merits and/or defects. It is also apparent that some re-adjustment of the evaluation of the significance of the changes in NK activity observed in the clinical studies, will take place in the near future, with our increasing understanding of the regulation and function of NK cells and N-CMC in humans. Two temperate and perceptive reviews on the clinical significance of N-CMC in humans can be found in references [313] and [314]; similarlyn reference [9] contains a wealth of information on human N-CMC effectors and clinical correlations. On the other hand, pleasant uninformative editorials [315] should be avoided. Further clinical studies will certainly tell us in the near future whether N-CMC and its different effector cells plays a role in any of three levels of tumor-host interaction described in sub-section 1.2 (i.e. IS which is the reaction against *in situ* incipient tumors; tumor immunity, which is the post-factum set of complex responses of the host to the developing, and usually clinically recognizable tumor; and the complex mechanisms which affect metastatic spread of the tumor); and even in a fourth level, in this case man-made, which is the possible manipulation of residual tumor after surgery-chemotherapy. On the other hand, the simple descriptive listings of NK levels in blood measured in short term assays against K-562 targets, in a myriad of different diseases, which appear almost daily in the literature, will not improve our understanding of the *in vivo* role of N-CMC.

A good example of the problems in evaluating the association of NK abnormalities with disease, is provided by multiple sclerosis, where a variety of conflicting results concerning NK activity and their possible role in the pathogenesis of the disease have been reported (the appropriate references in [316]) which, however could not be sustained when monozygotic twins discordant for multiple sclerosis were compared: both interferon production and NK activity were similar between the affected and the heatlhy twins [316]. Similarly, and more directly related to the subject of this review and the patients discussed in sub-section 2.2, although an initial study showed that 10 of 12 males with x-linked lymphoproliferative disorder had marked deficiency of NK activity [317], which was cited as good evidence for a possible role of NK cells in IS [36, 151, 300]; a subsequent study showed quite clearly that the NK defect is secondary to the development of the syndrome and the EBV infection [318]. It is obvious that whether primary or secondary to the syndrome, the possible role of low NK activity and subsequent lymphoma development [155], needs further study. The studies on NK activity in patients with the naturally occurring immunode-

ficiencies, which as was discussed in section 2.2 are at a higher risk of developing lymphomas [145], have given a mixed picture of results with a predominance of normal NK activity in x-linked agammaglobulinemia and other forms of hypogammaglobulinemia; a depressed NK activity in most patients with severe combined immunodeficiency, and a variable frequency of low or normal NK activity in patients with other immunodeficiencies such as ataxia telangiectasia, Wiskott-Aldrich syndrome, IgA deficiency, etc. [319-322]. Forty-seven patients with different types of immunodeficiencies have been studied [319-321]. The common feature, stressed by Peter and collaborators [313, 322], being the association with impaired function of the bone marrow and the hemopoietic system. Since, in addition to the immunodeficiencies with marrow involvement cited above, low NK activity was also observed in patients with leukemia, preleukemia, osteomyelofibrosis, polycythemia vera, myeloma [322] and Fanconi's anemia [323]. These studies support the murine data on the extreme marrow dependency of the NK branch of N-CMC [32-36]. In some cases [319, 323], the defect appears related to problems in interferon production. In trisomy-21/Down's syndrome, which does not show a defect of overall NK activity, the augmentation of NK activity by interferon seems abnormal [324]. Whether this subtle NK change is related to the increased risk of the trisomic patients to infections or lymphoma-leukemia (ref. [324] contains the appropriate references; see also ref. [21]) needs further study. A decreased and protracted depressed NK activity has been also described in the patients immunodepressed for kidney transplantation [325]. The patients with Chediak-Higashi syndrome (of which the beige mouse is one animal homologue) have decreased NK activity [326] and a certain propensity for development of lymphoproliferative disorders (see [326] or [21] for appropriate references). However, it is not yeat clear whether the NK defect is the permissive factor for malignant development or part of the same abnormality which favors malignant change. Conversely, lepromatous leprosy patients, which, as discussed in sub-section 2.2, do not have an increased risk for malignancy or lymphoma development in spite of their immunodeficiency [182], do have suppressed NK activity during episodes of erythema nodosum [327]. In the case of patients with acquired immunodeficiency syndrome (see sub-section 2.2) only one study showed that all 4 patients had low NK activity when tested against herpes virus-infected targets [174]. Of these four patients only one had Kaposi's sarcoma [174].

In summary, with the reservations concerning the increased risk for development of a very limited range of tumors in the patients with naturally occurring or induced immunodeficiencies discussed in sub-section 2.2; it appears that most of those patients show low levels of NK activity at some stages of their disease. However, whether low NK activity in these patients

is in any way related to the increased tumor risk or is a consequence of tumor development, is only conjectural at present.

Finally, I would like to discuss three more aspects of the studies of NK activity in cancer patients. Firstly, the observation of lower NK-like activity in patients and some relatives with familial melanoma [328], needs confirmation and extension to other types of familial cancer. As was indicated previously, only a few of the 16 males at risk in the case of the x-linked lymphoproliferative disorder, and none of the other family members, showed decreased levels of NK activity [318].

Secondly, the possible prognostic value of tumor-related changes in NK activity in patients, is beginning to be evaluated, unfortunately with complex results of difficult interpretation, which prompted one group of researchers to indicate that '... the question of prognostic significance of NK activity in melanoma patients is hence largely unresolved...' [329]. Although a positive correlation between NK levels and prognosis in melanoma patients was reported [330] which was not corroborated in a somewhat similar study [331], albeit using a different assay for N-CMC; a subsequent analysis [329, 332] showed correlations which are difficult to interpret, if indeed NK cells should be '... an important surveillance mechanism against tumors *in vivo*...' [332]. In these studies it was shown that the tumor bearing state reduced NK activity (as was observed in some of the animal studies, see [32–36] for appropriate references in murine models); that NK levels increased after removal of the melanoma; and that the prognostic value of the NK levels was influenced by stage of disease and tumor thickness, as well as age of the patient [332]. Thus, there was a correlation of NK activity measured shortly after removal of the primary, with thickness of the tumor, but the patients with *high* NK activity at this time had a worse prognosis, and the interval between removal of the primary and local recurrences was shorter in the patients with high NK activity [329, 332]. In another study, it was shown that there was no correlation whatsoever between NK levels (before or after interferon therapy) and the clinical response in a group of 39 myeloma patients treated with interferon [333]. In general it is agreed that the NK defect in the blood of patients with cancer becomes more pronounced with progression of disease and metastatic spread or untreated leukemia [334, 335]. NK activity in nodes draining a variety of solid tumors could not be correlated with level, size, histological type, hyperplasia or metastatic spread in the regional node [336]. The studies which simply measured NK activity in cancer patients showed a whole spectrum of responses which include: insignificant depression in melanoma patients when compared to controls [337]; no differences between breast cancer patients and controls [336]; depression of NK activity in more than one third of 83 patients with untreated breast cancer, with the same pattern in 25 patients

with benign breast lesions [338]; while 31% of 51 adult patients with a variety of solid epithelial tumors showed low NK activity, only 7% of 27 normal controls failed to kill the K-562 target, however, while NK activity in all the normal low-NK responders could be restored by *in vitro* treatment with interferon, only half of the low-NK cancer patients could be restored [339]; patients with leukemia have in general low NK activity in blood [322, 335, 340] which may get normalized during remission, as is the case with acute myelocytic leukemia [341]. In summary, a rather variable picture which suggests that during the early stages of tumor development, the impairment of NK activity in blood (and nodes, when studied) may be comparable to normal, with a decline in activity associated with progression and metastatic spread.

Thirdly, and finally, the effects of many of the adjuvant therapies, whether putative immunomodulators or not, on NK activities is in some cases different than expected, if the expectation is that NK cells are participating in the beneficial effects of such therapies. For example, levamisole has been shown to be inhibitory of NK activity *in vitro* [342]. On the other hand, some of the cis-platinum compounds used in the clinic seem to increase N-CMC effector activity [343]. DES in prostatic cancer patients has been shown to profoundly depress NK activity [344], as was observed in the murine studies [217]. Similarly, interferon; which has been shown to be a major booster of NK activity in animals and man [32–34, 36, 250, 251, 280], actually reduced NK activity in patients treated for protracted periods of time [345, 346]. It is obvious from the above listing of 'paradoxes' that our understanding of the *in vivo* role of N-CMC, and its possible function in IS and anti-tumor responses, is still fragmentary and that further detailed studies are required before we can take a firm stance concerning its possible role in IS or other forms of the tumor-host interaction in humans.

5. CONCLUDING REMARKS

It is apparent that due to some space constraints, this review has produced a peculiar hybrid text in which some subjects have been discussed with punctilious detail, while others have been addressed in more general terms. It certainly does not address the more general properties, functions and problems of the N-CMC field, which I hope will be covered in other chapters in this volume. However, several important areas of the N-CMC field have not been discussed in this text: areas which, in the long run, may actually define the true *in vivo* role of N-CMC, and which may have little to do with anti-tumor activities, as was proposed for the more conventional immunological responses [21, 25, 29]. These include the overall regulation

of lymphohemopoiesis [347, 348], of which resistance to hemopoietic grafts [349, 350] or hybrid resistance to tumor grafts [221, 351] may be just two related examples; and the possible role of N-CMC in the defense mechanisms against a variety of bacterial, viral or parasitic infections (see pages 1445 to 1526 in ref. [9] for pertinent studies). Whether these are the 'true' functions of N-CMC, and the anti-tumor component is just a by-product of the general functional properties of the NCMC system, needs further definition. I will not repeat here some of our past comments on the voluntaristic need to find and define a special defense mechanism against cancer [21, 25, 29], and not to assume that it may simply be the by-product of the functions of a more general defense mechanism (of which N-CMC is a branch) against infections. However, it is worth mentioning here that, as discussed in sub-section 4.1 (see also [29]), the N-CMC system has indeed the potential capacity to act as an anti-tumor surveillance mechanism in the sense of the traditional IS (see sub-section 1.1); a potential capacity which is not shared by most of the conventional immunological responses (see ref. [29, 49, 57]). As a matter of fact, as stressed in past reviews [21, 25, 29, 49], this is the first time that an operative mechanism which may act as surveillance has emerged. Whether NK cells have actually 'rescued' IS as proposed recently [8], is still too premature to announce. Although as discussed in the text, some studies have produced impressive data on the capacity of N-CMC to affect the growth of transplanted tumors, especially as artificially-induced metastasis; our discussion in sub-section 4.2 shows that the NK branch of N-CMC has not produced a good correlation between deficient NK activity and spontaneous or induced tumor development in animal models. This, after all, is still the main (and perhaps sole) prediction of the original IS theory [15, 54], i.e. that the deficiency of the putative effector mechanisms which mediates IS, should increase the risk for tumor development. Whether other branches of N-CMC effector mechanisms may act as anti-tumor surveillance devises [35, 219, 232] needs extensive study. Whether NK and other N–CMC effector cells are part of the overall anti-tumor response of the host (see sub-section 1.2), rather than the more restricted IS function, seems to be a possibility, based on the experimental and clinical data.

Thus, at present writing, it seems that IS remains as a limited event that may apply to a limited number of tumors, and which in some cases can be interpreted by alternative hypotheses (e.g. the case of the lymphomas in immunodepressed patients, see sub-section 2.2). Whether in a restricted or an expanded form (to cover other types of tumors), the possibility of an IS-like mechanism mediated by N-CMC, cannot be fully dismissed at present, and certainly merits further study. In general, the main prediction of IS still remains that of an increased risk for tumor development when the

putative IS mechanism is defective. It is possible that from a more incisive understanding of N-CMC we may develop models of N-CMC deficiency which may help clarify that question. It is also apparent from sub-section 4.2, that the partial NK deficiency in beige mice does not seem to represent the idea model for testing the role of NK cells in IS. Thus, the present status of IS seems to be one of guarded convalescence from the present day schizophrenic period, which followed the manic state of the early seventies.

ACKNOWLEDGEMENTS

I would like to thank Fred Mekeel (deceased), Robert Bohnenberger, Michael J. Cuttito and Mary Lou Devitt for the technical help in the animal experiments described here which were supported by grants from the National Institutes of Health, CA-08748 and CA-15988 and Linda Stevenson for preparation of the manuscript. This review was completed december 22, 1982.

REFERENCES

1. Howard JC: Immune surveillance. In: Neoplastic transformation: Mechanisms and consequences. Koprowski H (ed), Berlin, Dahlen Conferenzen, Life Sciences Research Report 7, 1977, pp 227–245.
2. Patek PQ, Collins JL, Cohn M: Transformed cell lines susceptible or resistant to *in vivo* surveillance against tumorigenesis. Nature 276:510–511, 1978.
3. Collins JL, Patek PQ, Cohn M: In vivo surveillance of tumorigenic cells transformed in vitro. Nature 299:169–171, 1982.
4. Moller G, Moller E: Immunologic surveillance revisited. Transplant Proc 11:1041–1046, 1979.
5. Mitchison NA, Kinlen LJ: Present concepts in immune surveillance. In: Immunology 80: Progress in immunology IV. Fougereau M, Dausset J (eds). New York: Academic Press, 1980, pp 641–650.
6. Mitchison NA: The immunology of leukemia: An overview. In: Advances in comparative leukemia research 1981, Yohn DS, Blakeslee JR (eds). New York: Elsevier Biomedical, 1982, pp 157–168.
7. Wigzell H: Immunosurveillance. In: Advances in comparative leukemia research 1981, Yohn DS, Blakeslee JR (eds). New York: Elsevier Biomedical, 1982, pp 173–179.
8. Bloom BR: Natural killers to rescue immune surveillance? *Nature* 300:214–215, 1982.
9. Herberman RB (ed): NK cells and other natural effector cells. New York: Academic Press, 1982.
10. Baldwin RW: Immune surveillance revisited. *Nature* 270:557, 1977.
11. Allison AC: Immunological surveillance of tumours. Cancer Immunol Immunother 2:151–155, 1977.
12. Klein G, Klein E: Immune surveillance against virus-induced tumors and non-rejectability of spontaneous tumors: Contrasting consequences of host versus tumor evolution. Proc Natl Acad Sci USA 74:2121–2125, 1977.

13. Herberman RB, Holden HT: Natural killer cells as antitumor effector cells. J Natl Cancer Inst 62:441–445, 1979.
14. Moller G (ed): Natural killer cells. Immunol Rev 44:1979.
15. Burnet FM: The concept of immunological surveillance. Prog Exp Tumor Res 13:1–27, 1970.
16. Stutman O: Immunologic studies on resistance to oncogenic agents in mice. Natl Cancer Inst Monogr 35:107–115, 1972.
17. Stutman O: Immunological aspects of resistance to the oncogenic effect of 3-methylcholanthrene in mice. Isr J Med Sci 9:217–228, 1973.
18. Stutman O: Tumor development after 3-methylcholanthrene in immunologically deficient athymic nude mice. Science 183:534–536, 1974.
19. Stutman O: Tumor development on immunologically deficient nude mice after exposure to chemical carcinogens. In: Proc of the First International Workshop on Nude Mice, Rygaard J, Poulsen (eds). Stuttgart: G. Fisher, 1974 pp 257–264.
20. Stutman O: Tumor development after polyoma infection in athymic nude mice. J Immunol 144:1213–1217, 1975.
21. Stutman O: Immunodepression and malignancy. Adv Cancer Res 22:261–422, 1975.
22. Stutman O: Immunodepression and malignancy. In: Proceedings of the XIth International Cancer Congress, Florence 1974. Bucalossi P, Veronesi U, Cascinelli (eds). Amsterdam, Excerpta Medica, 1975, Vol. 1, pp 275–279.
23. Outzen HC, Custer RP, Eaton GH, Prehn RT: Spontaneous and induced tumor incidence in germfree 'nude' mice. J Reticuloendothel Soc 17:1–9, 1975.
24. Rygaard J, Paulsen CO: The nude mouse vs the hypothesis of immunological surveillance. Transplant Rev 28:43–61, 1976.
25. Stutman O: Immunological surveillance. In: Origins of human cancer, Hiatt HH, Watson JD, Winsten JW (eds). Cold Spring Harbor, NY: Cold Spring Harbor Laboratories, 1977, pp 729–750.
26. Stutman O: Spontaneous, viral and chemically induced tumors in the nude mouse. In: The nude mouse in experimental and clinical research, Fogh J, Giovanella BC (eds) New York, Academic Press, 1978 p 411–435.
27. Stutman O: Spontaneous tumors in nude mice: Effect of the viable yellow gene. Exp Cell Biol 47:129–135, 1979.
28. Stutman O: Chemical carcinogenesis in nude mice: Comparisons between nude mice from homozygous matings and heterozygous matings and effect of age and carcinogen dose. J Natl Cancer Inst 62:353–358, 1979.
29. Stutman O: Immunological surveillance and cancer. In: The handbook of cancer immunology, vol. 7, Waters H (ed). New York: Garland STPM Press, 1981 pp 1–25.
30. Herberman RB: Concluding remarks. Natl Cancer Inst Monogr. 37:217–218, 1973.
31. Herberman RB, Gaylord CE (eds): Conference and workshop on cellular immune reactions to human tumor-associated antigens. Natl Cancer Inst Monogr 37:1973.
32. Herberman RB, Holden HT: Natural cell-mediated immunity. Adv Cancer Res 27:305–377, 1978.
33. Kiessling R, Haller O: Natural killer cells in the mouse: An alternative immune surveillance mechanism? Contemp Topics Immunobiol 8:171–201, 1978.
34. Kiessling R, Wigzell H: An analysis of murine NK cells as to structure, function and biological relevance. Immunol Rev 44:165–208, 1979.
35. Stutman O, Lattime EC, Figarella EF: Natural cytotoxic cells against solid tumors in mice: A comparison with natural killer cells. Fed Proc 40:2699–2704, 1981.
36. Roder JE, Karre K, Kiessling R: Natural killer cells. Prog Allergy 28:66–159, 1981.
37. Alexander P: Surveillance against neoplastic cells — It is mediated by macrophages? Br J Cancer 33:344–345, 1976.

38. Hibbs JB, Chapman HA, Weinberg JB: The macrophage as an antineoplastic surveillance cell: Biological perspectives. J Reticuloendothel Soc 24:549–570, 1978.
39. Chow DA, Greene MI, Greenberg AH: Macrophage-dependent, NK-cell independent 'natural' surveillance of tumors in syngeneic mice. Int J Cancer 23:788–797, 1979.
40. Adams DO, Snyderman R: Do macrophages destroy nascent tumors? J Natl Cancer Inst 62:1341–1345, 1979.
41. Keller R: Regulatory capacities of mononuclear phagocytes with particular reference to natural immunity against tumors. In: Natural cell-mediated immunity against tumors, Herberman R.B. (ed). New York: Academic Press, 1980, pp 1219–1269.
42. Menard S, Colnaghi MI, Della Porta G: Natural antitumor serum reactivity in BALB/c mice: I. Characterization and interference with tumor growth. Int J Cancer 19:267–274, 1977.
43. Chow DA, Wolosin LB, Greenberg AH: Murine natural anti-tumor antibodies: II. The contribution of natural antibodies to tumor surveillance. Int J Cancer 27:459–469, 1981.
44. Shin HS, Johnson RJ, Pasternak GR, Economu JS: Mechanisms of tumor immunity: The role of antibody and non-immune effectors. Prog Allergy 25:163–210, 1978.
45. Greenberg AH, Greene M: Non-adaptive rejection of small tumor inocula as a model of immune surveillance. Nature 264:356–359, 1976.
46. Stutman O: Natural anti-tumor resistance in immune-deficient mice. Proc 4th Int Workshop in Immune-deficient Animals in Experimental Research. Basel: S. Karger AG. 1983. In press.
47. Kripke ML: Immunologic mechanisms in UV radiation carcinogenesis. Adv. Cancer Res 34:69–106, 1981.
48. Urban JL, Burton RC, Holland JM, Kripke ML, Schreiber H: Mechanisms of syngeneic tumor rejection: Susceptibility of host-selected progressor variants to various immunological effector cells. J Exp Med 155:557–573, 1982.
49. Stutman O: Cellular regulation of tumor immunity an overview. Proc 13th Int Cancer Congress. Alan Liss, Publ. In press.
50. Klein G, Purtilo DT (eds): Symposium on Epstein-Barr virus-induced lymphoproliferative diseases in immunodeficient patients. Cancer Res 41:4209–4304, 1981.
51. Louie S, Schwartz RS: Immunodeficiency and the pathogenesis of lymphoma and leukemia. Seminars in Hematology 15:117–138, 1978.
52. Klein G: Immune and non-immune control of neoplastic development: Contrasting effects on host and tumor evolution. Cancer 45:2486–2499, 1980.
53. Klein G: Immunological surveillance against neoplasia. The Harvey Lectures 69:71–102, 1975.
54. Burnet FM: Immunological surveillance in neoplasia. Transplant Rev 7:3–25, 1971.
55. Woodruff MFA: The interaction of cancer and host: Its therapeutic significance. New York: Grune & Stratton, 1980.
56. Fidler IJ, Hart IR: Biological diversity in metastatic neoplasms: Origins and Implications. Science 217:998–1003, 1982.
57. Stutman O: Some thoughts on tumor immunology and immunotherapy. In: Proceedings on Immunology of the Eye, Workshop I, Steinberg GM, Gery I, Nussenblatt RB (eds). Virginia: Information Retrival Inc., 1980, pp 179–190.
58. Hellstrom KE, Hellstrom I: Lymphocyte mediated cytotoxicity and blocking serum activity to tumor antigens. Adv Immunol 18:209–277, 1974.
59. Plescia OJ, Smith AH, Grinwich K: Subversion of immune system by tumor cells and role of prostaglandins. Proc Natl Acad Sci USA 72:1848–1851, 1975.
60. Kamo I, Friedman H: Immunosuppression and the role of suppressive factors in cancer. Adv Cancer Res 25:271–321, 1977.

61. North RJ, Spitalny GL, Kirstein DP: Antitumor defense mechanisms and their subversion. In: The handbook of cancer immunology, Vol. 2, Waters H (ed). New York: Garland STPM Press, 1978, pp 187–223.
62. Naor D: Suppressor cells: Permitters and promoters of malignancy? Adv Cancer Res 29:45–125, 1979.
63. Greene MI: The genetic and cellular basis of regulation of the immune response to tumor antigens. Contemp Topics Immunobiol 11:81–116, 1981.
64. Nelson DS, Nelson M, Farram E, Inoue Y: Cancer and subversion of host defenses. Aust J Exp Biol Med Sci 59:229–262, 1981.
65. Klein G: Theme 10: Summary tumor immunology. In: Immunology 80: Progress of immunology, IV, Fougereau M, Dausset J (eds). New York: Academic Press, 1980 pp 680–687.
66. Prehn RT: Functions of depressed immunologic reactivity during carcinogenesis. J Natl Cancer Inst 31:791–805, 1963.
67. Thomas L: Discussion remarks. In: Cellular and humoral aspects of the hypersensitivity state. Lawrence HS (ed). New York: Hoeber-Harper, 1959, pp 529–532.
68. Brent L: Tissue transplantation immunity. Prog. Allergy 5:271–348, 1958.
69. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B: An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci USA 72:3666–3670, 1975.
70. Taverne J, Depledge P, Playfair JHL: Differential sensitivity *in vivo* of lethal and nonlethal malarial parasites to endotoxin-induced serum factor. Infect Immun 37:927–934, 1982.
71. Sado T, Hoshino H, Kamisaku H, Tanooka H: Absence of correlation between T-lymphocyte activity and development of carcinogen-induced skin tumor in mice. Gann 70:755–762, 1979.
72. Daynes RA, Harris CC, Connor RJ, Eichwald EJ: Skin cancer development in mice exposed chronically to immunosuppressive agents. J Natl Cancer Inst 62:1075–1981, 1979.
73. Curtis GL, Ryan WL, Stenback F: Antibody stimulation of benzo(a) pyrene carcinogenesis. Cancer Lett 4:223–228, 1978.
74. Fink MP, Parker CW, Shearer WT: Antibody stimulation of tumor growth in T-cell depleted mice. Nature 255:404–405, 1975.
75. Stenback F, Curtis G, Ryan W: Effect of ALS or ATS administration on tumor formation in two-stage carcinogenesis. Cancer Immunol Immunother 6:125–128, 1979.
76. Stenback F, Peto R, Shubik P: Initiation and promotion at different ages and doses in 2200 mice: II. Decrease in promotion by TPA with ageing. Br J Cancer 44:15–23, 1981.
77. Summerhayes IC, Franks ML: Effects of donor age on neoplastic transformation of adult bladder epithelium in vitro. J Natl Cancer Inst 62:1017–1023, 1979.
78. Gatti RA, Good RA: Occurrence of malignancy in immunodeficiency diseases. In: Cancer genetics, Lynch HT (ed). Springfield IL: Charles Thomas, 1976, pp 63–75.
79. Outzen HC: Development of carcinogen-induced skin tumors in mice with varied states of immune capacity. Int J Cancer 26:87–92, 1980.
80. Prehn RT, Lappe MA: An immunostimulation theory of tumor development. Transplant Rev 7:26–54, 1971.
81. Prehn RT, Outzen HC: Immunostimulation of tumor growth. In: Immunology 80: Progress in immunology IV, Fougereau M, Dausset J (eds). New York: Academic Press, 1980, pp 651–658.
82. Andrews EJ: Failure of immunosurveillance against chemically induced *in situ* tumors in mice. J Natl Cancer Inst 52:729–732, 1974.
83. Brodt P, Gordon J: Anti-tumor immunity in B lymphocyte-deprived mice: I. Immunity to a chemically-induced tumor. J. Immunol. 121:359–362, 1978.

84. Gidlund M, Ojo EA, Orn A, Wigzell H, Murgita RA: Severe suppression of the B-cell system has no impact on the maturation of natural killer cells in mice. Scand J Immunol 9:167–173, 1979.
85. Brodt P, Gordon J: Antitumor immunity in B-lymphocyte-deprived mice: II. In vitro studies. Cell Immunol 65:20–32, 1981.
86. Brodt P. Gordon J: Natural resistance mechanisms may play a role in protection against chemical carcinogens. Cancer Immunol immunother 13:125–127, 1982.
87. Pirofsky B, Dawson PJ, Reid RH: Lack of oncogenicity with immunosuppressive therapy. Cancer 45:2096–2101, 1980.
88. Merton RK: The sociology of science: Theoretical and empirical investigations. Chicago: The Univ Chicago Press, 1973, pp 381–418.
89. Cruse JP, Lewin MR, Clark CG: Corynebacterium parvum enhances colonic cancer in diemthylhydrazide-treated rats. Br J Cancer 37:639-643, 1978.
90. Rogers EA, Gildin J: Effect of BCG on dimethylhydrazine induction of colon tumors in rats. J Natl Cancer Inst 55:385–391, 1975.
91. Schmall D, Habs M, Diehl B: Experimental investigations on the influence upon the chemical carcinogenesis: II. Studies with 3,4-benzopyrene. Z. Krebsforsch 86:86–88, 1976.
92. Schmahl D, Danisman A, Habs M, Diehl B: Experimental investigations on the influence upon the chemical carcinogenesis: III. Studies with 1,2-dimethylhydrazine. Z Krebforsch 86:89–94, 1976.
93. Habs M, Schmahl D: Influence of five different postnatal lifelong treatments on the transplacental carcinogenicity of ethylnitrosourea in Sprague-Dawley rats. Cancer Lett 2:93–100, 1976.
94. Hard GC: Levemisole is without effect in modifying chemically-induced renal carcinogenesis. Cancer Lett 3:221–226, 1977.
95. Kripke ML, Borsos T: Accelerated development of benzo(a)pyrene-induced skin tumors in mice treated with pyran copolymer. J Natl Cancer Inst 53:1409–1410, 1974.
96. Schinitsky MR, Hyman LR, Blazkovec AA, Burkholder PM: Bacillus Calmette-Guerin vaccination and skin tumor promotion with croton oil in mice. Cancer Res 33:659–663, 1973.
97. Tokunaga T, Yamamoto S, Nakamura RM, Kataoka T: Immunotherapeutic and immunoprophylactic effects of BCG on 3-methylcholanthrene-induced autochtonous tumors in Swiss mice. J Natl Cancer Inst 53:459–563, 1974.
98. Ishibashi T, Yamada H, Harada S, Harada Y, Takamoto M, Sugiyama K: Comparison of the mode of immunopotentiating action of BCG and Wax D: II. Effect on the methylcholanthrene carcinogenesis. Jap J Exp Med 47:435–440, 1977.
99. Bekierkunst A, Levij IS, Yarkoni E, Vilkas E, Lederer E: Suppression of urethan-induced lung adenomas in mice treated with Trehalose-6,6-fimycolate (cord factor) and living Bacillus Calmette Guerin. Science 174:1240–1242, 1971.
100. Ikegami R, Rakatsu K, Ono S, Hamaoka T, Fujiwara H, Kitagawa M, Azuma I, Yamamura Y: Prophylactic effect of BCG cell-wall skeleton on the tumor induction by 7,12-dimethylbenz(a)anthracene in mice: Strain difference. Gann 70:101–107, 1979.
101. Piessens WF, Heimann R, Legros N, Heuson JC: Effect of Bacillus Calmette-Guerin on mammary tumor formation and cellular immunity in dimethylbenz(a)anthrene treated rats. Cancer Res 31:1061–1065, 1971.
102. Bruley-Rosset M, Florentin I, Kiger N, Schulz J, Davigny M, Mathe G: Age-related changes of the immune response and immunorestoration by stimulating agents. In: The immune system: Functions and therapy of dysfunction. Doris G, Eshkol A (eds). New York: Academic Press, 1980 pp 171–187.

103. Bruley-Rosset M, Hercend T, Martinez J, Rappaport H, Mathe G: Prevention of spontaneous tumors of aged mice by immunopharmacologic manipulation: Study of immune antitumor mechanisms. J Natl Cancer Inst 66:1113–1119, 1981.
104. Stutman O: Suppressor cells in tumor-host interactions. In Biological responses in cancer: Progress toward potential application, Mihich E (ed). New York: Plenum Press, 1982, pp 23–87.
105. Hellstrom I, Hellstrom KE: Cyclophosphamide delays 3-methylcholanthrene sarcoma induction in mice. Nautre 275:129–130, 1978.
106. Flood PM, Urban JL, Kripke ML, Schreiber H: Loss of tumor-specific and idiotype-specific immunity with age. J Exp Med 154:275–290, 1981.
107. Fisher MS, Kripke ML: Suppressor T lymphocytes control the development of primary skin cancers in ultraviolet-irradiated mice. Science 216:1133–1134, 1982.
108. Micklem HS, Loutit JF: Tissue grafting and irradiation. New York: Academic Press, 1966.
109. Stutman O: Natural and induced immunity to mouse mammary tumors and the mouse mammary tumor virus (MuMTV). Springer Sem Immunopathol 4:333–372, 1982.
110. Martinez C: Effect of early thymectomy on development of tumors in mice. Nature 203:1188, 1964.
111. Heppner GH: Neonatal thymectomy and mouse mammary tumorigenesis. In: Immunity and tolerance in oncogenesis, Severi L (ed). Perugia: Division of Cancer Res Publ, 1970, pp 503–524.
112. Prehn RT: Tumor progression and homeostasis. Adv Cancer Res 23:203–236, 1977.
113. Collins JL, Patek PQ, Cohn M: Tumorigenicity and lysis by natural killers. J Exp Med 53:89–106, 1981.
114. Fialkow PJ: Clonal origin of human tumors. Biochim Biophys Acta 458:283–321, 1976.
115. Reddy AL, Fialkow PJ: Multicellular origin of fibrosarcomas in mice induced by the chemical carcinogen 3-methylcholanthrene. J Exp Med 150:878–887, 1979.
116. Tanooka H, Tanaka K: Evidence for single-cell origin of 3-methylcholanthrene induced fibrosarcomas in mice with cellular mosaicism. Cancer Res 42:1856–1858, 1982.
117. Woodruff MFA, Ansell JD, Forbes GM, Gordon JC, Burton DI, Micklem HS: Clonal interaction in tumors. Nature 299:822–824, 1982.
118. Bartlett GL: Effect of host immunity on the antigenic strength of primary tumors. J Natl Cancer Inst 49:493–504, 1972.
119. Hewitt HB, Blake ER, Walder AS: A critique of the evidence for active host defense against cancer, based on personal studies of 27 murine tumors of spontaneous origin. Br J Cancer 33:241–259, 1976.
120. Prehn RT: Relationship of tumor immunogeneicity to concentration of the oncogen. J Natl Cancer Inst 55:189-190, 1975.
121. Woodruff M, Bard J, Ross A, Forbes G: Cellular basis for the loss of carcinogen from methylcholanthrene-impreganted Millipore membrane. Proc R Soc Lond B 211:269–286, 1981.
122. Lawler EM, Outzen HC, Prehn RT: Effect of different immunization and challenge procedures on *in vivo* tumor immunogenicity tests. Cancer Immunol Immunother 11:87–91, 1981.
123. Prehn RT: Immunological basis for differences in susceptibility to hydrocarbon oncogenesis among mice of a single genotype. Int J Cancer 24:789–791, 1979.
124. Prehn LM, Lawler EM: Rank order of sarcoma susceptibility among mouse strains reverses with low concentrations of carcinogen. Science 204:309–310, 1979.
125. Bernfeld P, Homburger F: Tumor susceptibility in two mouse strains with varying doses of carcinogen. Proc Soc Exp Biol Med 170:39–41, 1982.

126. Nebert DW, Felton JS: Importance of genetic factors influencing the metabolism of foreign compounds. Fed Proc 35:1133–1141, 1976.
127. Woodruff M, Forbes G, Gordon J: Immunogenicity, macrophage sensitivity and therapeutic response to *C. parvum* of fibrosarcomas induced in *C. parvum*-treated and untreated mice. Cancer Immunol Immunother 12:255–258, 1982.
128. Zwilling BS, Filippi JA, Choipenning FW, Koestner A, Rheims MS: Chemical carcinogenesis and immunity: Immunologic status of rats treated with methylnitrosourea. J Natl Cancer Inst 61:731–735, 1978.
129. Old LJ, Boyse EA, Clarke DA, Carswell EA: Antigenic properties of chemically induced tumors. Ann NY Acad Sci 101:80–106, 1962.
130. Prehn RT: Immunological surveillance: Pro and con. In: Clinical immunobiology, Vol. 2, Bach FH, Good RA (eds). New York: Academic Press, 1974, pp 191–205.
131. Bonmassar E, Menconi E, Goldin A, Cudkowicz G: Escape of small numbers of allogeneic lymphoma cells from immune surveillance. J Natl Cancer Inst 53:475–479, 1974.
132. Mengersen R, Schick R, Kolsch E: Correlation of 'sneaking through' of tumor cells with specific immunological impairment of the host. Eur J Immunol 5:532–537, 1975.
133. Gatenby PA, Basten A, Creswick P: 'Sneaking through': A T-cell dependent phenomenon. Br J Cancer 44:753–756, 1981.
134. Nelson DS: Immunity to infection, allograft immunity and tumor immunity: Parallels and contrasts. Transplant Rev 19:226–254, 1974.
135. Bloom BR: Games parasites play: How parasites evade immune surveillance. Nature 279:21–26, 1979.
136. Stutman P, Paige CJ, Feo Figarella E: Natural cytotoxic cells against solid tumors in mice: I. Strain distribution and target cell susceptibility. J Immunol 121:1819–1826, 1978.
137. Cudkowicz G, Hochman PS: Do natural killer cells engage in regulated reactions against self to ensure homeostasis? Immunol Rev 44:13–41, 1979.
138. Fraumeni JF, Hoover R: Immunosurveillance and cancer: Epidemiologic observations. Natl Cancer Inst Monogr 47:121–126, 1977.
139. Kinlen LJ, Sheil AGR, Peto J, Doll R: Collaborative United Kingdom Australasian study of cancer in patients treated with immunosuppressive drugs. Br Med J 2:1461–1466, 1979.
140. Anderson JL, Bieper CP, Fowles RE, Stinson EB: Idiopathic cardiomyopathy, age, and suppressor-cell dysfunction as resik determinants of lymphoma after cardiac transplantation. Lancet 2:1174–1177, 1978.
141. Krikorian JG, Anderson JL, Bieber CP, Penn I, Stinson E: Malignant neoplasms following cardiac transplantation. J Am Med Assoc 240:639–651, 1978.
142. Adamson RH, Sieber SM: Carcinogenic potential of drugs. J Environmental Path Toxicol 3:377–385, 1980.
143. Kinlen LJ: Immunosuppression and the aetiology of non-Hodgkin's lymphoma. In: Advances in comparative leukemia research 1981, Yohn D, Blakslee JR (eds). New York: Elsevier Biomedical, 1982, pp 17–21.
144. Kersey JH, Spector BD, Good RA: Cancer in children with primary immunodeficiency diseases. J Pediat 84:263–264, 1974.
145. Filipovich AH, Spector BD, Kersey J: Immunodeficiency in humans as a risk factor in development of malignancy. Preventive Med 9:252–259, 1980.
146. Kinlen LJ, Eastwood JB, Kerr DNS, Moorhead JF, Oliver DO, Robinson BHB, de Wardener HE, Wing AJ: Cancer in patients receiving dialysis. Br Med J 1:1401–1403, 1980.
147. Matas AJ, Simmons RL, Kjellstrand CM, Buselmeier TJ, Johnson TL, Najarian JS: Increased incidence of malignancy in uremic patients and its significance to transplanta-

tion. Transplant. Proc. 9:1137–1140, 1977.
148. Sutherland GA, Glass J, Gabriel R: Increased malignancy in chronic renal failure. Nephron 18:182–184, 1977.
149. Lindner A, Farewell VT, Sherrard DJ: High incidence of neoplasia in uremic patients receiving long-term dialysis. Nephron 27:292–296, 1981.
150. Jacobs C, Brunner FP, Brynger H, Chantler C, Donckerwolcke RA, Hathaway RA, Kramer P, Selwood NH, Wing AJ: Malignant diseases in patients treated by dialysis and transplantation in Europe. Transplant Proc 13:729–732, 1981.
151. Penn I: Depressed immunity and the development of cancer. Clin Exp Immunol 46:459–474, 1981.
152. Penn I: The occurrence of cancer in immune deficiencies. Current Problems in Cancer 6:7–64, 1982.
153. Frizzera G, Rosai J, Dehner LP, Spector BD, Kersey JH: Lymphoreticular disorders in primary immunodeficiencies: New findings based on an up-to-date histologic classification of 35 cases. Cancer 46:692–699, 1980.
154. Hanto D, Frizzera G, Gajl-Peczalska K, Purtilo D, Klein G, Simmons RL, Najarian JS: The Epstein-Barr virus (EBV) in the pathogenesis of post-transplant lymphoma. Transplant Proc 13:756–760, 1981.
155. Purtilo DT: The x-linked lymphoproliferative syndrome as a model of EBV-induced oncogenesis. Adv Cancer Res 34:279–312, 1981.
156. Saemundsen AK, Purtilo DT, Dakamoto K, Sullivan JL, Synnerholm AC, Hanto D, Simmons R, Anvret M, Collins R, Klein G: Documentation of Epstein-Barr virus infection in immunodeficient patients with life-threatening lymphoproliferative diseases by Epstein-Barr virus complementary RNA/DNA and viral DNA/DNA hybridization. Cancer Res 41:4237–4242, 1981.
157. Hanto DW, Frizzera G, Purtilo DR, Sakamoto K, Sullivan JL, Saemundsen AK, Klein G, Simmons RL, Najarian FS: Clinical spectrum of lymphoproliferative disorders in renal transplant recipients and evidence for the role of Epstein-Barr virus. Cancer Res 41:4253–4261, 1981.
158. Frizzera G, Hanto DW, Gajl-Peczalska GH, Rosai J, McKenna RW, Sibley RK, Holahan KP, Lindquist LL:: Polymorphic diffuse B-cell hyperplasias and lymphomas in renal transplant recipients. Cancer Res 41:4262–4279, 1981.
159. Weintraub J, Warke RA: Lymphoma in cardiac allotransplant recipients. Transplantation 33:347–351, 1982.
160. Robinson JE, Brown N, Andiman W, Halliday K, Francke U, Robert MF, Andersson-Anvert M, Horstmann D, Miller G: Diffuse polyclonal B-cell lymphoma during primary infections with Epstein-Barr virus. N Eng J Med 302:1293–1297, 1980.
161. Snydman DR, Rudders RA, Daoust P, Sullivan JL, Evans AS: Infectious mononucleosis in an adult progressing to fatal immunoblastic lymphoma. Ann Int Med 96:737–742, 1982.
162. Gossett TC, Gale RP, Fleischman H, Austin GE, Sparkes RS, Taylor CR: Immunoblastic sarcoma in donor cells after bone-marrow transplantation. N Eng J Med 300:904–907, 1979.
163. Schubach WH, Hackman R, Neiman PE, Miller G, Thomas ED: A monoclonal immunoblastic sarcoma in donor cells bearing Epstein-Barr virus genomes following allogeneic marrow grafting for acute lymphocytic leukemia. Blood 60:180–187, 1982.
164. Kersey JH, Filipovich AH, Spector BD, Frizzera G: Lymphoma after thymus transplantation. N Eng J Med 302:301, 1980.
165. Borzy MS, Hong R, Horowitz SD, Gilbert E, Kaufman D, De Mendonca W, Oxelius VA, Dichtor M, Packman L: Fatal lymphoma after transplantation of cultured thymus in children with combined immunodeficiency disease. N Eng J Med 301:565–568, 1979.

166. Reece ER, Gartner JG, Seemayer TA, Joncas JH, Pagano JS: Epstein-Barr virus in a malignant lymphoproliferative disorder of B-cells occurring after thymic epithelial transplantation for combined immunodeficiency. Cancer Res 41:4243–4247, 1981.
167. Harwood AR, Osoba D, Hofstader SL, Goldstein MB, Cardella CJ, Holecek MJ, Kunynetz R, Giammarco RA: Kaposi's sarcoma in recipients of renal transplants. Am J Med 67:759–765, 1979.
168. Klepp O, Dahl O, Stenwig JT: Association of Kaposi's sarcoma and prior immunosuppressive therapy: A 5-year-material of Kaposi's sarcoma in Norway. Cancer 42:2626–2630, 1978.
169. DiGiovanna JJ, Safai B: Kaposi's sarcoma: Retrospective study of 90 cases with particular emphasis on the familial occurrence, ethnic background and prevalence of other diseases. Am J Med 71:77–83, 1981.
170. Giraldo G, Beth E: The relationship of cytomegalovirus to certain human cancers, particularly to Kaposi's sarcoma. In: The role of virus in human cancer, Vol. 1, Giraldo G, Beth E (eds). Amsterdam: Elsevier North Holland, 1980, pp 57–73.
171. Hymes KB, Cheung T, Greene JB, Prose NS, Marcus A, Ballard H, William DC, Laubenstein LJ: Kaposi's sarcoma in homosexual men: A report of eight cases. Lancet 2:580–600, 1981.
172. Gottlieb MS, Schroff R, Schanker HM, Weisman JD, Fan PT, Wolf RA, Saxon A: Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: Evidence of a new acquired cellular immunodeficiency. N Eng J Med 305:1425–1431, 1981.
173. Masur H, Michelis MA, Greene JB, Onoratto I, Vande Stouwe RA, Holzman RS, Wormser G, Brettman L, Lange M, Marray HW, Cunningham-Rundles: An outbreak of community acquired pneumocystis carinii pneumonia. Initial manifestations of cellular immune dysfunction. N Eng J Med 305:1431–1438, 1981.
174. Siegal FM, Lopez C, Hammer CS, Brown AE, Kornfeld SJ, Gold J, Hasett J, Hirschman SZ, Cunningham-Rundles C, Adelsberg BR, Parham DM, Siegal M, Cunningham-Rundles S, Armstrong D: Severe acquired immunodeficiency in male homosexuals, manifested by chronic perianal ulcerative herpes simplex lesions. N Eng J Med 305:1439–1444, 1981.
175. Friedman-Kiem AE, Laubenstein LJ, Rubinstein P, Buimovici-Klein E, Marmor M, Stahl R, Spigland I, Kim KS, Zolla-Pazner S: Disseminated Kaposi's sarcoma in homosexual men. Ann Int Med 96:693–700, 1982.
176. Mildvan D, Mathur U, Enlow RW, Romain PL, Winchester RJ, Colp C, Singman H, Adelsberg BR, Spigland I: Opportunistic infections and immune deficiency in homosexual men. Ann Int Med 96:700–704, 1982.
177. Durack DT: Opportunistic infections and Kaposi's sarcoma in homosexual men. N Eng J Med 305:1465–1467, 1981.
178. Croopman JE, Gottlieb MS: Kaposi's sarcoma: An oncologic looking glass. Nature 299:103–104, 1982.
179. VerMeulen M: The gay plague. New York Magazine, 31 May 1982, p 50.
180. Wallis C: The deadly spread of AIDS. Time Magazine, 6 September 1982, p 55.
181. Fasth A: Primary immunodeficiency disorders in Sweden: Cases among children, 1974–1979. J Clin Immunol 2:86–92, 1982.
182. Oleinik A: Altered immunity and cancer risk: A review of the problem and analysis of the cancer mortality experience in leprosy patients. J Natl Cancer Inst 43:775–781, 1969.
183. Stutman O: Immunodeficiency and cancer. In: Mechanisms of tumor immunity, Green I, Cohen S, McCluskey RT (eds). New York: Wiley, 1977, pp 27–53.
184. Warner JF, Dennert G: Effects of a cloned cell line with NK activity on bone marrow transplants, tumor development and metastasis *in vivo.* Nature 300:31–34, 1982.

185. Rygaard J, Poulsen CO: The mouse mutant nude does not develop spontaneous tumors. Acta Pathol Microbiol Scand Sect B 82:99–106, 1974.
186. Gershwin ME, Merchant B, Gelfand MC, Vickers J, Steinberg AD, Hansen TC: The natural history and immunopathology of outbred athymic (nude) mice. Clin Immunol Immunopathol 4:324–340, 1975.
187. Sharkey FE, Fogh J: Incidence and pathological features of spontaneous tumors in athymic nude mice. Cancer Res 39:833–839, 1979.
188. Beattie G, Baird S, Lannom R, Slimmer S, Jensen FC, Kaplan NO: Induction of lymphoma in athymic mice: A model for study of the human disease. Proc Natl Acad Sci USA 77:4971–4974, 1980.
189. Baird SM, Beattie GM, Lannom RA, Lipsick JS, Jensen FC, Kaplan NO: Induction of lymphoma in antigenically stimulated athymic mice. Cancer Res 42:198–206, 1982.
190. Sharkey FE, Lozzio BB, Giovanella BC, Fogh J: Spontaneous tumors in nude mice. In: The nude mouse in experimental and clinical research, Vol. 2, Fogh J, Giovanella BC (eds). New York: Academic Press, 1982, pp 19–38.
191. Holland JM, Mitchell TJ, Gispon LC, Whitaker MS: Survival and cause of death in aging germfree athymic nude and normal inbred C3Hf/He mice. J Natl Cancer Inst 61:1357–1361, 1978.
192. Murphy ED: Characteristic tumors. In: Biology of the laboratory mouse, Green EL (ed). New York: McGraw-Hill, 1966, pp 521–562.
193. Tarone RE, Chu KC, Ward JM: Variability in the rates of some common naturally occurring tumors in Fischer 344 rats and (C57BL/6N × C3H/HeN)F_1 (B6c3F_1) mice. J Natl Cancer Inst 66:1175–1181, 1981.
194. Sher SP: Tumors in control mice: Literature tabulation. Toxicol Appl Pharmacol 30:337–359, 1974.
195. Parker JW, Joyce J, Pattengale P: Spontaneous neoplasms in aged athymic (nude) mice. In: Proc of the Third International Workshop on Nude Mice, Reed ND (ed). New York: Gustav Fischer, 1982, pp 347–357.
196. Andervont HB, Dunn TB: Efforts to detect a mammary tumor agent in strain C mice. J Natl Cancer Inst 8:235–240, 1948.
197. Deringer MK: Occurrence of mammary tumors, reticular neoplasms and pulmonary tumors in strain BALB/cAnDe breeding female mice. J Natl Cancer Inst 35:1047–1052, 1965.
198. Madison RM, Rabstein LS, Bryan WR: Mortality rate and spontaneous lesions found in 2,928 untreated BALB/cCr mice. J Natl Cancer Inst 40:683–685, 1968.
199. Heston WE: Genetic factors affecting tumorigenesis. In: RNA viruses and host genome oncogenesis, Emelot P, Bentveltzen P. (eds). Amsterdam: North Holland, 1972, pp 13–24.
200. Eaton GJ, Custer RP, Crayne AR: Spontaneous tumors of long-lived reconstituted nude mice. In: Proc of the Third International Workshop on Nude Mice, Reed ND (ed). New York: Gustav Fischer, 1982, pp 359–365.
201. Stutman O: Cell-mediated immunity and aging. Fed Proc 33:2028–2032, 1974.
202. Eaton GJ, Johnson FN, Custer RP, Crayne A: The Icr:Ha (ICR) mouse: A current account of breeding, mutations, diseases and mortality. Lab Anim Sci 14:17–29, 1980.
203. Gautsch JW, Knowles AF, Jensen FC, Kaplan NO: Human tumor-induced expression of endogenous murine leukemia viruses of athymic nude mice. In: Proc. of the Third International Workshop on Nude Mice, Reed ND (ed). New York: Gustav Fischer 1982, pp 601–609.
204. Dameshek W, Schwartz RS: Leukemia and auto-immunization: Some possible relationships. Blood 14:1151–1158, 1959.

205. Metcalf D: Reticular tumors in mice subjected to prolonged antigenic stimulation. Br J Cancer 15:769–779, 1961.
206. Krueger GRF: Chronic immunosuppression and lymphomagenesis in man and mice. Natl Cancer Inst Monogr 35:183–190, 1972.
207. Gleichmann E, Gleichmann H, Wilke W: Autoimmunization and lymphomagenesis in parent-F1 combination differing at the major histocompatibility complex: Model for spontaneous disease caused by altered self antigens? Transplant Rev 31:156–224, 1976.
208. Vandeputte M, Eysen H, Sobis H, DeSomer P: Induction of polyoma tumors in athymic nude mice. Int J Cancer 14:445–450, 1974.
209. Anderson LM, Budinger JM, Maropont RR, Good RA: Transplacental lung tumorigenesis in the athymic mouse. Cancer Res 38:137–141, 1978.
210. Gershwin ME, Ikeda R: DMBA induced papillomas in congenitally athymic (nude) and hereditarily asplenic (Dh/+) mice: Contrasts and comparisons with immunologically intact littermates. Devel Comp Immunol 2:529–538, 1978.
211. Outzen HC: A preliminary analysis of skin tumor induction in immunomodulated mice. In: Proc. of the Symposium on the Use of Athymic (Nude) Mice in Cancer Research, Houchens D, Ovejera A (eds). New York: Gustav Fischer, 1978, pp 93–99.
212. Yuspa SH, Spangler EF, Donahoe R, Geusz S, Ferguson E, Wenk M, Hennings H: Sensitivity of two-stage carcinogenesis of SENCAR mouse skin grafted to nude mice. Cancer Res 42:437–439, 1982.
213. Holland JM, Perkins EH: Resistance of germ-free athymic nude mice to two-stage skin carcinogenesis. In: Proc of the Third International Workshop on Nude Mice, Reed ND (ed). New York: Gustav Fischer, 1982, pp 423–433.
214. Anderson LM, Last-Barney K, Budinger JM: Sensitivity to carcinogenesis in nude mice: Skin tumors caused by transplacental exposure to ethylnitrosourea. Science 218:682–684, 1982.
215. Lappe MA: Evidence for the antigenicity of papillomas induced by 3-methylcholanthrene. J Natl Cancer Inst 40:823–846, 1968.
216. Andervont HB, Shinkim MB, Canter HY: Susceptibility of seven inbred strains and their F_1 hybrids to estrogen-induced testicular tumors and occurrence of spontaneous testicular tumors in strain BALB/c mice. J Natl Cancer Inst 25:1069–1081, 1960.
217. Kalland T: Effect of depression of NK activity by neonatal exposure to diethylstilbestrol on susceptibility to transplanted and primary-carcinogen induced tumors. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 1437–1444.
218. Stutman O: NK cells, antitumor surveillance and interleukins. Immunology Today 2:205–208, 1981.
219. Stutman O: Natural cell-mediated cytotoxicity against tumors in mice. In Human cancer immunology, Vol. 6, Serrou B (ed). Amsterdam: Elsevier Biomedical Press, 1982, pp 205–223.
220. Popper K: Conjectures and refutations: The growth of scientific knowledge. New York: Harper and Row, 1963, p 248.
221. Clark EA, Harmon RC: Genetic control of natural cell-mediated cytotoxicity and hybrid resistance. Adv Cancer Res 31:227–285, 1980.
222. Minato N, Reid L., Bloom BR: On the heterogeneity of murine natural killer cells. J Exp Med 154:750–762, 1981.
223. Lust JA, Kumar V, Burton RC, Bartlett SP, Bennett M: Heterogeneity of natural killer cells in the mouse. J Exp Med 154:306–317, 1981.
224. Burton RC: Alloantisera selectively reactive with NK cells: Characterization and use in defining NK cell classes. In: Natural cell-mediated immunity against tumors, Herberman RB (ed). New York: Academic Press, 1980, pp 19–35.

225. Henney CS, Kuribayashi K, Kern DE, Gillis SE: Interleukin-2 augments natural killer cell activity. Nature 291:335–358, 1981.
226. Lattime EC, Pecoraro GA, Stutman O: The activity of natural cytotoxic (NC) cells is augmented by interleukin-2 and interleukin-3. J Exp Med. 157:1070-1075, 1983.
227. Warner NL, Woodruff MF, Barton RL: Inhibition of growth of lymphoid tumors in syngeneic athymic (nude) mice. Int J Cancer 20:146–155, 1977.
228. Haller O, Hansson M, Kiessling R, Wigzell H: Role of non-conventional natural killer cells in resistance against syngeneic tumor cells *in vivo*. Nature 270:609–611, 1977.
229. Kasai M, Leclerc JC, McVay-Boudreau L, Shen FW, Cantor H: Direct evidence that natural killer cells in non-immune spleen populations prevent tumor growth *in vivo*. J Exp Med 149:1260–1264, 1979.
230. Riccardi C, Puccetti P, Santoni A, Herberman RB: Rapid *in vivo* assay of mouse natural killer cell activity. J Natl Cancer Inst 63:1041–1045, 1979.
231. Riccardi C, Santoni A, Barlozzari T, Puccetti P, Herberman RB: *In vivo* natural reactivity of mice against tumor cells. Int J Cancer 25:475–486, 1980.
232. Stutman O, Feo Figarella E, Paige CJ, Lattime EC: Natural cytotoxic (NC) cells against solid tumors in mice: General characteristics and comparison to natural killer (NK) cells. In: Natural cell-mediated immunity against tumors, Herberman RB (ed). New York: Academic Press, 1980, pp 187–229.
233. Reisenfeld I, Orn A, Gidlung M, Axberg I, Alm GV, Wigzell H: Positive correlation between *in vitro* NK activity and *in vivo* resistance towards AKR lymphoma cells. Int J Cancer 25:399–403, 1980.
234. Kedar E, Ikejiri BL, Gorelik E, Herberman RB: Natural cell-mediated cytotoxicity *in vitro* and inhibition of tumor growth *in vivo* by murine lymphoid cells cultured with T cell growth factor (TCGF). Cancer Immunol Immunother 13:14–23, 1982.
235. Habu S, Okumura K: Evidence for *in vivo* reactivity against transplantable and primary tumors. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 1323–1329.
236. Kawase I, Urdal DL, Brooks CG, Henney CS: The effect of selective NK cell depletion on the growth of NK sensitive and resistant lymphoma cell variants. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 1339–1345.
237. Hanna N: Natural-killer cell-mediated cytotoxicity against solid tumors: *In vitro* and *in vivo* studies. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 1359–1367.
238. Karre K, Klein GO, Kiessling R, Argov S, Klein GO, Kiessling R, Argov S, Klein G: The beige model in studies of natural resistance to syngeneic, semisyngeneic and primary tumors. In: NK cells and other natural effector Cells, Herberman RB (ed). New York: Academic Press, 1982, pp 1369–1378.
239. Becker S, Kiessling R, Lee N, Klein G: Modulation of sensitivity to natural killer (NK) cell lysis after *in vitro* explanation of a mouse lymphoma. J Natl Cancer Inst 61:1495–1498, 1978.
240. Auerbach R, Auerbach W: Regional differences in the growth of normal and neoplastic cells. Science 215:127-134, 1982.
241. Talmadge JE, Meyers KM, Prieur DJ, Starkey JR: Role of natural killer cells in tumor growth and metastasis in C57BL/6 normal and beige mice. J Natl Cancer Inst 65:929–935, 1980.
242. Talmadge JE, Meyers KM, Prieur DJ, Starkey JR: Role of NK cells in tumor growth and metastasis in beige mice. Nature 284:622–624, 1980.
243. Salomon JC, Creau-Goldberg N, Lynch NR: Cancer induction by methylcholanthrene and

metastatic spread of transplantable tumor in Chediak Higashi (beige) mice. Cancer Immunol Immunother 8:67–70, 1980.

244. Hanna H, Fidler IJ: Role of natural killer cells in the destruction of circulating tumor emboli. J Natl Cancer Inst 65:801–809, 1980.
245. Hanna N, Fidler IJ: Relationship between metastatic potential and resistance to natural killer cell-mediated cytotoxicity in three murine tumor systems. J Natl Cancer Inst 66:1183–1190, 1981.
246. Hanna N: Expression of metastatic potential of tumor cells in young nude mice is correlated with low levels of natural killer cell-mediated cytotoxicity. Int J Cancer 26:675–680, 1980.
247. Gorelik E, Wiltrout E, Okumura K, Habu S, Herberman RB: Acceleration of metastatic growth in anti-asialo GM1-treated mice. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 1331–1337.
248. Riccardi C, Barlozzari T, Santoni A, Herberman RB, Cesarini C: Transfer to cyclophosphamide-treated mice of natural killer (NK) cells and *in vivo* natural reactivity against tumors. J Immunol 126:1284–1289, 1981.
249. Carlson GA, Melnychuck D, Meeker MJ: H2-associated resistance to leukemia transplantation: Natural killing *in vivo*. Int J Cancer 25:111–122, 1980.
250. Herberman RB: Natural killer (NK) cells and their possible role in resistance against disease. Clin Immunol Rev 1:1–65, 1981.
251. Herberman RB, Ortaldo JR: Natural killer cells: Their role in defense against disease. Science 214:24–30, 1981.
252. Lattime EC, Pecoraro GA, Cuttito M, Stutman O: Solid tumors are killed by NK and NC cell populations: Interferon inducing agents do not augment NC cell activity under NK activating conditions. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 179–186.
253. Ehrlich R, Efrati M, Witz IP: Some characteristics of natural cytostatic mouse splenocytes. J Immunol Methods 40:193–208, 1981.
254. Saksela E, Timonen T, Ranki A, Hayry P: Morphological and functional characterization of isolated effector cells responsible for human natural killer activity to fetal fibroblasts and to cultured cell line targets. Immunol Rev 44:71–123, 1979.
255. Tagliabue A, Borasachi D, Alberti S, Luini W: Large granular lymphocytes as effector cells of natural killer activity in the mouse. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 25–30.
256. Roder JC, Kiessling R, Biberfeld P, Anderson B: Target-effector interaction in the natural killer cell system: II. The isolation of NK cells and studies on the mechanism of killing. J Immunol 121:2509–2517, 1978.
257. Lanza E, Djeu JY: Age-independent natural killer cell activity in murine peripheral blood. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 335–340.
258. Koo GC, Peppard JR, Hatzfeld A, Cayre Y, Ontogeny of NK-1$^+$ natural killer cells. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 325–328.
259. Brooks CG, Flannery GR: Quantitative studies of natural immunity to solid tumors in rats: Persistence of natural immunity throughout the reproductive life, and absence of suppressor cells in infant rats. Immunology 39:187–194, 1980.
260. Haller O, Kiessling R, Gidlund M, Wigzell H: Natural killer cells to leukemia: *In vitro* and *in vivo* studies. In: Tumor-associated antigens and their specific immune response, Spreafico F, Arnon R (eds). New York: Academic Press, 1979, pp 151–166.
261. Haller O, Kiessling R, Orn A, Wigzell H: Generation of natural killer cells: An autonomous function of the bone marrow. J Exp Med 145:1411–1416, 1977.

262. Gorelik E, Kedar E, Sredni B, Herberman RB: *In vivo* antitumor effect of local adoptive transfer of mouse and human cultured lymphoid cells. Int J Cancer 28:157–164, 1981.
263. Batory G, Benczur M, Varga M, Garam T, Onody C, Petrany GG: Increased killer cell activity in aged humans. Immunobiol 158:393–402, 1981.
264. Herberman RB, Nunn ME, Holden HT, Saal S, Djeu JY: Augmentation of natural cytotoxic reactivity of mouse lymphoic cells against syngeneic and allogeneic target cells. Int J Cancer 19:555–564, 1977.
265. Clark EW, Engel D, Windsor NT: Immune responsiveness of SM/J mice: Hyper NK cell activity mediated by NK 1^+ $Qa5^-$ cells. J Immunol 127:2391–2395, 1981.
266. Storer JB: Longevity and gross pathology at death in 22 inbred mouse strains. J Gerontol 30:404–409, 1966.
267. Clark EA, Schultz LD, Pollack SB: Mutations in mice that influence natural killer (NK) cell activity. Immunogenetics 12:601–613, 1981.
268. Zoller M, Matzku S: Characterization of natural cytotoxicity *in vitro* in a spontaneous rat tumor model. J Immunol 124:1683–1690, 1980.
269. Zoller M, Matzku S, Goerttler K: High incidence of spontaneous transplantable tumors in BD X rats. Br J Cancer 37:61–66, 1978.
270. Scott JL, Pal BK, Rasheed S, Gardner MB: Natural killer cell activity in a population of leukemia-prone wild mice (Mus Musculus). Int J Cancer 28:241–247, 1981.
271. Gardner MB: Type C viruses in wild mice: Characterization and natural history of amphotropic, ecotropic and xenotropic MuLV. Curr Top Microbiol Immunol 79:215–259,1978.
272. Klein E, Masucci MG, Masucci G, Vanky F: Natural and activated lymphocyte killers which affect tumor cells. In: Natural cell-mediated immunity against tumors, Herberman RB (ed). New York: Academic Press, 1980, pp 909–920.
273. Smith GS, Walford RL, Mickey MR: Lifespan and incidence of cancer and other diseases in selected long-lived imbred mice and their F_1 hybrids. J Natl Cancer Inst 50:1195–1213, 1973.
274. Duran-Reynals ML, Lilly F, Bosch A, Blank KJ: The genetic basis of susceptibility to leukemia induction in mice by 3-methylcholanthrene applied percutaneously. J Exp Med 147:459–469, 1978.
275. Mayer A, Lilly F, Duran-Reynals ML: Genetically dominant resistance in mice to 3-methylcholanthrene-induced lymphoma. Proc. Natl Acad Sci USA 77:2960–2963, 1980.
276. Lattime EC, Pecoraro G, Stutman O: Natural cytotoxic cells against solid tumors in mice: IV. Natural cytotoxic (NC) cells are not activated natural killer (NK) cells. Int J Cancer 30:471–477, 1982.
277. Brooks CG, Kuribayashi K, Sale GE, Henney CS: Characterization of five cloned murine cell lines showing high cytolytic activity against YAC-1 cells. J Immunol 128:2326–2335, 1982.
278. Beck BN, Gillis S, Henney CS: Display of the neutral glycolipid ganglio-N-tetraosylceramide (asialo GM 1) on cells of the natural killer and T lineages. Transplantation 33:118–122, 1982.
279. Gorelik E, Herberman RB: Inhibition of the activity of mouse natural killer cells by urethan. J Natl Cancer Inst 66:543–548, 1981.
280. Herberman RB, Timonen T, Ortaldo JR, Bonnard GD, Gorelik E: Natural cell-mediated cytotoxicity. In: Immunology 80. Progress in immunology IV, Fougereau M, Dausset J (eds). New York: Academic Press, 1980, pp 691–709.
281. Gorelik E, Herberman RB: Role of natural-cell mediated immunity in urethan-induced lung carcinogenesis. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 1415–1421.

282. Berenblum I, Boiato L, Trainin N: On the mechanism of urethan leukemogenesis in newborn C57BL mice: I. Influence of injections of syngeneic bone marrow cell suspensions. Cancer Res 26:357–380, 1966.
283. Della Porta G, Capitano J, Parmi L, Colnaghi MI: Cancerogenesi da uretano in topi neonati lattanti e adulti dei ceppi C57BL, C3H, BC3Fl, C3Hf e SWR. Tumori 53:81–102, 1967.
284. Heston WE: Inheritance of susceptibility to spontaneous pulmonary tumors in mice. J Natl Cancer Inst 3:79–82, 1942.
285. Yamamoto RS, Weisburger JH, Weisburger EK: Controlling factors in urethan carcinogenesis in mice: Effect of enzyme inducers and metabolic inhibitors. Cancer Res 31:483–486, 1971.
286. Ehrlich R, Efrati M, Witz IP: Cytotoxicity and cytostasis mediated by splenocytes of mice subjected to chemical carcinogens and of mice bearing primary tumors. In: Natural cell-mediated immunity against tumors, Herberman RB (ed). New York: Academic Press, 1980, pp 997–1010.
287. Ehrlich R, Efrati M, Bar-Eyal A, Wollberg M, Schiby G, Ran M, Witz IP: Natural cellular reactivities mediated by splenocytes from mice bearing three types of primary tumor. Int J Cancer 26:315–323, 1980.
288. Ehrlich R, Malatzky E, Efrati M, Wtiz IP: The suppression of NK activity by the chemical carcinogen dimethylbenzanthracene and its restoration by interferon or poly I:C. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 1431–1436.
289. Parkinson DR, Brightman RP, Waksal SD: Altered natural killer cell biology in C57BL/6 mice after leukemogenic split-dose irradiation. J Immunol 126:1460–1464, 1981.
290. Gorelik E, Rosen B, Herberman RB: Depression of NK reactivity in mice by leukemogenic doses of irradiation. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 1423–1430.
291. Haran-Ghera N, Peled A: Induction of leukemia in mice by irradiation and radiation leukemia virus variants. Adv Cancer Res 30:45–87, 1979.
292. Berenblum I, Boiato L, Trainin N: On the mechanism of urethan leukemogenesis in newborn C57BL mice: II. Influence of thymectomy and subsequent thymus reimplantation. Cancer Res 26:361–363, 1966.
293. Berenblum I, Chen L, Trainin N: A quantitative study of the leukemogenic action of whole body x-irradiation and urethane: I. In adult mice. Isr J Med Sci 4:1159–1163, 1968.
294. Chen L, Berenblum I: A quantitative study of the leukemogenic action of whole body x-irradiation and urethane: II. In newborn C57BL mice. Isr J Med Sci 4:1154–1168, 1968.
295. Kawamoto S, Ida N, Kirschbaum A, Taylor G: Urethan and leukemogenesis in mice. Cancer Res 18:725–729, 1958.
296. Karre K, Klein GO, Kiessling G, Roder JC: Low natural *in vivo* resistance to syngeneic leukemias in natural killer-deficient mice. Nature 284:624–626, 1980.
297. Loutit JF, Townsend KMS, Knowles JF: Tumor surveillance in beige mice. Nature 285:66, 1980.
298. Argov S, Cochran AJ, Karre K, Klein GO, Klein G: Incidence and type of tumors induced in C57BL bg/bg mice and +/bg littermates by oral administration of DMBA. Int J Cancer 28:739–746, 1981.
299. Haliotis T, Roder J, Dexter D: Evidence for *in vivo* NK reactivity against primary tumors. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 1399-1404.
300. Roder JC, Haliotis T: Do NK cells play a role in anti-tumor surveillance? Immunology Today 1:96–100, 1980.

301. Roder J, Duwe AK: The beige mutation in the mouse selectively impairs natural killer cell function. Nature 278:451–453, 1979.
302. Brunda MJ, Holden HT, Herberman RB: Augmentation of natural killer cell activity of beige mice by interferon and interferon inducers. In: Natural cell-mediated immunity against tumors, Herberman RB (ed). New York: Academic Press, 1980, pp 411–415.
303. Beck BN, Henney CS: An analysis of the natural killer cell defect in beige mice. Cell Immunol 61:343–352, 1981.
304. McKinnon KP, Hale AH, Ruebush MJ: Elicitation of natural killer cells in beige mice by infection with vesicular stomatitis virus. Infect Immun 32:204–219, 1981.
305. Stutman O, Cuttito MJ: Normal levels of natural cytotoxic cells against solid tumors in NK-deficient beige mice. Nature 290:254–257, 1981.
306. Roder JC, Lohmann-Mathes ML, Domzig W, Wigzell H: The beige mutation in the mouse: II. Selectivity of the natural killer (NK) cell defect. J Immunol 123:2174–2181, 1979.
307. Mahoney KH, Morse SS, Morahan PS: Macrophage function in beige (Chediak-Higashi syndrome) mice. Cancer Res 40:3934–3939, 1980.
308. Stutman O, Cuttito MJ: Genetic control of NC activity in the mouse: Three genes located in chromosome 17. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 218–289.
309. Lane PW, Murphy ED: Susceptibility to spontaneous pneumonitis in an inbred strain of beige and satin mice. Genetics 72:451–460, 1972.
310. Goodrick CL: Body weight change over the lifespan and longevity for C57BL/6J mice and mutations which differ in maximal body weight. Gerontology 23:405–413, 1977.
311. Lilly F, Mayer A: Genetic aspects of murine Type-C viruses and their hosts in oncogenesis. In: Viral oncology, Klein G (ed). New York: Raven Press, 1980, pp 89–108.
312. Pross HF, Baines MG: Studies of human natural killer cells: I. In vivo parameters affecting normal cytotoxic function. Int J Cancer 29:383–390, 1982.
313. Peter HH, Heidenreich W: Spontaneous cell-mediated cytotoxicity. Cancer Immunol Immunother 8:79–82, 1980.
314. Pross HF, Baines MG: Spontaneous human lymphocyte-mediated cytotoxicity against tumor target cells: VI. A brief review. Cancer Immunol Immunother 3:75–85, 1977.
315. Gupta S: Natural killer cells – Immunologist's fancy? Gastroenterology 78:865–867, 1980.
316. Abb J, Kaudewitz P, Zander H, Ziegler HWL, Deinhardt F, Riethmuller G: Interferon (IFN) production and natural killer (NK) cell activity in patients with multiple sclerosis: Influence of genetic factors assessed by studies of monoxygotic twins. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 1233–1240.
317. Sullivan JL, Byron KS, Brewster FE, Purtilo DT: Deficient natural killer cell activity in x-linked lymphoproliferative syndrome. Science 210:543–545, 1980.
318. Seeley JK, Bechtold T, Purtilo DT, Lindsten T: NK-deficiency in x-linked lymphoproliferative syndrome. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 1211–1218.
319. Lipinski M, Virelizier JL, Tursz T, Girscelli C: Natural killer and killer cell activities in patients with primary immunodeficiencies or defects in immune interferon production. Eur J Immunol 10:246–249, 1980.
320. Koren HS, Amos DB, Buckley RH: Natural killing in immunodeficient patients. J Immunol 120:796–799, 1978.
321. Pross HF, Gupta S, Good RA, Baines MG: Spontaneous human lymphocyte mediated cytotoxicity against tumor targets: VII. The effect of immunodeficiency disease. Cell Immunol 43:160–175, 1979.

322. Peter HH, Lange B, Serbin A, Euler S, Stangl W, Avenarius HJ, Deicher H: Natural killing (NK) in hemopoietic disorders and immunodeficiency syndromes: Evidence for the bone marrow dependecy of human NK effector cells. Immunobiology 156:206–219, 1979.
323. Hersey P, Edwards A, Lewis R, Kemp A, McInnes J: Deficient natural killer cell activity in a patient with Fanconi's anaemia and squamous cell carcinoma: Association with defect in interferon release. Clin Exp Immunol 48:205–212, 1982.
324. Nurmi T, Huttunen K, Lassila O, Henttonen M, Sakinen A, Linna SL, Tiliakainen A: Natural killer cell function in trisomy-21 (Down's syndrome). Clin Exp Immunol 47:735–741, 1982.
325. Lipinski M, Tursz T, Kreis H, Finale Y, Amiel JL: Dissociation of natural killer cell activity and antibody-dependent cell-mediated cytotoxicity in kidney allograft recipients receiving high dose immunosuppressive therapy. Transplantation 29:214–218, 1980.
326. Haliotis T, Roder JC, Klein M, Ortaldo J, Fauci AS, Herberman RB: Chediak-Higashi gene in humans: I. Impairment of natural killer function. J Exp Med 151:1039–1048, 1980.
327. Humphries RC, Gelber RH, Krahenbuhl JL: Suppressed natural killer cell activity during episodes of eythema nodosum leprosum in lepromatous leprosy. Clin Exp Immunol 59:500–508, 1982.
328. Hersey O, Edwards A, Honeyman M, McCarthy WH: Low natural killer cell activity in familial melanoma patients and their relatives. Br J Cancer 40:113–122, 1979.
329. Hersey P, Edwards A, McCarthy W, Milton G: Tumor related changes and prognostic significance of natural killer cell activity in melanoma patients. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 1167–1174.
330. Hersey P, Edwards A, Milton GW, McCarthy WH: Relationship of cell-mediated cytotoxicity against melanoma cells to prognosis in melanoma patients. Br J Cancer 37:505–514, 1978.
331. Kristensen E, Brandslund I, Nielsen H, Svehag SE: Prognostic value of assays for circulating complexes and natural cytotoxicity in malignant skin melanoma (Stages I and II). Cancer Immunol Immunother 9:31–36, 1980.
332. Hersey P, Edwards A, McCarthy WH: Tumor-related changes in natural killer cell activity in melanoma patients: Influence of stage of disease, tumor thickness and age of patients. Int J Cancer 25:187–194, 1980.
333. Einhorn S, Ahre A, Blomgren H, Johansson B, Mellstedt H, Strander H: Enhanced NK activity in patients treated by interferon-alpha: Relation to clinical response. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 1259–1263.
334. Takasugi M, Ramseyer A, Takasugi J: Decline of natural non-selective cell-mediated cytotoxicity in patients with tumor progression. Cancer Res 37:413–418, 1977.
335. Pross HF, Baines MG: Spontaneous human lymphocyte-mediated cytotoxicity against tumor target cells: I. The effect of malignant disease. Int J Cancer 18:593–604, 1976.
336. Eremin O, Ashby J, Stephens JP: Human natural cytotoxicity in the blood and lymphoid organs of healthy donors and patients with malignant disease. Int J Cancer 21:35–41, 1978.
337. Peter HH, Knoop F, Kalden JR: Spontaneous and antibody-dependent cellular cytotoxicity in melanoma patients and healthy control persons. Z Immun Forsch 151:263–281, 1976.
338. Cunningham-Rundles S, Filippa DA, Braun DW, Antonelli P, Ashikari H: Natural cytotoxicity of peripheral blood lymphocytes and regional lymph node cells in breast cancer in women. J Natl Cancer Inst 67:585–590, 1981.

339. Kadish AS, Doyle AT, Steinhauer EH, Ghossein NA. Natural cytotoxicity and interferon production in human cancer: Deficient natural killer activity and normal interferon production in patients with advanced disease. J Immunol 127:1817–1822, 1981.
340. Ziegler HW, Kay NE, Zarling JM: Deficiency of natural killer cell activity in patients with chronic lymphocytic leukemia. Int J Cancer 27:321–327, 1981.
341. Stupp Y, Rosenkovitch E, Izak G: Natural killer activity in patients with acute myelocytic leukemia. Isr J Med Sci 14:1212–1215, 1978.
342. Shau H, Sawson JR: Regulation of human natural killing by levamisole. Cancer Immunol Immunother 13:24–29, 1982.
343. Kleinerman ES, Zwelling LA, Muchmore AV: Enhancement of naturally occurring human spontaneous monocyte-mediated cytotoxicity by cis-diaminedichloroplatinum (II). Cancer Res 40:3099–3102, 1980.
344. Haukaas S, Kalland T: Effect of diethylstilbestrol and estramustine phosphate (Estracyt^{R}) on natural killer activity in patients with carcinoma of the prostate. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 1317–1321.
345. Golub SH: NK cytotoxicity in interferon treated melanoma patients. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 1265–1271.
346. Maluish AE, Ortaldo JR, Herberman RB: Modulation of NK activity by recombinant leukocyte interferon in advanced cancer patients. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 1279–1283.
347. Hansson M, Kiessling R, Andersson B, Karre K, Roder J: NK cell-sensitive T cell subpopulation in thymus: Inverse correlation to host NK activity. Nature 278:174–176, 1979.
348. Hansson M, Kiessling R, Beran M: Natural killing of hematopoietic cells. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 1077–1084.
349. Bennett M, Baker EE, Eastcott JW, Kumar V, Yonkosky D: Selective elimination of marrow precursors with the bone-seeking isotope ^{89}Sr: Implications for hemopoiesis, lymphopoiesis, viral leukemogenesis and infection. J Reticuloendothel Soc 20:71–87, 1976.
350. Kiessling R, Hochman PS, Haller O, Shearer GM, Wigzell H, Cudkowicz G: Evidence for a similar common mechanism for natural killer cell activity and resistance to hemopoietic grafts. Eur J Immunol 7:655–663, 1977.
351. Klein G, Klein GO, Karre K, Kiessling R: 'Hybrid resistance' against parental tumors: One or several genetic patterns? Immunogenetics 7:391–404, 1978.

2. Role of Macrophages in Host Resistance Against Tumors

WILLIAM E. FOGLER and ISAIAH J. FIDLER

1. HOST-TUMOR INTERACTIONS IN THE PATHOGENESIS OF CANCER METASTASIS

The most devastating aspect of cancer is the propensity of malignant neoplasms to spread from their primary site of growth to distant organs where secondary tumors, metastases, can develop. Despite remarkable advances in aggressive adjuvant therapy and improvements in general patient care, most deaths of patients with solid cancers are caused by metastases. There are several reasons for the current failure to eradicate metastasis. First, by the time of surgical excision of the primary neoplasm, metastases may have already occurred. Second, even when metastases are diagnosed, their location and number may limit the effective dose of therapeutic agent that can be delivered to the lesion without being toxic to the host. Third, the most formidable obstacle to successful treatment of metastasis is the heterogeneous nature of malignant neoplasms and the rapid emergence of metastases that are resistant to conventional therapeutic regimens [1–3].

The pathogenesis of cancer metastasis depends on the interaction of tumor cells with the host and can be divided, somewhat arbitrarily, into several sequential steps: (1) invasion of cells from the primary tumor into the surrounding tissue, with penetration of blood and/or lymph vessels, (2) release of single or multiple tumor cell emboli into the circulation, (3) arrest of the circulating emboli in the small vascular beds of organs, (4) tumor cell extravasation through the wall of the arresting vessel and infiltration into adjacent tissue, and (5) multiplication of tumor cells and the development of vascularized stroma into the new tumor focus [4–6].

The pathogenesis of metastasis is influenced by many host factors that include immune reactivity. During the process of metastasis, tumor cells come in direct contact with various elements of the immune system such as

R.B. Herberman (ed), Basic and Clinical Tumor Immunology. ISBN-13: 978-1-4613-3875-8
© 1983. Martinus Nijhoff Publishers, Boston.

macrophages [7, 8], T lymphocytes [9, 10], natural killer lymphocytes [11, 12], and natural cytotoxic cells [13, 14]. Moreover, tumor metastasis can be inhibited by soluble effector molecules such as induced or naturally occurring antibodies and complement [15-18], tumor necrosis factor [19, 20], lymphotoxin [21, 22], and tumor-derived growth inhibition factor [23].

Experimental studies on the role of host immunity in cancer metastasis have yielded contradictory results. Highly immunogenic methylcholanthrene (MCA)-induced mammary carcinomas did not metastasize in the rat, whereas weakly immunogenic tumors produced extensive metastases [24]. In an experimental metastasis model, tumor cells injected intravenously were arrested primarily in the lungs, and mice bearing small local (subcutaneous) tumors were more resitant to the development of pulmonary metastases than normal mice [25, 26] or mice bearing large primary tumors [25, 27]. The development of metastatic foci was enhanced by treatment of tumor-bearing mice with antithymocyte serum [28-30] or X-radiation [31], and the resistance of normal mice to intravenous challenge with tumor cells was augmented by the transfer of spleen cells [32] or peritoneal cells [30] from immune donors. Serum alone had no effect on the resistance of X-irradiated recipients to intravenous tumor challenge unless normal or immune spleen cells were adoptively transferred into these mice before challenge [32]. Collectively, these results suggest that cellular and humoral immune responses can destroy hematogeneously disseminated tumor cells. Thus, suppression of host immunity was shown to increase the metastatic incidence of malignant neoplasms.

The finding that immune suppression leads to increased metastasis is not a generalized phenomenon. In many other tumor systems, depression of immunologic reactivity actually was associated with a decrease in metastasis [33-38] or had no influence whatsoever on the growth of a local or disseminated tumor [39]. The basis for these discrepancies could have been the differences in the histology and etiology of the tumors and in the species in which they originated. In order to study the importance of host immunity to the incidence of metastasis by tumors of different antigenicities, Fidler et al. [39] minimized these experimental variables; they examined three murine fibrosarcomas with different degrees of immunogenicity for their metastatic activity in normal, immunosuppressed and sham-suppressed mice, and in immunosuppressed mice whose immunity was reconstituted immediately prior to the studies. A highly immunogenic fibrosarcoma grew locally and formed more pulmonary tumor colonies in immunosuppressed mice than in normal, sham-suppressed, or reconstituted animals. A fibrosarcoma of intermediate immunogenicity grew locally and also formed more pulmonary metastases in immunosuppressed recipients, but this increase could

not be reversed by reconstitution with lymphocytes from normal mice. In contrast, the third and least immunogenic fibrosarcoma produced fewer pulmonary tumor colonies in immunosuppressed mice than it did in normal, sham-suppressed, or immunoreconstituted mice. Thus, even under relatively uniform laboratory conditions, the influence of the immune system on experimental cancer metastasis varied for the three syngeneic tumors. These data demonstrate that tumor immunogenicity is an important factor in the relationship between host immunity and tumor dissemination but that no generalizations about this relationship can be drawn from a single tumor system [39].

The interaction of circulating metastatic tumor cells with host tumoricidal factors represents a highly selective process and the subsequent growth of metastatic cells into visible foci is the end-point of many destructive events which few tumor cells survive.

2. BIOLOGIC HETEROGENEITY OF MALIGNANT NEOPLASMS AND IMPLICATIONS FOR HOST RESISTANCE AGAINST TUMORS

There is now a large body of data to indicate that at the time of diagnosis, malignant neoplasms are heterogeneous and contain cells with diverse phenotypic characteristics such as antigenicity and/or immunogenicity [40–57], growth rate [58–62], protein production [63–65], karyotypes [66–72], cell surface receptors [73–76], hormone receptors [77–81], response to a variety of cytotoxic drugs [82–95], and metastatic potential [96–101]. The recognition that malignant neoplasms are composed of cells with diverse biologic behaviors has initiated studies into the nature of this heterogeneity and its implications for therapy.

Heterogeneity in sensitivity to cytotoxic drugs exists among tumor cell subpopulations populating primary neoplasms. Cells isolated from a rat hepatocellular carcinoma [90], a methylcholanthrene-induced murine sarcoma [84], and a murine mammary tumor [89] have been shown to have different *in vitro* and *in vivo* sensitivities to a variety of chemotherapeutic agents. These observations are not restricted to experimental tumor systems. Various human neoplasms (such as melanoma [91], adenocarcinomas isolated from colon [102], stomach [86], breast [91], and ovary [94], and lymphoma [103]) also have been shown to be heterogeneous for drug response. Moreover, even within the same patient, different metastases can exhibit different susceptibilities to chemotherapeutic agents [95]. The emergence of drug-resistant tumor cell variants in clinical oncology is well documented. For example, small-cell carcinoma of the lung is usually initially sensitive to chemotherapy with or without radiotherapy. In contrast, recur-

Table 1. Immunomodulators capable of activating tumoricidal properties in mononuclear phagocytes.

Agent	Reference(s)
Endotoxin, lipopolysaccharide, lipid A	106–110
ds RNA (synthetic or viral origin)	106
Aggregated IgG, immune complexes	111
Lysolecithin analogs	112, 113
Chronic infection of host with bacteria, protozoa, nematodes	114–116
Maleic anhydride divinyl ether	117, 118
Muramyl dipeptide (MDP)	119–121
γ-Interferon	110, 122, 123
Lymphokines	
Specific macrophage-arming factor (SMAF)	7, 8, 124
Macrophage-activating factor (MAF)	107, 109, 110, 123, 125–127

rences, which are a common feature of this neoplasm, are resistant to chemotherapy regardless of the magnitude of the initial response to therapy [104, 105].

Antigenic and/or immunogenic heterogeneity of tumor cells also presents problems for treatment of metastasis. Analysis of several AKR murine leukemias demonstrated that these tumors were immunologically polyclonal [57]. Immunization of tumor-bearing animals with a vaccine prepared from the original tumor proved unsuccessful because only the dominant population was rejected, allowing the minor subpopulation to proliferate. The minor subpopulation(s) in the vaccine did not offer a significant immunologic challenge for stimulation [57]. Because tumor cell variants resistant to chemotherapy and/or immunotherapy can proliferate unchecked following the destruction of the sensitive populations, the successful treatment of metastases will be one that can circumvent the problem of cellular diversity and that does not induce resistance.

3. *IN VITRO* INTERACTION OF TUMORICIDAL MONONUCLEAR PHAGOCYTES WITH TARGET CELLS

Although tumor cell populations are heterogeneous with regard to many characteristics, they appear to be susceptible to destruction by activated (tumoricidal) macrophages. Mononuclear phagocytes can be activated to the tumoricidal state by various natural and synthetic materials as summarized in Table 1. The cellular and molecular processes by which a normal, nontumoricidal macrophage is rendered tumoricidal are now receiving wide

attention and will not be discussed here. Current concepts of macrophage activation suggest that a series of phenotypic alterations accompanying activation are acquired in a stepwise fashion, culminating in the development of tumoricidal activities. The rate of the appearance and/or loss of specific phenotypic alterations depends on the nature and duration of activating factors [107, 128, 129].

Activated macrophages acquire the ability to recognize and destroy neoplastic cells while leaving nonneoplastic cells unharmed [130, 132]. The susceptibility of tumor cells to destruction by tumoricidal macrophages is independent of the *in vivo* biological behavior of the tumor cell. Melanoma variant cell lines that have a low or high metastatic potential, that have invasive or noninvasive characteristics, and that are either susceptible or resistant to lysis mediated by syngeneic T-cells are lysed *in vitro* by lymphokine-activated macrophages [132]. Similarly, several cloned cell lines that were isolated from a murine fibrosarcoma induced by ultraviolet (UV) radiation and that vary in their degree of immunogeneicity and/or invasive and metastatic potential *in vivo* [52] are all susceptible to destruction *in vitro* by tumoricidal macrophages [99]. The nature of target cell susceptibility to destruction by activated macrophages has also been examined with virus-transformed cell lines in which various characteristics of the transformed phenotype are temperature dependent [133]. These studies demonstrated that the tumor cells were lysed by macrophages regardless of whether they expressed cell surface LETS protein or Forssman antigens, displayed surface charges that permitted agglutination by low doses of plant lectins, expressed SV-40 T antigen, had a low saturation density, or exhibited density-dependent inhibition of DNA synthesis [133]. Moreover, recent attempts to select *in vitro* tumor cell variant lines that are resistant to macrophage-mediated lysis have proved unsuccessful [134]; the techniques were similar to those used previously to successfully select tumor cell lines resistant to lysis by syngeneic T lymphocytes [46] or natural killer cells [12]. Recently we attempted to select for macrophage-resistant tumor variants using 11 mouse tumors of different histologic classification, etiology, and histocompatibility. Despite rigorous selection pressures we were unable to isolate macrophage-resistant tumor cell populations.

Much of our knowledge regarding the mechanism(s) by which mononuclear phagocytes destroy susceptible target cells has been obtained from a variety of *in vitro* assays, including light microscopy [135], inhibition of tumor growth (cytostasis) [136–138], cleared zones of tumor cell monolayers [115, 139], release of radioactive labels from target cells [140–142], cinemicrographic analysis [143], and sequential scanning and transmission electron microscopy [144]. Morphologic studies of the interaction of murine macrophages with susceptible target cells suggested to Hibbs that a direct

macrophage-target cell contact involving the transfer of lysosomal enzymes is responsible for target lysis [130, 145]. Evidence to support this concept was provided by the ultrastructural studies of a guinea pig system reported by Bucana et al. [144]. Tumor cell destruction by syngeneic peritoneal macrophages stimulated by bacillus Calmette-Guérin was associated with direct exocytosis of lysosomes from macrophages followed by lysosome endocytosis by the target cells. Both Hibbs [130] and Bucana et al. [144] emphasized that destabilization of the target cell membrane constitutes an integral step of the cytolytic process. In studies with murine peritoneal macrophages activated by *Corynebacterium parvum,* Puvion et al. [146] observed that lysosomes of activated macrophages disappear following contact with target cells. Moreover, the contact zone was characterized by an alteration in membrane enzyme localization. Macrophage-mediated cytotoxicity *in vitro* does not always correlate with the transfer of lysosomes and destabilization of the target cell membrane. Miller et al. examined murine peritoneal macrophages treated with perfluorochemicals and did not find evidence for the transfer of lysosomes from the activated macrophage into the target cell or alterations in the structure of adjacent macrophage-tumor membranes [147]. Another potential mechanism of macrophage-mediated tumor cell cytotoxicity following cell-cell interaction was suggested by the findings of Kaplan et al. [148]. Lewis lung carcinoma cells were found to undergo a reductive cell division in the absence of DNA synthesis following interaction with murine macrophages activated by *C. parvum,* suggesting that this aberrant division may be related to the lethal event mediated by activated macrophages.

Not all investigators agree that direct cell-to-cell contact is mandatory for destruction of target cells by macrophages. The release of soluble macrophage secretory products at contact sites with their targets has been proposed as one mechanism of target lysis [149–151]. Other agents thought to be involved in lysis of target cells include toxins released by macrophages [152–154], the third component of complement (C3a) [155], tumor cell growth inhibitory products such as excess thymidine [156], hydrogen peroxide [157, 158], and heat-labile neutral serine proteases [159, 160]. Although the exact mechanism(s) by which macrophages recognize and kill tumor cells is still unclear, most investigators do agree that, at least *in vitro,* activated macrophages discriminate between tumorigenic and nontumorigenic cells.

4. INVOLVEMENT OF THE MONONUCLEAR PHAGOCYTE SYSTEM IN CARCINOGENESIS AND PROGRESSION OF CANCER

Functions of the mononuclear phagocyte system in the maintenance of

Table 2. The role of mononuclear phagocytes in maintenance of homeostasis.

Function	Reference(s)
Erythrophagocytosis	161
Iron metabolism	162
Lipid metabolism	163
Secretory products involved in inflammatory and immunologic cascades	164
Inflammatory response	165, 166
Phagocytosis of senescent cells	
Wound healing	
Remodeling of tissue	
Immunologic response	165–167
Catabolism of antigen that prevents induction of tolerance	
Presentation of antigen in highly immunogenic form to lymphocytes	
Mediator of T-B lymphocyte interaction	
Host defense against bacterial, fungal, and parasitic infection	165, 166
Host defense against neoplasia	168, 169

homeostasis have been identified by various investigators, as summarized in Table 2. The most recognized physiologic role of macrophages is the clearance and catabolism of debris, which include effete red blood cells (erythrophagocytosis). In the latter process, hemoglobin is converted to an iron-free pigment, which is subsequently utilized in erythropoiesis. Macrophages are, therefore, involved in the controlled recycling of iron. In addition, the macrophage contains several esterases which are involved in the metabolism of lipids and the dissolution of atheromas. When host homeostatic mechanisms are stressed, the mononuclear phagocyte system can participate in complex interactions that involve cellular and humoral aspects of the inflammatory and immunologic response. In this regard, the macrophage is recognized as an important component of host defense against bacterial, fungal, and parasitic infections. Mononuclear phagocytes also affect the pathogenesis and development of neoplasms.

The mononuclear phagocyte may provide surveillance in the detection and destruction of neoplastic cells [170, 171]. Using tumor systems of rabbits, Lurie [172] noticed that the incidence of uterine cancer, dependent on age and strain, was parallel to the natural resistance to infection by tuberculosis (the most resistant strain having the lowest incidence of cancer). In addition, the resistance of the rabbits to tuberculosis was directly related to the bactericidal capacity of their mononuclear phagocytic system. These studies suggested a correlation between the activity of the phagocytes and the observed resistance to neoplasia. Studies by Droller and Remington supported this concept [173]. In their investigations, mice infected with the

intracellular protozoa *Toxoplasma gondii* were more resistant to viral induction of neoplasms and to tumors resulting from the transplantation of syngeneic tumor cells. The macrophages harvested from the protozoa-infected mice were cytotoxic *in vitro* to the transplanted tumor cells [173]. The importance of macrophages in carcinogenesis was revealed from studies by Norbury and Kripke [174], who determined whether treatment of mice with either a macrophage stimulant (pyran copolymer) or macrophage toxins (trypan blue or silica) would influence the latency and/or incidence of skin carcinogenesis induced by UV radiation. Treatment of mice with pyran copolymer lengthened the latent period of tumor development and reduced the incidence and number of the skin tumors that resulted from suboptimal exposures to UV radiation. Conversely, treatment of mice with macrophage toxins shortened the latent period for induction of skin cancer by UV radiation. Similarly, in transplantable tumor systems, the impairment of macrophage function by agents such as carrageenan or silica was found to be associated with an increase in the incidence of spontaneous [175, 176] and experimental metastases [177]. There are also several reports regarding the efficacy of macrophages in the inhibition of metastasis. In an adoptive transfer study, intravenous injections of syngeneic murine macrophages that had been rendered tumoricidal by *in vivo* or *in vitro* manipulation reduced the incidence of experimental metastasis of the B16 melanoma [178]; similarly activated macrophages have also been shown to eradicate metastases in two other murine tumor systems [179, 180]. *In vitro* activated murine macrophages also were shown to inhibit the growth of tumors at primary sites [181].

Progressively growing tumors can induce several alterations in macrophage function, such as enhanced carbon clearance *in vivo* [182, 183], increased expression of monocyte Fc receptors [184, 185], suppressed migration of macrophages into the peritoneal cavity or the site of subcutaneously growing tumors, and suppressed chemotactic response of macrophages in these sites [186–191]. In this context, Sone and Fidler recently investigated whether the presence of progressively growing metastases in lung parenchyma of rats influenced the number and function of lung macrophages [192]. The presence of pulmonary metastases (produced by a syngeneic mammary adenocarcinoma) did not result in a decrease in the number of lung macrophages, and the macrophages harvested from tumor-bearing rats were functionally intact. Furthermore, the macrophages sharvested from rats with metastases could be rendered cytotoxic to syngeneic tumor cells in response to activation stimuli administered *in vitro* or *in vivo*. Such findings suggest that the presence of a large number of tumor cells in organ parenchyma need not interfere with the function of macrophages in that organ.

Although the presence of macrophages at the periphery of and within

neoplasms is well recognized, their exact role in the tumors is unclear. Differences in the cytotoxic activity of macrophages isolated from nonmetastasizing and metastasizing tumors have been reported. Mononuclear phagocytes isolated from a nonmetastatic sarcoma demonstrated cytotoxicity *in vitro* [193]. In contrast, intratumoral macrophages of a weakly immunogenic, nonmetastasizing tumor were not demonstrably cytotoxic. Similar data have been reported for progressing and regressing murine sarcomas [194]. Macrophage-mediated cytotoxicity *in vitro* could not always be correlated with the *in vivo* behavior of the tumor from which the macrophages were isolated [195].

Eccles and Alexander [196, 197] found that the macrophage content of six carcinogen-induced rat fibrosarcomas correlated directly with their immunogenicity and inversely with their metastatic potential, suggesting that some tumors are nonmetastatic because they contain high numbers of macrophages. However, this is not generally true of other tumors. We recently examined the macrophage content of 16 different rodent tumors and did not find a correlation between the extent of macrophage infiltration into neoplasms and the metastatic behavior of the tumors [198]. Moreover, we did not find a correlation between the macrophage content of UV-radiation-induced murine fibrosarcomas growing in normal or immunosuppressed syngeneic mice and the immunogenic potential of the tumors.

Several factors in influence the extent of macrophage infiltration into tumors. One factor, tumor cell immunogenicity, was not correlated with macrophage content in our study. This observation is in agreement with studies by Evans and coworkers [199, 200], who examined the macrophage content of 33 different methylcholanthrene-induced murine fibrosarcomas and rhabdomyosarcomas and concluded that there was no relationship between macrophage content and the immunogenicity of the tumors. The factors influencing macrophage infiltration into tumors are poorly understood, but both immune and nonimmune factors are clearly involved [200]. In fact, many tumors appear to be nonimmunogenic under conditions of progressive growth, and in these tumors, macrophage infiltration may depend more on nonimmunologic factors such as inflammation and necrosis [201].

Whether tumors regress or progress is apparently determined by the degree to which tumoricidal activity of macrophages is generated *in situ,* rather than by the number of macrophages within the tumor [194]. This finding could explain why progressively growing spontaneous metastases often contain as many macrophages as or more macrophages than the parent tumor [202]. Two recent independent studies reported that the macrophage content of pooled metastases was similar to that of primary tumors [203] and that the metastases were not resistant to macrophage-mediated lysis [204].

Clearly, the role of the mononuclear phagocyte system in metastasis varies for different tumors and does not correlate with tumor cell immunogenicity and/or metastatic properties. In some tumors, numerous infiltrating macrophages can inhibit metastasis, but the absence of macrophages in a benign neoplasm will not lead to metastasis. The absence of macrophages is unlikely to compensate for inability of tumor cells to invade the host stroma and enter the circulation to produce distant growths. Therefore, neoplasms with low macrophage content may or may not be metastatic, as demonstrated in studies in which nonmetastatic clones isolated from a highly metastatic neoplasm also exhibited low macrophage content when growing subcutaneously [202].

5. MANIPULATIONS OF THE MONONUCLEAR PHAGOCYTE SYSTEM FOR TREATMENT OF METASTASES

As discussed in the previous section, the intravenous injection of nonspecifically activated mononuclear phagocytes into mice bearing metastases has been shown to inhibit tumor growth both in the primary site [181] and in metastases [178-180]; therefore, intravenous administration of activated macrophages might be useful in augmenting host resistance to disseminated neoplastic disease. For clinical use, however, this strategy has two serious shortcomings: it requires the transfusion of a large number of histocompatible or autologous macrophages, and most intravenously injected macrophages are likely to become arrested in the capillary bed of the lung and not reach other relevant sites. A more promising approach is to develop methods whereby autologous macrophages can be activated *in situ.*

One of the major pathways for macrophage activation *in vivo* is believed to result from the action of the lymphokine component (macrophage-activating factor [MAF]) released from sensitized lymphocytes (Table 1). Therapeutic use of MAF is hindered by the lack of purified preparations of this mediator, and efforts to activate the tumoricidal properties of macrophages *in vivo* by parenteral injection of crude preparations of lymphokines have proved unsuccessful. Injection of lymphokines into skin [205] or skin tumors [206, 207] provoked local inflammatory reactions and histologic changes suggestive of macrophage activation that resulted in the regression of small cutaneous tumors; however, systemic activation of macrophages is more difficult for several reasons: (1) Lymphokines injected into the venous circulation have a short half-life, probably because they bind with plasma proteins [208]. (2) Only a small fraction of the mononuclear phagocyte system may be capable of responding to MAF. (3) Macrophages can be activated by lymphokines only within a relatively short period following their

emigration into tissues from the circulation [208]. (4) The tumoricidal properties of macrophages are short-lived (2–3 days), and macrophages are refractory to reactivation by lymphokines [208].

A second major pathway for the activation of macrophages *in vivo* involves their direct interaction with microorganisms and/or their products. Because such biologic agents often cause undesirable side effects, such as granuloma formation and allergic reactions [209], it is preferable to use synthetic compounds that are relatively nontoxic yet possess immune-potentiating activity to activate macrophages *in vivo*. N-acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide [MDP]) is the minimum structural unit (mw 492) with immune-potentiating activity that can replace mycobacteria in Freund's complete adjuvant. MDP influences many macrophage functions including cytotoxic activity [210–211]; however, following parenteral administration, this water-soluble agent is rapidly cleared (<60 minutes) from the body and excreted in the urine [212], which limits its therapeutic potential. We recently showed [213–215] that the phagocytic uptake by macrophages of liposomes (concentric phospholipid vesicles separated by aqueous compartments) containing MDP produces highly efficient activation of rodent macrophages. Most liposomes injected intravenously are taken up by cells of the reticuloendothelial system. This 'passive targeting' of liposomes to macrophages offers a means of enhancing uptake of therapeutic agents that stimulate macrophage activity. Furthermore, liposomes are nonimmunogenic and do not elicit allergic reactions commonly associated with the systemic administration of other immune adjuvants [216].

We have demonstrated that both lymphokines [208, 213–215, 217–224] and MDP [121, 222, 223] when encapsulated in liposomes are highly effective in activating macrophages *in vitro* and *in vivo*. Dose-response measurements indicate that liposome-encapsulated preparations of these agents induce maximum activation of tumoricidal properties in macrophages at significantly lower doses than that needed for equivalent activation by the nonencapsulated (free) preparation. Activation of liposomes to the tumoricidal state requires phagocytic uptake of liposomes; this is followed by an undefined lag period before cytotoxic activity is expressed [218]. Participation of macrophage surface receptors is not required, suggesting that activation results from the interaction of MAF or MDP with an intracellular target(s). Unlike activation by free MAF, which requires binding of MAF to a fucoglycolipid receptor on the macrophage surface [224], liposome-encapsulated MAF can activate macrophages that lack functional receptors for MAF [213]. Moreover, liposome-encapsulated MAF can induce activation of subpopulations of tissue macrophages and intratumoral macrophages [208, 213] that are completely refractory to activation by free MAF.

These findings raised the possibility that macrophage-activating agents encapsulated within liposomes could be similarly efficient in activating mononuclear phagocytes *in vivo* and provide a therapeutic modality for enhancing host resistance against metastases. To test this possibility, we inoculated mice bearing spontaneous pulmonary metastases intravenously with either MAF or MDP encapsulated within multilamellar liposomes composed of phosphatidylcholine and phosphatidylserine. This type of liposome was chosen for several reasons. First, studies of body distribution of liposomes of different size and phospholipid composition demonstrated that these negatively charged liposomes localize and are retained in the lungs (in addition to organs rich with reticuloendothelial cell activity) [215]. Second, toxicity studies in which these liposomes containing MAF were injected intravenously into mice or beagle dogs revealed no adverse reactions in recipient animals even after repeated injections [219]. Finally, another study has shown that intravenous injection of these liposomes activates murine or rat lung macrophages to become tumoricidal [214].

In our studies, multiple intravenous injections of liposome-encapsulated MDP, but not free MDP or control liposome preparations, eradicated spontaneous visceral metastases in C57BL/6 mice from which a syngeneic melanoma had been surgically removed. At the start of therapy, the metastatic tumor burden in the lungs and lymph nodes may have exceeded a total-body burden of 10^7 cells. Nonetheless, 65% of mice treated with liposome-encapsulated MDP and 70% of mice treated with liposomes containing MAF survived at least 200 days. The tumor burden in these successfully treated mice was probably reduced to fewer than 10 viable cells, as evidenced by their survival of more than 40 days, which is the median life span of mice implanted with 10 viable B16 cells [225]. Thus, the intravenous injection of liposomes containing macrophage-activating agents brought about the complete regression of established pulmonary and lymph node metastases.

The mechanism(s) responsible for the regression of established metastases after the systemic administration of liposomes containing MAF or MDP probably involved the activation of macrophages to become tumoricidal. Several lines of evidence tend to support this conclusion. First, administration of macrophage-activating agents encapsulated within liposomes that are not retained in the lung fails to activate lung macrophages and there is no regression of lung metastases [220]. Second, the pretreatment of tumor-bearing animals with agents that are toxic for macrophages (silica, carrageenan, hyperchlorinated drinking water) before systemic therapy with liposome-encapsulated lymphokines or MDP abrogates the response to liposome therapy and such animals rapidly die of metastatic disease [220]. Third, systemic activation of macrophages by liposome-encapsulated MDP can be accomplished in athymic nude mice, eliminating the possibility that

T lymphocytes are the effector cells [222]. Finally, intravenous injection of macrophages activated *in vitro* by incubation with liposomes containing MAF or MDP produces a reduction in metastatic burden comparable to that achieved by systemic administration of liposome-encapsulated activators [220].

6. SUMMARY AND CONCLUSIONS

The emergence of metastases that are resistant to conventional therapy could be the major reason for the failure to treat cancer metastasis. Tumors are heterogeneous with regard to many characteristics, including metastatic potential, and the proliferation of a minor subpopulation of cells within the primary tumor could cause treatment-resistant metastases to emerge; therefore, the successful approach to destruction of metastases will be one that circumvents tumor cell heterogeneity and also against which resistance is unlikely to develop.

At least *in vitro*, appropriately activated macrophages appear able to recognize and destroy any neoplastic cells regardless of their different characteristics. Moreover, cells do not appear to develop resistance to macrophage-mediated cytotoxicity. A significant effort is now under way in many laboratories to develop effective agents that will stimulate the antitumor activities of macrophages. Liposomes offer a most suitable carrier system for delivering agents to macrophages *in vivo*. When injected intravenously, the majority of liposomes are taken by phagocytic reticuloendothelial cells in the liver and spleen and by circulating monocytes. This provides a highly effective mechanism for 'targeting', albeit passively, of liposome-encapsulated materials to macrophages. We have exploited this mechanism to deliver lymphokines and/or synthetic MDP to macrophages *in situ*. Intravenous administration of lymphokines or MDP encapsulated in multilamellar liposomes activates macrophages *in vivo* and augments host resistance to metastases. No beneficial effects are obtained with the same materials administered in unencapsulated form. The enhanced destruction of established metastases produced by therapy with liposome-encapsulated macrophage activation agents is mediated via activated macrophages. Treatments that impair macrophage activity abrogate the response to liposome administrations. Neither functional T lymphocytes nor NK cells are necessary for stimulation of host antitumor responses by liposome-encapsulated lymphokines or MDP.

Not all mice treated with intravenous injections of liposomes containing MDP survived; however, the metastases of treatment-failure animals are not populated by macrophage-resistant tumor cells, indicating that macro-

phage activation could overcome the fundamental problem of phenotypic heterogeneity among tumor cells. However, regardless of their state of activation, macrophage destruction of large tumor burden is not feasible. Thus, the most likely role for tumoricidal macrophages is in the destruction of micrometastases and/or the few tumor cells that remain after treatment with conventional adjuvant therapies such as chemotherapy.

REFERENCES

1. Fidler IJ: Tumor heterogeneity and the biology of cancer invasion and metastasis. Cancer Res 37:2481–2486, 1978.
2. Poste G, Fidler IJ: The pathogenesis of cancer metastasis. Nature 283:139–146, 1979.
3. Fidler IJ, Kripke ML: Biological variability within murine neoplasms. Antibiot Chemother 28:123–129, 1980.
4. Hart IR, Fidler IJ: The implications of tumor heterogeneity for studies on the biology and therapy of cancer metastasis. Biochim Biophys Acta 651:37–50, 1981.
5. Fidler IJ, Gersten DM, Hart IR: The biology of cancer invasion and metastasis. Adv Cancer Res 28:149–250, 1978.
6. Sugarbaker EV: Cancer metastasis: A product of tumor-host interactions. Curr Probl Cancer 7:3–59, 1979.
7. Evans R, Alexander P: Rendering macrophages specifically cytotoxic by a factor released from immune lymphoid cells. Transplantation 12:227–229, 1971.
8. Evans R, Grant CK, Cox H, Steele K, Alexander P: Thymus-derived lymphocytes produce an immunologically specific macrophage-arming factor. J Exp Med 136:1318–1322, 1972.
9. Henney C: On the mechanism of T-cell mediated cytolysis. Transplant Rev 17:37–70, 1973.
10. Hellstrom KE, Hellstrom I: Lymphocyte-mediated cytotoxicity and blocking serum activity to tumor antigens. Adv Immunol 18:209–277, 1974.
11. Herberman RB, Holden HT: Natural cell-mediated immunity. Adv Cancer Res 27:305–377, 1978.
12. Hanna N, Fidler IJ: Role of natural killer cells in the destruction of circulating tumor emboli. JNCI 65:801–809, 1980.
13. Stutman O, Paige CJ, Figarella EF: Natural cytotoxic cells against solid tumors in mice. I. Strain and age distribution and target cell susceptibility. J Immunol 121:1819–1826, 1978.
14. Paige CJ, Figarella EF, Cuttito MJ, Cahan A, Stutman O: Natural cytotoxic cells against solid tumors in mice: II. Some characteristics of the effector cells against solid tumors in mice. J Immunol 121:1827–1835, 1978.
15. Gorer PA, O'Gorman P: The cytotoxic activity of isoantibodies in mice. Transplant Bull 3:142–145, 1956.
16. Gorer P, Amos DB: Passive immunity of mice against C57BL leukosis EL4 by means of 180 immune serum. Cancer Res 16:338–343, 1956.
17. Irie K, Irie RF, Morton DL: Evidence for *in vivo* reaction of antibody and complement to surface antigens of human cancer cells. Science 186:454–456, 1974.
18. Bodurtha AJ, Chee DO, Laucius JF, Mastrangelo MJ, Prehn RT: Clinical and immunological significance of human melanoma cytotoxic antibody. Cancer Res 35:189–193, 1975.

19. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B: An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci USA 72:3666–3670, 1975.
20. Green S, Dobrjansky A, Chiasso MA, Carswell E, Schwartz MK, Old LJ: *Corynebacterium parvum* as the priming agent in the production of tumor necrosis factor in the mouse. J Natl Cancer Inst 59:1519–1522, 1977.
21. Kolb WP, Granger GA: Lymphocyte *in vitro* cytotoxicity: Characterization of mouse lymphotoxin. Cell Immunol 1:122–132, 1970.
22. Vailleur D, Donner M, Vailleur J, Burg C: Release of lymphotoxins by spleen cells sensitized against mouse tumor associated antigens. Cell Immunol 6:466–472, 1973.
23. Heppner G, Miller BE: Biological variability of mouse mammary neoplasms. In: Design of models for testing cancer therapeutic agents, Fidler IJ, White RJ (eds). New York: Van Nostrand, 1981, pp 37–49.
24. Kim U: Metastasizing mammary carcinomas in rats: Induction and study of their immunogenicity. Science 167:72–74, 1970.
25. Wexler H, Chretien PB, Ketcham AS, Sindelar WF: Induction of pulmonary metastasis in both immune and nonimmune mice: Effect of the removal of a transplanted primary tumor. Cancer 36:2042–2047, 1975.
26. Fidler IJ, Gersten DM, Riggs CW: Relationship of host immune status to tumor cell arrest, distribution, and survival in experimental metastasis. Cancer 40:46–54, 1977.
27. Milas L, Hunter N, Mason K, Withers HR: Immunological resistance to pulmonary metastases in C3Hf/Bu mice bearing syngeneic fibrosarcoma of different sizes. Cancer Res 34:61–71, 1974.
28. Gershon RK, Carter RL, Kondo K: On concomitant immunity in tumour-bearing hamsters. Nature 213:674–676, 1967.
29. Deodhar SD, Crile G, Jr.: Enhancement of metastases by antilymphocyte serum in allogeneic murine tumor system. Cancer Res 29:776–779, 1969.
30. Gershon RK, Carter RL: Facilitation of metastatic growth by antilymphocyte serum. Nature 226:368–370, 1970.
31. Vaage J, Chen K, Merrick S: Effect of immune status on the development of artificially induced metastases in different anatomical locations. Cancer Res 31:496–500, 1971.
32. Vaage J: Humoral and cellular immune factors in the systemic control of artifically induced metastases in C3Hf mice. Cancer Res 33:1957–1965, 1973.
33. Fisher ER: Role of thymus in skin and tumor transplantation in the rat. Lab Invest 14:546–552, 1965.
34. Fidler IJ: Immune stimulation-inhibition of experimental cancer metastasis. Cancer Res 34:491–498, 1974.
35. Umiel T, Trainin N: Immunological enhancement of tumor growth by syngeneic thymus-derived lymphocytes. Transplantation 18:244–250, 1974.
36. Hewitt HB, Blake ER, Walder AS: A critique of the evidence for active host defence against cancer, based on personal studies of 27 murine tumours of spontaneous origin. Br J Cancer 33:241–259, 1976.
37. Vaage J: A survey of the growth characteristics of, and host reactions to, one hundred C3H/He mammary carcinomas. Cancer Res 38:331–338, 1978.
38. Hager C, Miller FR, Heppner GH: Influence of serial transplantation on the immunological-clinical correlates of BALB/cf C3H mouse mammary tumors. Cancer Res 38:2492–2498, 1978.
39. Fidler IJ, Gersten DM, Kripke ML: Influence of immune status on the metastasis of three murine fibrosarcomas of different immunogenicities. Cancer Res 39:3816–3821, 1979.
40. Prehn RT: Cancer antigens in tumors induced by chemicals. Fed Proc 24:1018–1028, 1965.

41. Prehn RT: Analysis of antigenic heterogeneity within individual 3-methylcholanthrene-induced mouse sarcomas. J Natl Cancer Inst 45:1039–1044, 1970.
42. Sugarbaker EV, Cohen AM: Altered antigenicity in spontaneous pulmonary metastases from an antigenic murine sarcoma. Surgery 72:155–166, 1972.
43. Faraci RP: *In vitro* demonstration of altered antigenicity of metastases from a primary methylcholanthrene-induced sarcoma. Surgery 76:469–473, 1974.
44. Goldman LI, Flaxman BA, Wernick G, Zabriski JB: Immune surveillance and tumor dissemination: *In vitro* comparison of the B16 melanoma in primary and metastatic form. Surgery 76:50–56, 1974.
45. Killion JJ, Kollmorgen GM: Isolation of immunogenic tumor cells by cell-affinity chromatography. Nature 259:674–676, 1976.
46. Fidler IJ, Gersten DM, Budman MB: Characterization *in vivo* and *in vitro* of tumor cells selected for resistance to syngeneic lymphocyte-mediated cytotoxicity. Cancer Res 36:3160–3165, 1976.
47. Byers WS, Johnston JO: Antigenic differences among osteogenic sarcoma tumor cells taken from different locations in human tumors. Cancer Res 37:3173–3183, 1977.
48. Biddison WE, Palmer JC: Development of tumor cell resistance to syngeneic cell-mediated cytotoxicity during growth of ascitic mastocytoma P815Y. Proc Natl Acad Sci USA 74:329–333, 1977.
49. Fidler IJ, Bucana C: Mechanism of tumor cell resistance to lysis by syngeneic lymphocytes. Cancer Res 37:3945–3956, 1977.
50. Fugi H, Michich E, Pressman D: Differential tumor immunogenicity of L1210 and its sublines. J Immunol 119:983–986, 1977.
51. Killion JJ: Immunotherapy with tumor subpopulations: I. Active, specific immunotherapy of L1210 leukemia. Cancer Immunol Immunother 4:115–119, 1978.
52. Kripke ML, Gruys E, Fidler IJ: Metastatic heterogeneity of cells from an ultraviolet light-induced murine fibrosarcoma of recent origin. Cancer Res 38:2962–2967, 1978.
53. Miller FR, Heppner GH: Immunologic heterogeneity of tumor cell subpopulations from a single mouse mammary tumor. JNCI 63:1457–1463, 1979.
54. Kolb E, Muller E: Local responses in primary and secondary human lung cancers: II. Clinical conclusions. Br J Cancer 40:410–416, 1979.
55. Kerbel RS: Implications of immunological heterogeneity of tumors. Nature 280:358–360, 1979.
56. Schirmacher V, Bosslet K, Shantz G, Claver K, Hubsch D: Tumor metastases and cell-mediated immunity in a model system in DBA/2 mice: IV. Antigenic differences between a metastasizing variant and the parental tumor line revealed by cytotoxic T lymphocytes. Int J Cancer 23:245–252, 1979.
57. Olsson L, Ebbesen P: Natural polyclonality of spontaneous AKR leukemia and its consequence for so-called specific immunotherapy. JNCI 62:623–627, 1979.
58. DeWys WD: Studies correlating the growth rate of a tumor and its metastases and providing evidence for tumor-related systemic growth-retarding factors. Cancer Res 32:374–379, 1972.
59. Schabel FM: Concepts for systemic treatment of micrometastases. Cancer 35:15–24, 1975.
60. Cifone MA, Kripke ML, Fidler IJ: Growth rate and chromosome number of tumor cell lines with different metastatic potential. J Supramol Struct 11:467–476, 1979.
61. Cifone MA, Fidler IJ: Correlation of patterns of anchorage-independent growth with *in vivo* behavior of cells from a murine fibrosarcoma. Proc Natl Acad Sci USA 77:1039–1043, 1980.
62. Miller BE, Miller FR, Leith J, Heppner GH: Growth interaction *in vivo* between tumor

subpopulations derived from a single mouse mammary tumor. Cancer Res 40:3977–3981, 1980.

63. Gray JM, Pierce GB: Relationship between growth rate and differentiation of melanoma *in vivo*. J Natl Cancer Inst 32:1201–1211, 1964.
64. Niles RM, Makarski JS: Hormonal activation of adenylate cyclase in mouse melanoma metastatic variants. J Cell Physiol 96:351–359, 1978.
65. Fidler IJ, Gruys E, Cifone MA, Barnes Z, Bucana C: Demonstration of multiple phenotypic diversity in a murine melanoma of recent origin. JNCI 67:947–956, 1981.
66. Ito E, Moore GE: Characteristic differences in clones isolated from an S37 ascites tumor *in vitro*. Exp Cell Res 48:440–447, 1967.
67. Mittleman F: The chromosomes of fifty primary Rous rat sarcomas. Hereditas 69:155–186, 1971.
68. Ohno S: Genetic implication of karyological instability of malignant somatic cells. Physiol Rev 51:496–526, 1971.
69. Bohm N, Sondritter W: DNA in human tumors: A cytophotometric study. Curr Top Pathol 60:152–219, 1975.
70. Vindelov LV: Flow microfluoremetric analysis of nuclear DNA in cells from solid tumors and cell suspensions. Virchows Arch [Cell Pathol] 24:227–242, 1977.
71. Siracky J: An approach to the problem of heterogeneity of human tumor-cell populations. Br J Cancer 39:570–577, 1979.
72. Vindelov LV, Hansen HH, Christensen IJ, Spange-Thomsen M, Hirsch FR, Hansen M, Nissen NI: Clonal heterogeneity of small-cell anaplastic carcinoma of the lung demonstrated by flow cytometric DNA analysis. Cancer Res 40:4295–4300, 1980.
73. Tao TW, Burger MM: Nonmetastasizing variants selected from metastasizing melanoma cells. Nature 270:437–438, 1977.
74. Brunson KW, Nicolson GL: Selection and biologic properties of malignant variants of a murine lymphosarcoma. JNCI 61:1499–1503, 1978.
75. Raz A, McLellan WE, Hart IR, Bucana CD, Hoyer LC, Sela BA, Dragsten P, Fidler IJ: Cell surface properties of B16 melanoma variants with differing metastatic potential. Cancer Res 40:1645–1651, 1980.
76. Reading CL, Belloni DN, Nicolson GL: Selection and *in vivo* properties of lectin-attachment variants of malignant murine lymphosarcoma cell lines. JNCI 64:1241–1249, 1980.
77. Franks LM: Estrogen-treated prostatic cancer. Cancer 13:490–501, 1960.
78. Sluyser M, Van Nie R: Estrogen receptor content and hormone-responsive growth of mouse mammary tumors. Cancer Res 34:3252–3257, 1974.
79. Baylin SB, Abeloff MD, Wieman KC, Tomford JW, Ettinger DS: Elevated histaminase (diamine oxidase) activity in small-cell carcinoma of the lung. N Engl J Med 293:1286–1290, 1975.
80. Sluyser M, Evers SG, DeGoey CC: Sex hormone receptors in mammary tumours of GR mice. Nature (Lond) 263:386–389, 1976.
81. Baylin SB, Weisburger WR, Eggleston JC, Mendelshon G, Beamen MA, Abeloff MD, Ettinger DS: Variable content of histaminase, L-dopa decarboxylase and calcitonin in small-cell carcinoma of the lung: Biologic and clinical implications. N Engl J Med 299:105–110, 1978.
82. Barranco SC, Ho D, Derwinko B, Romsdahl MM, Humphrey RM: Differential sensitivities of human melanoma cells grown *in vitro* to arabinosylcytosine. Cancer Res 32:2733–2736, 1972.
83. Barranco SC, Derwinko B, Humphrey RM: Differential response by human melanoma cells to 1,3-bis-(2-chloroethyl)-1-nitrosourea and bleomycin. Mutat Res 19:277–280, 1973.

84. Hakansson L, Trope C: On the presence within tumors of clones that differ in sensitivity to cytostatic drugs. Acta Pathol Microbiol Scand [A] 82:35–40, 1974.
85. Hakansson L, Trope C: Cell clones with different sensitivity to cytostatic drugs in methylcholanthrene-induced mouse sarcomas. Acta Pathol Microbiol Scand [A] 82:41–47,
86. Trope C, Hakansson L, Dencker H: Heterogeneity of human adenocarcinomas of the colon and the stomach as regards sensitivity to cystostatic drugs. Neoplasma 22:423–430, 1975.
87. Trope C: Different sensitivity to cytostatic drugs of primary tumor and metastasis of the Lewis carcinoma. Neoplasma 22:171–180, 1975.
88. Fugmann RA, Anderson JC, Stoli R, Martin DS: Comparison of adjuvant chemotherapeutic activity against primary and metastatic spontaneous murine tumors. Cancer Res 37:496–500, 1977.
89. Heppner GH, Dexter DL, DeNucci T, Miller FR, Calabresi P: Heterogeneity in drug sensitivity among tumor cell subpopulations of a single mammary tumor. Cancer Res 38:3758–3763, 1978.
90. Barranco SC, Hanenelt BR, Gea EL: Differential sensitivities of five rat hepatoma cell lines to anticancer drugs. Cancer Res 38:656–660, 1978.
91. Lotan R: Different susceptibilities of human melanoma and breast carcinoma cell lines to retinoic acid-induced growth inhibition. Cancer Res 39:1014–1019, 1979.
92. Lotan R, Nicolson GL: Heterogeneity in growth inhibition by β-trans-retinoic acid of metastatic B16 melanoma clones and *in vivo*-selected cell variant lines. Cancer Res 39:4767–4771, 1979.
93. Calabresi P, Dexter DL, Heppner GH: Clinical and pharmacological implications of cancer cell differentiation and heterogeneity. Biochem Pharmacol 28:1933–1941, 1979.
94. Trope C, Aspergen K, Kullander S, Astredt B: Heterogeneous response of disseminated human ovarian cancers to cytostasis *in vitro*. Acta Obstet Gynecol Scand 58:543–546, 1979.
95. Tsuruo T, Fidler IJ: Differences in drug sensitivity among tumor cells from parental tumors, selected variants, and spontaneous metastases. Cancer Res 41:3058–3064, 1981.
96. Fidler IJ, Kripke ML: Metastasis results from preexisting variant cells within a malignant tumor. Science 197:893–895, 1977.
97. Nicolson GL, Brunson KW: Organ specificity of malignant B16 melanoma; *in vivo* selection for organ preference of blood-borne metastasis. Cancer Res 20:15–24, 1977.
98. Nicolson GL, Brunson KW, Fidler IJ: Specificity of arrest, survival, and growth of selected metastatic variant cell lines. Cancer Res 38:4105–4111, 1978.
99. Fidler IJ, Cifone MA: Properties of metastatic and nonmetastatic cloned subpopulations of an ultraviolet-light-induced murine fibrosarcoma of recent origin. Am J Pathol 97:633–648, 1979.
100. Liotta LA, Tryggvason K, Gabrisa A, Hart IR, Foltz CM: Metastatic potential correlated with enzymatic degradation of basement membrane collagen. Nature 284:67–68, 1980.
101. Fidler IJ, Hart IR: The origin of metastatic heterogeneity in tumors. Eur J Cancer 17:487–494, 1981.
102. Trope C: Different susceptibilities of tumor cell subpopulations to cytotoxic agents. In: Design of models for testing cancer therapeutic agents, Fidler IJ, White RJ (eds). New York: Van Nostrand, Inc., 1981, pp 64–79.
103. Baylin SB: Clonal selection and heterogeneity of human solid neoplasms. In: Design of models for testing cancer therapeutic agents, Fidler IJ, White RJ (eds). New York: Van Nostrand, Inc., 1981, pp 50–63.
104. Abeloff MD, Ettinger DS, Khouri NF, Lenhard RE: Intensive induction therapy for small cell carcinoma of the lung. Cancer Treat Rep 63:519–524, 1979.

105. Livinston RB: Treatment of small cell carcinoma: Evaluation and future directions. Semin Oncol 5:299–308, 1979.
106. Alexander P, Evans R: Endotoxin and double stranded RNA render macrophages cytotoxic. Nature 232:76–78, 1971.
107. Hibbs JB Jr, Taintor RR, Chapman HA Jr, Weinberg JB: Macrophage tumor killing: Influence of the local environment. Science 197:279–282, 1977.
108. Doc WF, Henson PM: Macrophage stimulation by bacterial lipopolysaccharides. J Exp Med 148:544–556, 1978.
109. Pace JL, Russell SW: Activation of mouse macrophages for tumor cell killing: I. Quantitative analysis of interactions between lymphokine and lipopolysaccharide. J Immunol 126:1863–1868, 1981.
110. Peu G, Polentarutti N, Sessa C, Mangioni C, Mantovani A: Tumoricidal activity of macrophages isolated from human ascitic and solid ovarian carcinomas: Augmentation by interferon, lymphokines and endotoxin. Int J Cancer 28:143–152, 1981.
111. Evans R: Specific and nonspecific activation of macrophages. In: Activation of macrophages: Proceedings of the 2nd Workshop Conference, Hoechst, Schloss Reisensburg, 25-26 October, 1972. Amsterdam: Excerpta Medica, 1974, pp 305–317.
112. Munder PG, Weltzien HV, Modolell M: Lysolecithin analogs: A new class of immunopotentiators. In: Immunopathology, Miescher PA (ed). New York: Grune & Stratton , 1977, pp 411–424.
113. Tarnowski GS, Mountain IM, Stock CC, Munder PG, Weltzier HL, Westphal O: Effects of lysolecithin and analogs on mouse ascites tumors. Cancer Res 38:339–344, 1978.
114. Keller R, Jones VE: Role of activated macrophages and antibody in inhibition and enhancement of tumor growth in rats. Lancet 2:847–849, 1971.
115. Hibbs JB, Lambert LH, Remington JS: Macrophage-mediated non-specific cytotoxicity: Possible role in tumor resistance. Nature New Biol 235:48–50, 1972.
116. Cleveland RP, Meltzer MS, Lbau B: Tumor cytotoxicity *in vitro* by macrophages from mice infected with *Mycobacterium bovis* strain BCG. J Natl Cancer Inst 52:1887–1895, 1974.
117. Kaplan AM, Morachan PS, Regelson W: Induction of macrophage-mediated tumor-cell cytotoxicity by pyran copolymer. J Natl Cancer Inst 52:1919–1927, 1974.
118. Chirigos MA, Stylos WA: Immunomodulatory effect of various molecular-weight maleic anhydride-divinyl ether and other agents *in vivo*. Cancer Res 40:1967–1972, 1980.
119. Juy D, Chedid L: Comparison between macrophage activation and enhancement of nonspecific resistance to tumors by mycobacterial immunoadjuvants. Proc Natl Acad Sci USA 72:4105–4109, 1975.
120. Taniyama T, Holden HT: Direct augmentation of cytolytic activity of tumor-derived macrophages and macrophage cell lines by muramyl dipeptide. Cell Immunol 48:369–374, 1979.
121. Sone S, Fidler IJ: *In vitro* activation of tumoricidal properties in rat alveolar macrophages by synthetic muramyl dipeptide encapsulated in liposomes. Cell Immunol 57:42–50, 1981.
122. Schultz RM, Papamatheakis JD, Chirigos MA: Interferon: An inducer of macrophage activation by polyanions. Science 197:674–676, 1977.
123. Boraschi D, Tagliabui A: Interferon-induced enhancement of macrophage-mediated tumor cytolysis and its difference from activation by lymphokines. Eur J Immunol 11:110–114, 1981.
124. Kripke ML, Budmen MB, Fidler IJ: Production of specific macrophage-activating factor by lymphocytes from tumor-bearing mice. Cell Immunol 30:341–352, 1976.
125. David JR: Macrophage activation by lymphocyte mediators. Fed Proc 34:1730–1736, 1975.

126. Churchill WH, Piessens WF, Sulis CA, David JR: Macrophages activated as suspension cultures with lymphocyte mediators devoid of antigens become cytotoxic for tumor cells. J Immunol 115:781–786, 1975.
127. Fidler IJ, Raz A: The induction of tumoricidal capacities in mouse and rat macrophages by lymphokines. In: Lymphokines, Pick E (ed). New York: Academic Press, 1981, pp 345–363.
128. Cohn ZA: The activation of mononuclear phagocytes: Fact, fancy and future. J Immunol 121:813–816, 1978.
129. North RJ: The concept of the activated macrophage. J Immunol 121:806–809, 1978.
130. Hibbs JB Jr: Discrimination between neoplastic and non-neoplastic cells *in vitro* by activated macrophages. J Natl Cancer Inst 53:1487–1492, 1974.
131. Piessens WF, Churchill WH Jr: Macrophages activated *in vitro* with lymphocyte mediators kill neoplastic but not normal cells. J Immunol 114:293–299, 1975.
132. Fidler IJ: Recognition and destruction of target cells by tumoricidal macrophages. Isr J Med 14:177–191, 1978.
133. Fidler IJ, Roblin RO, Poste G: *In vitro* tumoricidal activity of macrophages against virus-transformed lines with temperature-dependent transformed phenotypic characteristics. Cell Immunol 38:131–146, 1978.
134. Fidler IJ, Poste G: Macrophage-mediated destruction of malignant tumor cells and new strategies for the therapy of metastatic disease. In: Seminars in immunopathology, Baldwin R (ed). In press, 1982.
135. Granger GA, Weiser RS: Homograft target cells: Specific destruction *in vitro* by contact interaction with immune macrophages. Science 145:1427–1429, 1964.
136. Evans R, Alexander P: Mechanism of immunologically specific killing of tumor cells by macrophages. Nature 236:168–170, 1972.
137. Keller R: Cytostatic elimination of syngeneic rat tumor cells *in vitro* by nonspecifically activated macrophages. J Exp Med 138:625–644, 1973.
138. Keller R: Susceptibility of normal and transformed cell lines to cytostatic and cytocidal effects exerted by macrophages. J Natl Cancer Inst 56:369–376, 1976.
139. Hibbs JB Jr: Macrophage nonimmunologic recognition: Target cell factors related to contact inhibition. Science 180:868–869, 1973.
140. Meltzer MS, Tucker RW, Sanford KK, Leonard EJ: Interaction of BCG-activated macrophages with neoplastic and non-neoplastic cell lines *in vitro*: Quantitation of the cytotoxic reaction by release of initiated thymidine from prelabeled target cells. J Natl Cancer Inst 54:1177–1186, 1975.
141. Norbury K, Fidler IJ: *In vitro* tumor cell cytotoxicity by syngeneic mouse macrophages: Methods for assaying cytotoxicity. J Immunol Methods 7:109–121, 1975.
142. Raz A, Fogler WE, Fidler IJ: The effects of experimental conditions on the expression of *in vitro* mediated tumor cytotoxicity. Cancer Immunol Immunother 7:157–163, 1979.
143. Meltzer MS, Tucker RW, Brever AC: Interaction of BCG-activated macrophages with neoplastic and nonneoplastic cell lines *in vitro*: Cinemicrographic analysis. Cell Immunol 17:30–42, 1975.
144. Bucana C, Hoyer LC, Hobbs B, Breesman S, McDaniel M, Hanna MG Jr: Morphological evidence for the translocation of lysosomal organelles from cytotoxic macrophages into the cytoplasm of tumor target cells. Cancer Res 36:4444–4458, 1976.
145. Hibbs JB Jr: Heterocytolysis by macrophages activated by bacillus Calmette-Guerin: Lysosome exocytosis into tumor cells. Science 184:468–471, 1974.
146. Puvion F, Fray A, Halpern B: A cytochemical study of the *in vitro* interaction between normal and activated mouse peritoneal macrophages and tumor cells. J Ultrastruct Res 54:95–108, 1976.

147. Miller ML, Stimmett JD, Clark LC Jr: Ultrastructure of tumoricidal peritoneal exudate cells stimulated *in vivo* by perfluorochemical emulsions. J Reticuloendothel Soc 27:105–117, 1980.
148. Kaplan AM, Brown J, Collins JM, Morahan PS, Snodgrass MJ: Mechanism of macrophage-mediated tumor cell cytotoxicity. J Immunol 121:1781–1789, 1978.
149. Allison AC: Macrophage activation and non-specific immunity. Int Rev Exp Pathol 18:303–312, 1978.
150. Aksamit RR, Kim KJ: Macrophage cell lines produce a cytotoxin. J Immunol 122:1785–1790, 1979.
151. Currie GA, Basham C: Differential arginine dependence and the selective cytotoxic effects of activated macrophages for malignant cells *in vitro*. Br J Cancer 39:653–659, 1978.
152. McIvoc KL, Weiser RS: Mechanisms of target cell destruction by alloimmune peritoneal macrophages: II. Release of a specific cytotoxin from interacting cells. Immunology 20:315–322, 1971.
153. Pipen CE, Molvoc KL: Alloimmune peritoneal macrophages as specific effector cells: Characterization of specific macrophage cytotoxin. Cell Immunol 17:423–430, 1975.
154. Sharma SF, Piessens WF, Middlebrook G: *In vitro* killing of tumor cells by soluble products of activated guinea pig peritoneal macrophages. Cell Immunol 49:379–383, 1980.
155. Ferluga J, Schorlemmer HU, Baptista CC, Allison AC: Production of the complement cleavage product, C3a, by activated macrophages and its tumorolytic effects. Clin Exp Immunol 31:512–517, 1978.
156. Stadecker MJ, Calderon J, Kainovsky MJ, Unanue ER: Synthesis and release of thymidine by macrophage. J Immunol 119:1738–1743, 1978.
157. Nathan CF, Brukner LH, Silverstein SC, Cohn LA: Extracellular cytolysis by activated macrophages and granulocytes: I. Pharmacologic triggering of effector cells and the release of hydrogen peroxide. J Exp Med 149:84–99, 1979.
158. Nathan CF, Silverstein SC, Brukner LH, Cohn LA: Extracellular cytolysis by activated macrophages and granulocytes: II. Hydrogen peroxide as a mediator of cytotoxicity. J Exp Med 149:110–113, 1979.
159. Adams DO: Effector mechanisms of cytolytically activated macrophages: I. Secretion of natural proteases and effect of protease inhibitors. J Immunol 124:286–292, 1980.
160. Adams DO, Kao KJ, Farb R, Pizzo SV: Effector mechanisms of cytolytically activated macrophages: II. Secretion of a cytolytic factor by activated macrophages and its relationship to secreted neutral proteases. J Immunol 124:293–300, 1980.
161. Kay MMB: Mechanism of removal of senescent cells by human macrophages *in situ*. Proc Natl Acad Sci USA 72:3521–3525, 1975.
162. MacDonald RA, MacSween RNM, Pechet GS: Iron metabolism by reticuloendothelial cells *in vitro*: physical and chemical conditions, lipotrope deficiency, and acute inflammation. Lab Invest 21:236–241, 1969.
163. Day AJ: The macrophage system, lipid metabolism and atherosclerosis. J Atherosclerosis Res 4:117–131, 1964.
164. Nathan CF, Murray HW, Cohn LA: Current concepts: The macrophage as an effector cell. N Engl J Med 303:622–626, 1980.
165. Cohn ZA: The structure and function of monocytes and macrophages. Adv Immunol 9:163–189, 1968.
166. Van Furth R: Mononuclear phagocytes in immunity, infection and pathology, Oxford, Blackwell, 1975.
167. Nelson DS: Immunobiology of the macrophage. New York: Academic Press, 1976.
168. Fink MA: The macrophage in neoplasia. New York: Academic Press, 1976.
169. James K, McBride B, Stuart A: The macrophage and cancer. Edinburgh: University of Edinburgh Press, 1977.

170. Hibbs JB, Lambert LH, Remington JS: Control of carcinogenesis: A possible role for the activated macrophage. Science 177:998–1000, 1972.
171. Evans R, Alexander P: Mechanisms of extracellular killing of nucleated mammalian cells by macrophages. In: The immunobiology of the macrophage, Nelson DS (ed). New York: Academic Press, 1976, pp 562–576.
172. Lurie H: Resistance to tuberculosis: experimental studies in native and acquired defense mechanisms. Cambridge: Harvard University Press, 1964.
173. Droller MJ, Remmington JS: A role for the macrophage *in vivo* and *in vitro* resistance to murine bladder tumor cell growth. Cancer Res 35:49–53, 1975.
174. Norbury K, Kripke ML: Ultraviolet-induced carcinogenesis in mice treated with silica, trypan blue or pyran copolymer. J Reticuloendothel Soc 26:827–837, 1979.
175. Jones PDE, Castro JE: Immunological mechanisms in metastatic spread and the antimetastatic effects of *C. parvum*. Br J Cancer 35:519–527, 1977.
176. Sadler TE, Jones DDE, Castro JE: The effects of altered phagocytic activity on growth of primary and metastatic tumors. In: The macrophage and cancer, McBride JF, Stuart A (eds). Edinburgh: Econoprint, 1979, pp 115–163.
177. Mantovani A, Giavazzi R, Polentanitti N, Spreafico F, Gavattinni S: Divergent effects of macrophage toxins on growth of primary tumors and lung metastases in mice. Int J Cancer 25:617–624, 1980.
178. Fidler IJ: Inhibition of pulmonary metastasis by intravenous injection of specifically activated macrophages. Cancer Res 34:1074–1078, 1974.
179. Liotta LA, Gattozzi C, Kleinerman J, Saidel G: Reduction of tumor cell entry into vessels by BCG-activated macrophages. Br J Cancer 36:639–641, 1977.
180. Fidler IJ, Fogler WE, Connor J: The rationale for the treatment of established experimental micrometastases with the injection of tumoricidal macrophages. In: Immunobiology and immunotherapy of cancer, Terry WD, Yamamura Y (eds). New York: Elsevier, 1979, pp 361–375.
181. Den Otter W, Dullens Hub FJ, Van Lovern H, Peils E: Anti-tumor effects of macrophages injected into animals: A review. In: The macrophage and cancer, James K, McBride B, Stuart A (eds). Edinburgh: Econoprint, 1977, pp 119–141.
182. Blamey RW, Crosby DL, Baker JM: Reticuloendothelial activity during the growth of rat sarcomas. Cancer Res 29:335–337, 1969.
183. Old LJ, Clarke DA, Benacerraf B, Goldsmith M: The reticuloendothelial system and the neoplastic process. Science 88:164–180, 1960.
184. Rhodes J: Altered expression of human monocyte Fc receptors in malignant disease. Nature 265:253–255, 1977.
185. Rhodes J: Resistance of tumor cells to macrophages. Cancer Immunol Immunother 7:211–215, 1980.
186. Bernstein ID, Zbar B, Rapp HJ: Impaired inflammatory response in tumor-bearing guinea pigs. J Natl Cancer Inst 49:1641–1647, 1972.
187. Eccles SA, Alexander P: Sequestration of macrophages in growing tumors and its effect on the immunological capacity of the host. Br J Cancer 30:42–49, 1974.
188. Meltzer MS, Stevenson MM: Macrophage function in tumor-bearing mice: Tumoricidal and chemotactic response of macrophages activated by infection with Mycobacterium bovis, strain BCG. J Immunol 118:2176–2181, 1977.
189. Meltzer MS, Stevenson MM: Macrophage function in tumor-bearing mice: Dissociation of phagocytic and chemotactic responsiveness. Cell Immunol 35:99–111, 1978.
190. Normann SJ, Sorkin E: Cell-specific defect in monocyte function during tumor growth. J Natl Cancer Inst 57:135–140, 1976.
191. Snyderman R, Pike MC, Blaylock BL, Weinstein P: Effect of neoplasms on inflammation:

Depression of macrophage accumulation after tumor implantation. J Immunol 116:585–589, 1976.
192. Sone S, Fidler IJ: Activation of rat alveolar macrophages to the tumoricidal state in the presence of progressively growing pulmonary metastases. Cancer Res 41:2401–2406, 1981.
193. Mantovani A: Effects on *in vitro* tumor growth of murine macrophages isolated from sarcoma lines differing in immunogenicity and metastasizing capacity. Int J Cancer 22:741–747, 1978.
194. Russell SW, McIntosh AT: Macrophages isolated from regressing Moloney sarcomas are more cytotoxic than those recovered from progressing sarcomas. Nature 268:69–71, 1977.
195. Evans R: Tumor macrophages in host immunity to malignancies. In: The macrophage in neoplasia, Fink MA (ed). New York: Academic Press, 1976, pp 27–42.
196. Eccles SA: Macrophages and cancer. In: Immunological aspects of cancer, Castro JE (ed). Lancaster, England: MTP Press, 1978, pp 123–154.
197. Eccles SA, Alexander P: Macrophage content of tumors in relationship to metastatic spread. Nature 250:667–669, 1974.
198. Talmadge JE, Key M, Fidler IJ: Macrophage content of metastatic and nonmetastatic rodent neoplasms. J Immunol 126:2245–2248, 1981.
199. Evans R, Lawler EM: Macrophage content and immunogenicity of C57BL/6J and BALB/cByJ methylcholanthrene-induced sarcomas. Int J Cancer 26:831–835, 1980.
200. Evans R, Eidler DM: Macrophage accumulation in transplanted tumors is not dependent on host immune responsiveness or presence of tumor-associated rejection antigens. J Reticuloendothel Soc 30:425–437, 1981.
201. Dvorak HR, Dickersin GR, Dvorak AM, Manseau EJ, Pyne K: Human breast carcinoma: Fibrin deposits and desmoplasia. Inflammatory cell type and distribution in microvasculature and infarction. JNCI 67:335–345, 1981.
202. Key M, Talmadge JE, Fidler IJ: Lack of correlation between the progressive growth of spontaneous metastases and their content of infiltrating macrophages. J Reticuloendothel Soc, 32:387–396, 1982.
203. Nash JRG, Price JE, Tarin D: Macrophage content and colony-forming potential in mouse mammary carcinomas. Br J Cancer 43:478–485, 1981.
204. Mantovani A: *In vitro* effects on tumor cells of macrophages isolated from an early-passage chemically-induced murine sarcoma and from its spontaneous metastases. Int J Cancer 27:221–228, 1981.
205. Yoshida T, Cohen S: Lymphokine activity *in vivo* in relation to circulating monocyte levels and delayed skin reactivity. J Immunol 122:1540–1546, 1972.
206. Papermaster BW, Holtermann OA, Rosner D, Klein E, Dao T, Djerassi I: Regressions produced in breast cancer lesions by a lymphokine fraction from a human lymphoid cell line. Res Commun Chem Pathol Pharmacol 8:413–428, 1974.
207. Salvin SB, Younger JS, Nishio J, Neta R: Tumor suppression by a lymphokine released into the circulation of mice with delayed hypersensitivity. J Natl Cancer Inst 55:1233–1236, 1975.
208. Poste G, Kirsh R: Rapid decay of tumoricidal activity and loss of responsiveness to lymphokines in inflammatory macrophage. Cancer Res 39:2582–2590, 1979.
209. Allison AC: Model of action of immunological adjuvants. J Reticuloendothel Soc 26:619–630, 1979.
210. Chedid L, Caulli L, Audibut F: Recent developments concerning muramyl dipeptide, a synthetic immunoregulating molecule. J Reticuloendothel Soc 26:631–641, 1979.
211. Matter A: The effects of muramyl dipeptide (MDP) in cell-mediated immunity: A com-

parison between *in vitro* and *in vivo* systems. Cancer Immunol Immunother 6:201–210, 1979.

212. Parant M, Parant F, Chedid L, Yapo A, Petit JF, Lederer E: Fate of the synthetic immunoadjuvant, muramyl dipeptide (14C-labelled) in the mouse. Int J Immunopharmacol 1:35–41, 1978.
213. Poste G, Kirsh R, Fogler WE, Fidler IJ: Activation of tumoricidal properties in mouse macrophages by lymphokines encapsulated in liposomes. Cancer Res 39:881–891, 1979.
214. Fogler WE, Raz A, Fidler IJ: In situ activation of murine macrophages by liposomes containing lymphokines. Cell Immunol 53:214–219, 1980.
215. Fidler IJ, Raz A, Fogler WE, Kirsh R, Bugelski P, Poste G: Design of liposomes to improve delivery of macrophage-augmenting agents to alveolar macrophages. Cancer Res 40:4460–4466, 1980.
216. Allison AC, Gregoriadis G: Liposomes as immunological adjuvants. Nature 252:252–254, 1979.
217. Fidler IJ: Therapy of spontaneous metastases by intravenous injection of liposomes containing lymphokines. Science 208:1469–1471, 1980.
218. Fidler IJ, Raz A, Fogler WE, Hoyer LC, Poste G: The role of plasma membrane receptors and the kinetics of macrophage activation by lymphokines encapsulated in liposomes. Cancer Res 41:495–504, 1981.
219. Hart IR, Fogler WE, Poste G, Fidler IJ: Toxicity studies of liposome-encapsulated immunomodulators administered intravenously to dogs and mice. Cancer Immunol Immunother 10:157–166, 1981.
220. Fidler IJ, Barnes Z, Fogler WE, Kirsh R, Bugelski P, Poste G: Involvement of macrophages in the eradication of established metastases produced by intravenous injection of liposomes containing macrophage activators. Cancer Res 42:496–501, 1982.
221. Sone S, Poste G, Fidler IJ: Rat alveolar macrophages are susceptible to free and liposome-encapsulated lymphokines. J Immunol 124:2197–2202, 1980.
222. Fidler IJ: The *in situ* induction of tumoricidal activity in alveolar macrophages by liposomes containing muramyl dipeptide is a thymus-independent process. J Immunol 127:1719–1720, 1981.
223. Fidler IJ, Sone S, Fogler WE, Barnes Z: Eradication of spontaneous metastases and activation of alveolar macrophages by intravenous injection of liposomes containing muramyl dipeptide. Proc Natl Acad Sci USA 78:1680–1684, 1981.
224. Poste G, Kirsh R, Fidler IJ: Cell surface receptors for lymphokines. Cell Immunol 44:71–87, 1979.
225. Schabel FM Jr, Griswold DR Jr, Corbett TH, Lloyd HH: Quantitative evaluation of anticancer agent activity in experimental animals. Pharmacol Ther A 1:411–435, 1977.

3. Specific Antitumor Immunity and its Role in Host Resistance to Tumors

ROBERT W. BALDWIN

1. INTRODUCTION

The repertoire of tumors in which there is conclusive evidence for the expression of antigens which can be manipulated to produce tumor rejection responses has grown considerably since the early studies reported some 25 to 30 years ago with 3-methylcholanthrene (MCA)-induced rodent sarcomas [1–3]. It now includes tumors of many types such as carcinomas and sarcomas induced with a broad spectrum of chemical carcinogens [4] many of these belonging to the classes (polycyclic hydrocarbons, nitrosamines, aromatic amines and aminoazo dyes) which are known, or suspected to be, human carcinogens [5]. Rodent tumors induced with DNA- and RNA-containing viruses also express antigens able to induce tumour rejection responses in the host [6, 7].

Faced with this wealth of evidence from rodent tumor studies, that the adaptive immune system can respond to tumors, it is not surprising that many attempts have been made, and continue to be made, to introduce specific immunotherapy for cancer treatment [8]. To date there is little evidence to suggest that these approaches have been successful and it is now evident that clinical immunotherapy trials were introduced far too prematurely. Basically, there are a number of conditions which must be fulfilled before manipulation of host immune responses can be considered as a realistic approach to therapy. Foremost of these, it must be conclusively shown that human tumors are specifically recognised by the host's immune system and procedures for enhancing immune responses are effective. The influence of immunostimulation, either specifically or non-specifically in a patient who already may have responded immunologically to a developing tumor must also be taken into consideration since these responses may function to 'down-regulate' antitumor immunity. Finally, the pathways whereby antibody and sensitized lymphocytes are generated following recognition of

R.B. Herberman (ed), Basic and Clinical Tumor Immunology. ISBN-13: 978-1-4613-3875-8
© 1983. Martinus Nijhoff Publishers, Boston.

tumor-associated antigens and the mechanisms involved in eliciting anti-tumor responses needs to be defined.

2. SPECIFIC IMMUNE RECOGNITION OF MALIGNANT CELLS

For a tumor to generate specifically sensitized lymphocytes and/or initiate antibody production, it must express antigens which are immunogenic in the host. This requirement is met by many experimental animal tumors induced by chemical carcinogens or oncogenic viruses. Tumors induced by DNA- or RNA-containing viruses may express an array of plasma membrane associated antigens [6, 7]. This is reflected in the specificity of immunity elicited to these tumors which is 'virus-related' although not simply due to viral antigens. These characteristics basically differentiate virus-induced tumors from carcinogen-induced tumors where tumor-associated antigens exhibit a high degree of polymorphism so that tumors appear to express individually-distinct antigens [4]. There is still, however, little known about the nature of the antigens expressed upon carcinogen-induced tumors. It has been argued that one set of tumor specific antigens on MCA-induced murine sarcomas is retrovirus-derived, being carried on the envelope protein of gp70. According to this hypothesis the tumor antigens probably arise by the recombination events that yield diverse gp70s of the MCF viruses [9]. This intriguing hypothesis remains to be evaluated with other tumors and it is by no means the only mechanism proposed to account for neoantigen expression in carcinogen transformed cells, one view being that they represent mutation products [4, 10].

In contradistinction to the findings with many rodent tumors induced with chemical carcinogens or oncogenic viruses, naturally arising (spontaneous) tumors are less frequently able to elicit tumor rejection reactions [11, 12]. These findings have been used to argue that many, if not all, of the experimental animal tumors are irrelevant in relation to human cancer [11]. It is not true, however, that *all* tumors lack immunogenicity as defined by their capacity to induce resistance in normal immunocompetent hosts against a challenge with viable tumor cells at doses sufficient to produce progressively growing tumor in controls. It was established more than 12 years ago in experiments showing that a number of naturally arising rat mammary carcinomas were immunogenic [13]. Since then the total number of 'spontaneous' tumors studied has increased to 44 and of these 7 had demonstrable immunogenicity [12]. Clearly, however, the majority of the tumors were not immunogenic and this finding must be borne in mind in considering specific immunotherapy of human cancer. In this context it must also be recognized that all carcinogen-induced tumors are not immu-

nogenic. For example, about 70% of sarcomas induced in WAB/Not rats with doses of MCA ranging from 1 to 10 mg and almost all the aminoazo dye induced hepatocellular carcinomas have proved to be immunogenic [14]. In contrast, the frequency of immunogenic hepatocellular carcinomas, ear duct carcinomas and mammary carcinomas induced with N-2-fluorenylacetamide FAA in WAB/Not rats was quite low [15]. For example only 2 of 11 FAA-induced mammary carcinomas were able to induce tumor immunity [16].

These findings indicate that the distinction drawn between non-immunogenic 'spontaneous' tumors and the immunogenicity of tumors induced by carcinogens or oncogenic viruses, is too extreme. Basically animal studies show that some tumors do express tumor rejection antigens and other do not. This may be because the antigens were never expressed or they may have been selected against during tumor progression leading to 'deletion' or 'masking'.

Some of the non-immunogenic murine tumors used in arguments on the relevance of animal tumors to human cancer [11] have been shown to yield stable tumor cell variants expressing tumor rejection antigens following exposure to potent mutagens [17]. For example, applying this technique to a 'non-immunogenic' murine thymic leukaemia yielded variants which conferred protection against the original tumor. These variants arose with a frequency far greater than that expected from mutational events so that the mutagen treatment may reverse a process of tumor antigen modulation [18]. This is but one example of a condition whereby tumor antigens may become 'silent' through immunomodulation [19].

However, one may conclude from the many studies with rodent tumors that the frequency of expression of antigens which can be manipulated to induce immune rejection responses is quite variable. Quite clearly tumors induced by extrinsic agents are more frequently more immunogenic than those arising naturally. This implies that tests for measuring this characteristic are necessary before embarking upon specific immunotherapy. With animal tumors this can be established to some extent by studying the rejection of tumor transplanted into syngeneic recipients. But there is as yet no satisfactory *in vitro* correlate which can be used for defining the immunogenicity and immunosensitivity of tumors.

3. GENERATION OF ANTI-TUMOR IMMUNITY

Augmentation of immune responses to tumor-associated antigens is potentially one of the most effective ways for manipulating the tumor-host relationship in therapy. But, this approach must take into consideration the

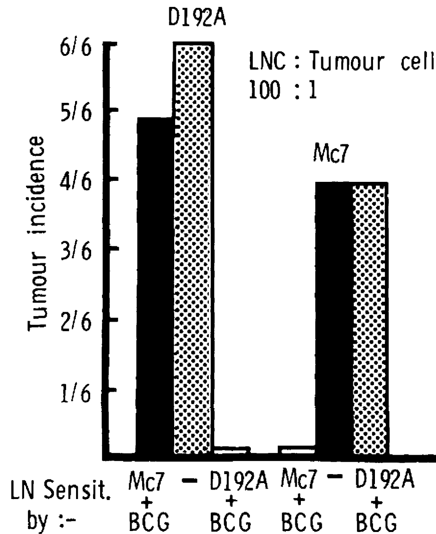

Figure 1. Inhibition of growth of rat hepatoma D192A and sarcoma Mc7 by lumbar lymph node cells from WAB/Not rats immunized by intramuscular inoculation of tumor cells admixed with BCG (Glaxo percutaneous vaccine). Lymph node cells (LNC) from tumor immune donors mixed with tumor cells at a ratio of 100:1 were inoculated intramuscularly into normal recipients and tumor growth assayed in comparison with rats receiving tumor cells alone. Growth of hepatoma D192A was suppressed by LNC from rats immune to this tumor but not sarcoma Mc7-immune rats. Conversely LNC from sarcoma Mc7-immune donors suppressed sarcoma Mc7 but not hepatoma D192A.

complex tumor-host interactions elicited by a developing tumor which broadly tend to down-regulate anti-tumor responses [20–24]. Added to this, therapy by surgery, radiotherapy and chemotherapy all induce some degree of immunosuppression. Furthermore, there is now considerable evidence to support the view that tumors may contain heterogeneous populations of tumor cells even expressing distinct tumor antigens [25–29] and this could prove an obstacle in developing immunotherapy for the treatment of recurrent or metastatic disease.

3.1 *Effector cells involved in tumor recognition*

A role for specifically sensitized T lymphocytes in tumor rejection was originally deduced from studies with many rodent tumors showing that immunity could not be induced to transplanted tumors following immunization of recipients deprived of T cells by thymectomy, whole body irradiation and reconstitution with T cell-depleted bone marrow cells. Also, immunization of congenitally athymic mice does not lead to the development of a tumor-specific immune response [29].

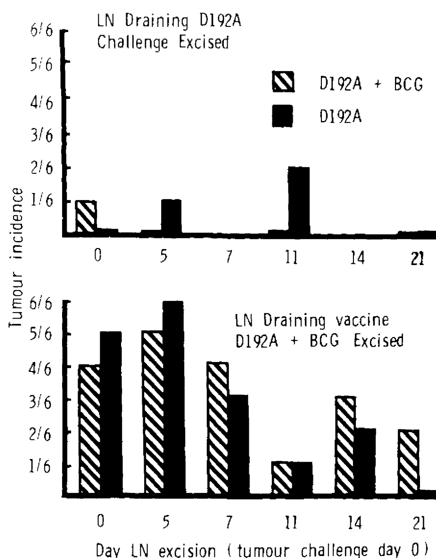

Figure 2. (bottom) Influence of lymphadenectomy on the inhibition of intramuscular growth of hepatoma D192A following stimulation of a tumor-specific immune response by a contralateral intramuscular inoculation of a vaccine containing viable hepatoma D192A cells together with BCG. Excision of the lymph node draining the vaccine (D192A cells + BCG) within 7 days abrogated the development of tumor immunity and the contralateral challenge with hepatoma D192A developed in almost all rats. Furthermore the hepatoma D192A cells admixed with BCG for immunostimulation also produced whereas tumor cells in this type of vaccine are normally suppressed (see below). (Top) Excision of the lymph node draining the site of hepatoma D192A challenge had no significant effect upon the development of tumor immunity to hepatoma D192A. In these experiments the challenge inoculum was rejected in most rats. Moreover, the mixed tumor cell-BCG vaccine only produced tumors in one rat whereas (as shown in the top figure) this vaccine produced actively growing tumors when the vaccine draining lymph node was excised.

Adoptive transfer experiments have also been used to demonstrate the requirement of specifically sensitized lymphocytes in tumor rejection. This is illustrated by the experiments summarized in Figure 1 showing that lumbar lymph node cells draining an intramuscular challenge of viable hepatoma D192A cells together with BCG suppressed growth of this tumor when transferred to normal immunocompetent WAB/Not rats in admixture with tumor cells. Hepatoma D192A-stimulated effector cells were ineffective against an immunologically unrelated tumor (sarcoma Mc7) and conversely lymph node cells sensitized to sarcoma Mc7 suppressed growth of this tumor, but not hepatoma D192A [30]. In agreement with these experiments, immunization of normal WAB/Not rats with hepatoma D192A-BCG vaccines generates a systemic immunity so that a contralateral challenge with this tumor, but not others, is rejected. One interpretation of these experiments is that specifically sensitized lymphocytes generated by immu-

nization with tumor-cell-containing vaccines function systemically perhaps with the capacity to localize in tumor deposits. In support of this, excision of the lymph node draining the tumor cell-BCG vaccine inoculation site abrogated the tumor immune response so that a contralateral challenge with hepatoma D192A cells produced tumours (Figure 2). In comparison, excision of the lymph node draining the tumor challenge site had no effect upon the capacity of tumor cell-BCG vaccines at a contralateral site to elicit tumor immunity [30]. Inoculation of tumor cell vaccines, especially when incorporated with BCG, also results in the generation of activated macrophages and augmentation of natural killer cells. So the responses obtained following immunization with hepatoma D192A-BCG vaccines could conceivably be due to the differential sensitivity of the rat tumors to NK cells or macrophages. This is not likely, however, since sarcoma Mc7 cells are more susceptible to NK cells than hepatoma D192A [31].

3.2 *Function of effector cells*

Numerous studies have established that cytotoxic T lymphocytes are generated following immunological recognition of tumors and it has been concluded that these effector cells mediated tumor rejection [32–34]. It is now clear, however, that distinct subsets of T cells are involved in tumor cell recognition and in the mouse these can be phenotypically classified by their expression of Lyt differentiation antigens [35, 36]. Analysis of T cell subsets using these markers indicates in several systems that the Lyt 1^-2^+ cytolytic T cell is not primarily involved in anti-tumor responses [37–39]. Instead, the functionally active cell in the Lyt $1^+2's-$ T cell (delayed type hypersensitivity effector cells, TDTH). This is illustrated by studies showing that Lyt $1^+2,3^-$ T cells derived from mice immune to sarcoma S1509a suppressed growth of this tumor when injected in admixture with tumor cells (Winn assay) into normal mice. In comparison Lyt $1^-,2,3,^+$ T cells were ineffective in Winn assays [39]. It was also concluded that the cytotoxic T cell was not the main effector cell mediating anti-tumor responses to Moloney virus-induced sarcomas in the rat [40, 41]. Expansion of spleen cells from rats in which the tumor had regressed by culturing together with Mitomycin C-inactivated-tumor cells generated a population which when injected intravenously into tumor-bearing rats produced tumor rejection. Fractionation of stimulated spleen cells in a fluorescence activated cell sorter following reaction with the anti-rat T cell monoclonal antibody W3/25 yielded one population (W3/25 positive) which was poorly cytotoxic *in vitro* for tumor cells, but induced tumor rejection when infused intravenously into tumor-bearing rats. Conversely, the W3/25 negative subset of splenic lymphocytes was cytotoxic *in vitrc* but produced slight enhancement rather than suppression of tumor growth [40].

In addition to these two examples, there are several other reports showing that TDTH cells rather than cytotoxic cells are the effector cell predominantly involved in the rejection of syngeneic tumor transplants. It is also pertinent to note that murine T cells mediating skin graft rejection in the mouse have been reported to be Lyt 1^+ with the Lyt-2.3^+ and Lyt-$1,2,3^+$ cells being ineffective [42, 43]. Also rejection of skin allografts in T cell deficient rats could be induced by injection of thoracic duct lymphocytes and this response was abolished by removal of the subset labelled by W3/25 monoclonal antibody [44]. These findings again support the view that the ability to reject skin allografts in T cell deprived rats can be restored by injection of TDTH cells and that cytotoxic T cells have no discernible effect.

These studies on rejection of syngeneic tumor transplants and the related finding on skin and tumor allograft rejection strongly suggest that the cytotoxic T cell identified by its effect *in vitro* on target tumor cells is not the main effector cell *in vivo*. Rather the T cell which generates an anti-tumor response has characteristics of a TDTH cell.

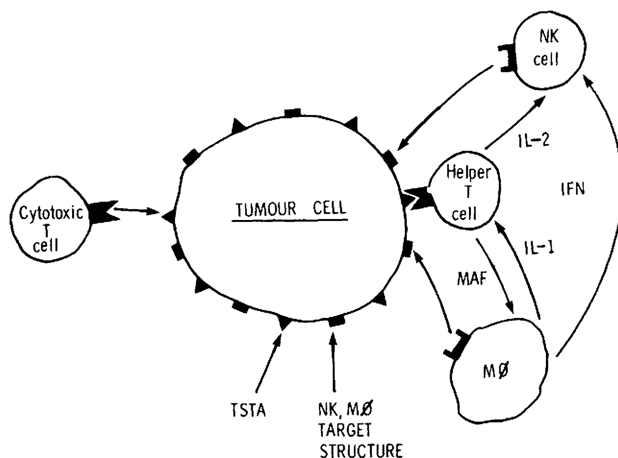

Figure 3. Diagrammatic representation of the function of sensitized T cells in mediating anti-tumor responses. Cytotoxic T cells may be generated which directly exert an anti-tumor response. Another T cell subset (TDTH) may release soluble factors (interleukins, interferon) following target cell recognition. These factors may act upon tumor infiltrating macrophages leading to activation to a state where they produce a direct (or indirect) effect upon tumor cells. TDTH-initiated factors may also augment tumor cytotoxicity of tumor infiltrating lymphocytes, eg. via interferon production. These populations will include NK cells and also 'activated' T cells [48]. Activated lymphocytes may attack tumor cells indiscriminately or following recognition of target cell structures (NK/MQ receptors?). The other function of factors released following TDTH interaction with tumor antigens is to increase the infiltration of host cells (NK cells, macrophages, most cells) so enhancing the potential of in situ anti-tumor responses.

Table 1. The phenotype of rat lymphocytes producing T cell growth factor following mixed lymphocyte reaction (MLR).

Cell population used to generate MLR supernatants [a]	Proliferation of T cell blasts in the presence of supernatants (cpm ^3H thymidine incorporation) [b]		
	Exp 1	Exp 2	Exp 3
–	606±39	739±62	629±29
Unfractioned spleen	3666±54	5292±162	2621±19
W3/25 – spleen	740±30	672±101	470±13
W3/25 + spleen	6537±26	9412±83	4135±20
–	1120±30	1035±22	893±22
Unfractionated spleen	4958±60	4249±34	2337±89
OX8 – spleen	6621±82	6496±69	4715±71
OX8 + spleen	987±39	1014±22	911±16
–	544±67	621±22	NT
Unfractioned spleen	2392±52	4453±54	NT
W3/13 – spleen	627±87	718±39	NT
W3/13 + spleen	2712±77	4767±79	NT

[a] Irradiated WAB/KX F_1 lymph node cells were used as stimulators. Spleen cells separated into $W3/25^+$ and $W3/25^-$ antibody binding populations in the fluorescence activated cell sorter [45].

[b] The proliferation of T cell blasts was assessed over a 3 day culture period. Proliferative responses from day 2 (maximal activity) are presented.

The exact function of TDTH cells in mediating tumor rejection is not clear but it is likely that they promote activation and/or infiltration into tumors of non-specific effector cells including NK cells and macrophages [23]. This could result from the release of soluble factors such as lymphokines and interferon following tumor cell – TDTH interactions (Figure 3). As shown in Table 1, rat spleen cells cultured with semi-allogeneic lymphocytes generate growth promoting factors (TCGF) for T-cell blasts. The phenotype of the TVGF-producing cells assessed using antibody labelled cell populations separated in the fluorescence activated cell sorter [45] indicates that they are W3/25 and W3/13 positive. In additional experiments, the blocking effect of these antibodies on the production of TCGF in the mixed lymphocyte reaction was examined. These showed that addition of W3/25 antibody at the beginning, but not the end of an MLR, results in the loss of TCGF activity in rat MLR-conditioned medium. Addition of W3/13 and 0X8 antibodies had no effect. Taken together these experiments indicate that the T cell lymphokine is a product of $W3/25^+$ T cells.

The production of immune interferon (γ-IFN) following antigen stimulation of sensitized T cell is well established but the role of T cell subsets has not been fully elucidated. In murine systems γ-IFN appears to be a product of the Lyt $2,3^+$ T cell subset possibly requiring the cooperation of B cells or macrophages [46]. In comparison, human γ-IFN produced following lymphocyte stimulation with PHA involved subsets identified as $T\gamma$, T non μ, OKM 1^+ $OKT4^-$, $OXT8^-$ and $OKT11a^+$ [47].

If, as argued above, a non-cytotoxic T cell plays a critical role in tumor rejection through the generation of soluble factors, this implies that the final cytotoxic response is produced by non-specific effector cells including activated macrophages and NK cells (Figure 3). One must also include the effect of soluble factors leading to the generation of activated T cells which unlike those generated by antigen recognition may exhibit an indiscriminate lytic effect. This concept recently proposed by Klein and co-workers [48] is based upon the view that T cells in certain differentiation states are able to lyse target cells on the basis of two types of interactions. One is due to epitope recognition and requires clonal amplification of specific lymphocyte populations. The other involves T cell differentiation so that these activated cells may not express antigen receptors. Expansion of these populations is considered to be polyclonal and they kill target cells indiscriminately.

4. MODULATION OF ANTI-TUMOR IMMUNITY

Many immunogenic tumors grow progressively when transplanted into normal immunocompetent recipients, even when the dose of tumor cells is only just sufficient to produce consistent tumor growth. In comparison, preimmunization, for example by injection of radiation-attenuated tumor cells, confers solid immunity to a subsequent challenge with up to 10–100 times the tumor cell dose producing growth in untreated recipients. This is but one of the many observations which suggests that a developing tumour may 'escape' from host controls in a positive manner rather than by simply failing to develop a sufficiently effective tumor immunity and a number of pathways for this have been proposed [20–24]. 'Blocking factors' such as circulating immune complexes of tumor antigen with specific antibody and even tumor antigen have been postulated to be involved, since these serum factors modified lymphocyte cytotoxicity for tumor cells. But, probably a more important pathway is the generation of suppressor cells, especially suppressor T lymphocytes (Ts) which inhibit the generation of effective tumor immunity [21, 22, 39]. An extreme example of the powerful influence of Ts cells is illustrated by studies on UV-induced murine tumors which are highly immunogenic, but are prevented from being rejected by impairment

of host responses through Ts cell induction [49]. A further example of the powerful influence of Ts cells in 'down-regulating' tumor immunity is provided by studies on the adoptive transfer of resistance to the highly immunogenic murine fibrosarcoma Meth A [50]. Tumors growing in immunodeprived mice (thymectomy, lethal irradiation and bone marrow reconstitution, TXB) were rejected following intravenous infusion of spleen cells from tumor immune donors. However, prior infusion of spleen cells from mice bearing established (12 to 14 day old) Meth A tumors as a source of Ts cells inhibited the regression of tumor by immune spleen cells [50]. Adoptive transfer of splenocytes from mice bearing another tumor, the P815 mastocytoma also abrogated the tumor suppressive effect observed following infusion of immune spleen cells into TBX-mice bearing this tumor. The suppressor cells were identified as T cells by their susceptibility to treatment with anti Thy 1-2 antibody and complement [51] but identification of the T cell subset has not yet been reported and the investigation of the specificity of the response is still incomplete. Analysis of the temporal changes following adoptive transfer of tumor-immune splenocytes and the subsequent treatment with Ts cells indicated, however, that it is likely that the suppressor cells inhibit the generation of a secondary response. This is illustrated in experiments showing that infusion of immune cells resulted in the production in the node draining the test tumor of T cells cytotoxic for P815 target cells; peak levels occurring coincidently with the onset of tumor regression. Also production of cytotoxic T cells was greatly reduced in recipients receiving immune spleen cells and suppressor spleen cells. From the previous discussion it may be that the cytotoxic T cells are not the effector cells which produce tumor rejection, but they may by an indication of the specific T cell response generated to the tumour. For example, in tests with murine sarcoma S1509a the Lyt $1^{+}2^{-}$ cells, which are predominantly involved in tumor rejection rather than the cytotoxic Lyt $1^{-}2^{+}$ cell, were shown to proliferate *in vitro* in response to tumor antigen. The proliferative response of this Lyt $1^{+}2^{-}$ subset could be specifically blocked by administration of Ts cells prior to assay [39].

There is as yet only limited understanding of the pathways involved in tumor antigen recognition, especially with respect to the generation of sensitized T lymphocyte populations, although antigen presentation seems crucial, with cell membrane bound or soluble antigen favouring Ts production [39]. For example, in numerous studies with carcinogen-induced rat hepatomas and sarcomas, tumor-specific immunity was induced following immunization with attenuated tumor cells [2, 4]. In contrast immunization with tumor cell membranes or tumor antigen preparations isolated by a variety of procedures including 3MKCl or papain extraction were completely ineffective in generating anti-tumor immunity [52]. This may be

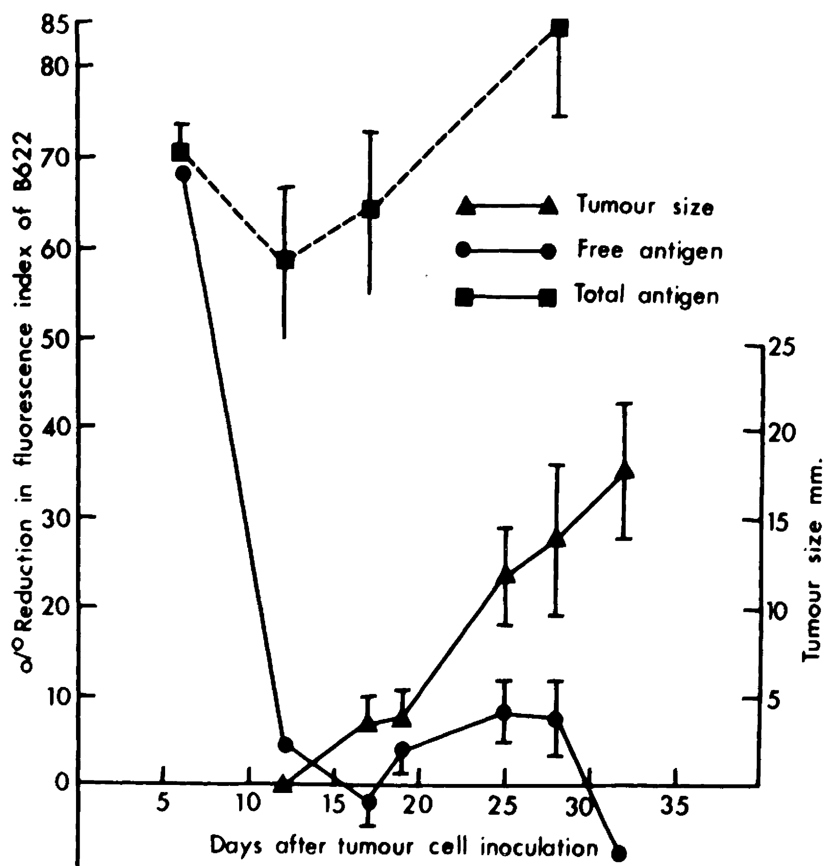

Figure 4. Serum levels of tumor antigen and specific immune complexes during intramuscular growth of hepatoma D23 from an inoculum of 10^2 tumor cells. Free hepatoma D23 antigen was determined by the capacity of serum to neutralize tumor-specific antibody in syngeneic immune serum (B622) as measured by reduction in membrane immunofluorescence staining of hepatoma D23 cells. Total D23 antigen was measured in serum following low pH dissociation of immune complexes and chromatographic isolation of antigen-containing products.

attributed to inappropriate processing of antigens since pre-treatment of rats with cyclophosphamide (40 mg/kg) to eliminate suppressor cell precursors allowed tumor immunity to be detected following immunization with 3MKC1 extracts of rat hepatoma [30]. Related to these findings, tumor antigen shedding occurs very early during growth of transplanted rat hepatomas and sarcomas [53]. For example, sequential analysis of serum samples from rats implanted intramuscularly with 10^2 hepatoma D23 cells revealed that circulating tumor antigen could be detected within 5 days of tumor challenge, this being well before tumor was detectable (Figure 4). Initially tumor antigen was present in free form but then this was rapidly lost to be replaced by immune-complexed products [54]. This sequence of

events obtained with hepatoma D23 and sarcoma Mc7 implanted into different anatomical sites suggests that tumour antigen shedding occurs as a very early consequence of tumor growth and before the host elicits a tumor immune response and these are conditions which favour the induction of Ts cells [53, 54].

5. ACTIVE SPECIFIC IMMUNOTHERAPY

Specific immunostimulation in which animals implanted with tumor cells receive a *simultaneous* treatment at a separate site with tumor cell vaccines leads with many rodent tumors to complete rejection or partial control of tumor growth. In general, the most effective vaccine contains intact tumor cells and the response is enhanced by including immunomodulating agents such as BCG or C. parvum in the vaccines [55]. There are only a few systems, however, in which therapy is successful if this type of treatment is delayed until several days after tumor challenge [55]. To take an example, injection of hepatoma D192A cells admixed with BCG elicits a specific antitumor immune response which is sufficiently powerful to induce rejection of a simultaneous contralateral intramuscular challenge of the same tumor (Figure 5). Tumor growth was also suppressed when treatment was delayed until 5 days after tumor challenge, but thereafter the therapeutic response was reduced so that tumors developed in 4/6 rats when immunotherapy was given on day 8 [56]. Experiments with another immunogenic rat tumor,

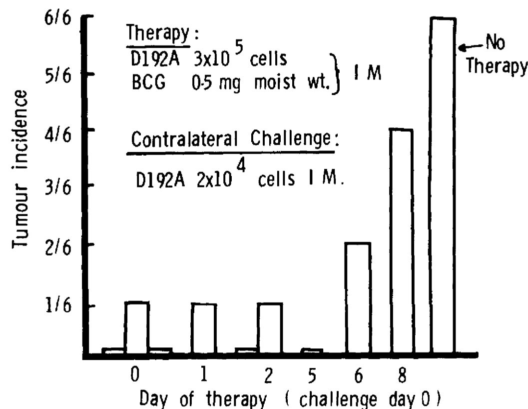

Figure 5. Active specific immunotherapy of intramuscular (IM) implants of rat hepatoma D192A. Hepatoma D192A cells injected IM were treated up to 8 days later by a contralateral IM inoculation of hepatoma D192A cells admixed with BCG. This immunostimulating inoculum does not produce a progressive tumor and the systemic host response inhibits the contralateral tumor.

sarcoma Mc7 also showed that early treatment with tumor cell-BCG vaccines was effective but this did not influence tumor growth after 6 days [57].

These experiments emphasize that additional manipulations are necessary when attempts are made to immunostimulate tumor-bearing hosts since, as already discussed, they may be in a state of T cell mediated immunosuppression directed towards 'down-regulating' concomitant tumor immunity [22]. One approach has been to eliminate the immunosuppressive component in the tumor-bearing host and in early studies this was attempted using procedures to remove 'blocking factors' from serum [58]. Although this approach still has some support from those who advocate plasmaphoresis in cancer therapy there is little evidence to show that this type of manipulation can significantly alter the immunological status of tumor-bearing animals [59]. In this respect, however, most of the experimental studies have been attempted with tumor-bearing animals. It cannot be excluded, therefore, that procedures designed to remove circulating immunosuppressive substances either alone of in combination with secondary immunotherapy may not influence the growth of recurrent tumors and/or metastases.

Another approach involves the administration of agents which will eliminate Ts lymphocytes and/or their precursors. As already described cyclophosphamide (Cy) treated rats are able to elicit a tumor-specific rejection response to soluble hepatoma-antigen preparations, this response not being obtained in normal immunocompetent rats [30]. This effect of Cy treatment is further illustrated in experiments on the adoptive transfer of tumor immunity to the murine Meth A sarcoma [60]. Infusion of tumor-immune spleen cells into normal mice did not elicit rejection of tumor implanted 4 days earlier, but treatment was effective when spleen cells were administered one hour after Cy treatment (100 mg/kg) of the recipients. Further analysis of this system revealed that the tumor regression produced by combination therapy with Cy and immune spleen cells could be abrogated by intravenous infusion of tumor-bearer spleen cells as a source of suppressors. The suppressor cell population was defined as Ts cells since they were susceptible to anti-thy 1-2 antibody and complement. Moreover, this suppressor cell population could be completely eliminated from tumor-bearer spleen cells by treatment of the donor with Cy 24 hours before harvesting spleen cells [60].

In extending these studies, it was found that the P815 mastocytoma in DBA/2 mice and SA1 sarcoma in A/J mice were also susceptible to combination therapy with Cy and immune cells although the sensitivity of these tumors to Cy alone complicates the test. Cy treatment alone caused a temporary arrest of tumor growth and then they grew rapidly. Combination therapy ensured complete suppression of the tumors.

There are many other examples where cyclophosphamide has enhanced tumor immunotherapy. Transfer of splenic Lyt 1^+2^- lymphocytes from immune donors in combination with Cy 5 days after inoculation of Friend virus-induced leukaemic cells significantly increased survival in comparison with Cy alone [38]. Conversely a role for anti-tumor immunity has been reported in experiments showing that Cy cured mice bearing large (12 to 16 day old), but not small (4 day old) growths of MOPC-315 [61, 62]. Cyclophosphamide treatment has also been used to enhance the effect of specific immunostimulation with 1-butanol-extracted tumor antigen preparations in mice with subcutaneously growing implants of an MCA-induced murine sarcoma MCA-F [63]. This tumor was susceptible to Cy therapy (25 mg/kg intraperitoneally twice weekly) but whilst survival was increased from 15% in untreated mice to 63% in Cy-treated mice, there were no tumor regressions. In comparison, combination treatment of Cy with tumor antigen vaccination led to complete tumor regression in 20% of the tested mice.

Another approach to eliminating Ts cells involves their specific immunological depletion. Suppressor cells in the mouse express surface structures encoded in the I-J subregion of the H-2 complex and this has led to the use of antisera against these determinants for controlling immune suppression [64]. For example, administration of low doses of anti I-J antisera, prepared by immunizing mice of a given strain with lymphoid cells differing only at the I-J region, inhibited growth of the murine sarcoma S1509a [65]. Serum reactivity could not be removed by absorption of sera with tumor cells suggesting that the effect observed in tumor-bearing mice was mediated by abrogation of the Ts effector cell response.

This type of serotherapy has been effective against other tumor systems [66]. In particular anti I-J serotherapy markedly inhibited the growth of UV-induced tumors in UV-irradiated mice which, as already described, are extremely effective in developing Ts cells causing enhancement of tumor growth [67].

The recent development of I-J specific monoclonal antibodies [68–70] provides yet another approach for controlling Ts effects in tumor-bearing hosts. Studies reported by Drebin et al. [39] show, for example, that anti-$I\text{-}J^k$ antibodies significantly inhibit growth of sarcoma S1509a in AJ ($H\text{-}2^a$, $I\text{-}J^k$) mice whereas anti-$I\text{-}J^b$ antibodies had no effect. But in [A.BY-γAJ]F_1 ($H\text{-}2^{a/b}$, $I\text{-}J^{k/b}$) mice, both anti $I\text{-}J^k$ and anti-$I\text{-}J^b$ monoclonal antibodies inhibited tumor S1509a growth.

6. IMMUNOTHERAPY OF METASTATIC TUMORS

6.1 *Specific immunotherapy*

The limited effectiveness of specific immunotherapy against even small

ANTIGENICITY CROSS TESTS WITH PRIMARY AND METASTATIC SUBLINES FROM RAT SARCOMA Mc97

IMMUNISING TUMOUR	SUBLINE SOURCE	IMMUNITY TO CHALLENGE WITH SUBLINE:- Mc97A	Mc97B	Mc97G	Mc97R	Mc97P
Mc97A	PRIMARY	+	+	+	-	N.T.
Mc97B	PRIMARY	N.T.	+	+	N.T.	N.T.
Mc97G	PERITONEAL SECONDARY	+	N.T.	+	N.T.	-
Mc97R	RENAL SECONDARY	-	-	N.T.	+	+
Mc97P	PULMONARY SECONDARY	-	-	-	+	+

+ RESISTANT TO CHALLENGE; - NO RESISTANCE; N.T. NOT TESTED

Figure 6. Tumor rejection antigens expressed on rat sarcoma Mc97 and its metastases. Tumor cell lines were established from the primary MCA-induced sarcoma (Mc97A and Mc97B) and from metastases developing in the peritoneum (Mc97a), kidney (Mc97R) and lung (Mc97p). WAB/Not rats were immunized against each tumor and the spectrum of immunity to the immunizing tumor and various sublines established [26].

localized tumors is now well appreciated, but this type of therapy may be effective against tumor metastases. Visceral tumor deposits produced following intravenous injection of the guinea pig line 10 hepatoma were susceptible to treatment following multiple inoculations of irradiated tumor cells mixed with heat killed BCG or *M. Phlei* [71, 72]. Also, tumors developing at pulmonary and extrapulmonary sites following intravenous injection of an MCA-induced murine sarcoma were suppressed following immunization with tumor antigen-containing extracts prepared in 1-butanol [63]. These more recent studies further extend early reports showing that pulmonary tumor growth can be controlled by active immunization with tumor antigen-containing vaccines [73–75]. But the experimental approach used in this type of investigation does not take into account the antigenic heterogeneity of primary tumors and more particularly the finding that metastases may express antigens which differ from those of the primary. To investigate this, sarcomas were induced in WAB/Not rats by subcutaneous injection of MCA and primary tumors as well as metastases taken from the peritoneum, kidney and lung established as tumor lines by transplantation into syngeneic recipients. Rats were then immunized against each of the separate sublines and their resistance to challenge with the immunizing tumor and other sublines determined [25]. The pattern of antigenic cross-reactivities with one sarcoma Mc97 and its sublines is illustrated in Figure 6. Tumor lines established from opposite poles of the primary showed cross-reactivity and the antigen expressed on these tumors was also detected on the subline established from the peritoneal metastasis, but not the renal

metastasis. Tumors derived from the renal metastasis and the pulmonary metastasis expressed cross-reacting tumor rejection antigens, but these were not detected in tumor lines derived from the primary tumor. The heterogeneity of tumor cell populations with regard to a number of characteristics including neoantigen expression and immunosensitivity has been reported by other investigators particularly with respect to determining whether metastasis development represents a random or selective event [4, 27, 28]. Although much of this research is still at an early stage of development it may be concluded that the immunogenicity of metastases is quite variable and many metastases may develop from cell populations which probably only form a minor component of the original tumor.

It has also been shown that the specificity of tumor rejection antigens on recurrent tumors may differ from those on primary tumors [25]. This was established again using MCA-induced sarcomas in experiments showing that tumor rejection antigens expressed on tumor lines established from the primary tumor were different from those on lines derived from rapidly developing recurrent tumors. Again therefore it is highly unlikely that growth of recurrent tumors will be suppressed by enhancing the immune response of the host to primary tumors. This was then established in studies with a limited number of tumors where treatment of rats with vaccines containing cells derived from primary MCA-induced sarcomas and BCG did not influence the rapid re-growth of tumors at the tumor excision site [77]. It should be emphasized that there are other contributory factors which must be considered in relation to these studies, including the influence of trauma associated with the surgical resection of primary sarcomas. Also, unlike tests with transplanted tumors, it is not possible to control the amount of tumor being treated. Nevertheless these tests with primary tumors are more relevant than experiments with transplanted tumors and further research on these systems is necessary in relation to the development of immunotherapy of human cancer.

6.2 *Regional immunotherapy*

Several approaches are being developed for generating specific and/or non-specific anti-tumor responses in tumor deposits for treating metastases. These include targeting of immunomodulating agents to tumors. This is exemplified by the intravenous injection of liposomes for targeting muramyl dipeptide (MDP) or lymphokines in the treatment of pulmonary tumor deposits [78-80]. Liposomes are taken up by alveolar macrophages and this leads to their activation so producing an anti-tumor response. It is also possible to mimic the intratumor responses generated by T cell recognition of tumor associated antigens. This is illustrated by experiments showing that growth of naturally-arising rat mammary carcinomas Sp4 and Sp15 is

suppressed when tumor cells are injected together with the purified protein derivative of tuberculin (PPD) in BCG-sensitized WAB/Not rats [81]. Tumor rejection was initiated by the local DTH response elicited and BCG sensitization itself did not modify tumor growth. Furthermore growth of mammary carcinoma Sp4 was suppressed when tumor cells together with PPD were injected into WAB/Not rats which had received lymph node cells from BCG-sensitized donors.

From the experience gained in the use of liposomes for targeting chemotherapeutic agents, it is evident that this approach will have limited application. In one study, for example, liposomes injected intravenously were found to be extensively (80–90%) taken up in the reticuloendothelial cells in the liver and spleen and by circulating monocytes [80]. It is evident, therefore, that other methods must be developed for targeting immunomodulating agents and monoclonal antibodies reacting with tumor associated antigens may be used for this purpose [82]. This approach is exemplified by studies showing that a murine monoclonal antibody produced against human osteogenic sarcoma cells specifically localizes in osteogenic sarcoma xenografts maintained in immunodeprived mice [83]. This antibody has been coupled to interferon and the conjugates used to activate peripheral blood NK activity against K563 and osteogenic sarcoma target cells [84]. These experiments show that interferon can be directed to tumor cells using antibody conjugates which taken together with the *in vivo* antibody localization studies suggest that this represents a novel approach for targeting immunomodulating agents.

Monoclonal antibodies reacting at least preferentially with many types of human tumors are now available or under development [82, 85]. There is also evidence that these monoclonal antibodies localize in human tumors. For example one antibody (791T/36) has been found to localize in primary and metastatic deposits of human colorectal carcinomas [86]. Other monoclonal antibodies have been shown to localize in colon carcinoma [87] and malignant melanoma [85].

However, most of these monoclonal antibodies are of mouse origin and whilst their administration to patients has not produced adverse responses, they may have limited value for repeated administration. This is emphasized by a phase 1 clinical trial of colorectal cancer patients receiving monoclonal antibody 17-1A where it was found that antibodies against mouse immunoglobulin developed in three of the four patients [88]. Consequently, the development of monoclonal antibodies for targeting immunomodulating agents will depend upon the production of antibodies by human hybridomas.

ACKNOWLEDGEMENTS

These studies were supported by a grant from the Cancer Research Campaign. I thank Dr. J.A. Jones for permission to reproduce her data on the treatment of rat hepatoma D129A.

REFERENCES

1. Foley EJ: Antigenic properties of methylcholanthrene-induced tumors in mice of the strain of origin. Cancer Res 13:835–837, 1953.
2. Baldwin RW: Immunity to methylcholanthrene-induced tumours in inbred rats following atrophy and regression of implanted tumours. Brit J Cancer 9:652–657, 1955.
3. Prehn RT, Main JM: Immunity to methylcholathrene induced sarcomas. J Natl Cancer Inst 18:769–778, 1957.
4. Baldwin RW, Price MR: Neoantigen expression in chemical carcinogenesis. In: Cancer: A comprehensive treatise: 1. Etiology: Chemical and physical carcinogenesis, Becker FF (ed). New York: Plenum Press, 1982, pp 507–548.
5. IACR Working Group: An evaluation of chemicals and industrial processes associated with cancer in humans based on human and animal data. IACR Monogr Vols 1-20. Cancer Res 40:1-12, 1980.
6. Kurth R, Fenyo EM, Klein E, Essex M: Cell surface antigens induced by RNA tumour viruses. Nature 279:197–201, 1979.
7. Ito Y: Immunology of DNA virus-induced tumours. In: Immunology 80, Fougereau M, Dauset J (eds). London: Academic Press, 1980, pp 668–679.
8. Terry WD, Rosenberg SA: Immunotherapy of human cancer. Amsterdam: Elsevier, pp 1–520, 1982.
9. Lennox ES: The antigens of chemically induced tumours. In: Immunology 80, Fougereau M, Dausset J (eds). London: Academic Press, 1980, pp 659–667.
10. Baldwin RW, Embleton MJ, Gunn B, Price MR, Reeve JG: Neoantigens on tumours induced by chemical carcinogens: Regulation and characterisation. In: Genes and tumor genes, Winnacker E, Schoene H-H (eds). New York: Raven Press, 1982, pp 109–114.
11. Hewitt HB: The choice of animal tumors for experimental studies of cancer therapy. Adv Cancer Res 27:149–200, 1979.
12. Baldwin RW, Embleton MJ, Pimm MV: Host response to spontaneous rat tumours. In: Antiviral mechanisms in the control of neoplasia, Chandra P (ed). New York: Plenum Press, 1979, pp 333–353.
13. Baldwin RW, Embleton MJ: Immunology of spontanously acising rat mammary adenocarcinomas. Int J Cancer 4:430–439, 1969.
14. Baldwin RW, Embleton MJ, Pimm MV: Neoantigens in chemical carcinogenesis. In: Carcinogens: Identity and mechanisms of action, Griffin AC, Shaw CR (eds). New York: Raven Press, 1979, pp 365–379.
15. Baldwin RW, Embleton MJ: Tumour-specific antigens in 2-acetylaminofluorene-induced rat hepatomas and related tumors. Israel J Med Sci 7:144–153, 1971.
16. Baldwin RW, Embleton MJ: Immunology of 2-acetylaminofluorene-induced rat mammary adenocarcinomas. Int J Cancer 4:47–53, 1969.
17. Boon T, Von Snick J, Van Pel A, Uyttenhove C, Marchand M: Immunogenic variants obtained by mutagenesis of mouse mastocytoma P815: II. T lymphocyte-mediated cytolysis. J Exp Med 152:1184–1193, 1980.

18. Baldwin RW, Klein G: Escape of tumor cells from immune controls. Cancer Res 42:1608–1609, 1982.
19. Wheelock EF, Weinhold KJ, Levich J: The tumor dormant state. Adv Cancer Res 34:107–140, 1981.
20. Hellström KE, Hellström I, Nelson K: Antigen specific suppressor (blocking) factors in tumor immunity. In: Pathological membranes, Nowotny A (ed). New York: Plenum Press. 1982, In press.
21. Naor D: Suppressor cells: Permitters and promoters of malignancy. Adv Cancer Res 29:45–125, 1979.
22. North RJ, Dye ES, Mills CD, Chandler JP: Modulation of antitumor immunity: Immunobiological approaches. Seminars Immunopathol 512:193–220, 1982.
23. Baldwin RW: Mechanisms of immunity in cancer. Pathobiol Annual Ioachim HL (ed). New York: Raven Press, 1981, pp 155–175.
24. Ozer H: Tumor immunity and escape mechanisms in humans. In: Immunological approaches to cancer therapeutics, Mihich E (ed). New York: Wiley-Interscience, pp 39–73.
25. Pimm MV, Baldwin RW: Antigenic differences between primary methylcholanthrene-induced rat sarcomas and post-surgical recurrences. Int J Cancer 20:37–43, 1977.
26. Pimm MV, Embleton MJ, Baldwin RW: Multiple antigenic specificities within primary 3-methylcholanthrene-induced rat sarcomas and metastases. Int J Cancer 25:621–629, 1980.
27. Schmitt M, Daynes RA: Heterogeneity of tumorigenicity phenotype in murine tumors. Transplant 33:387–392, 1982.
28. Schirrmacher V, Bosslet K: Clonal analysis of expression of tumor-associated transplantation antigens and metastatic capacity. Cancer Immunol Immunother 1311:62–68, 1982.
29. Baldwin RW, Pimm MV: Human tumor xenograft in athymic nude mice: Non-specific host rejection responses. In: Immunodeficient animals for cancer research, S. Sparrow (ed). London: Macmillan, 1980, pp 125–133.
30. Baldwin RW, Byers VS, Hannant D, Jones JA, Pimm MV, Price MR: Cellular interactions modulating host resistance to tumours. Recent Res in Cancer Res 80. Berlin: Springer-Verlag, 1982, pp 338–345.
31. Brooks CG, Wayner EA, Webb PJ, Dixon Gray J, Kenwrick S, Baldwin RW: The specificity of rat natural killer cells and cytotoxic macrophages on solid tumor-derived target cells and selected variants. J Immunol 127:2477–2483, 1981.
32. Berke G: Interaction of cytotoxic T lymphocytes and target cells. Prog Allergy 27. Basel: Karger, 1980, pp 69–133.
33. Plata F: Specificity studies of cytolitic T lymphocytes against murine leukemia virus-induced tumors. J Exp Med 155:1050–1062, 1982.
34. Yamauchi K, Fujito S, Tada T: Differential activation of cytotoxic and suppressor T cells against syngeneic tumors in the mouse. J Immunol 123:1653–1658, 1979.
35. Cantor H, Boyse EA: Regulation of cellular and humoral immune responses by T cell subclasses. Cold Spring Harbor Symp 41:23–31, 1977.
36. McKenzie IFC, Potter T: Murine lymphocyte surface antigens. Adv Immunol 27:79–338, 1979.
37. Nelson M, Nelson DS, McKenzie IFC, Blanden RV: Thy and Ly markers on lymphocytes initiating tumor rejection. Cell Immunol 60:34–42, 1981.
38. Greenberg PD, Cheever MA, Fefer A: Eradication of disseminated murine leukemia by chemoimmunotherapy with cyclophosphamide and adoptively transferred immune Lyt 1^+2^- lymphocytes. J Exp Med 154:952–963, 1981.
39. Drebin JA, Perry LL, Carter R and Greene MI: Regulation of the immune response to antigens on the malignant cell surface. Seminars in Immunopathol 512:175–192, 1982.

40. Fernandez-Cruz E, Woda BA, Feldman JD: Elimination of syngeneic sarcoma in rats by a subset of T lymphocytes. J Exp Med 52:823–841, 1980.
41. Fernandez-Cruz E, Gilman SC, Feldman JD: Immunotherapy of a chemically-induced sarcoma in rats: Characterization of the effector T cell subset and nature of suppression. J Immunol 128:1112–1117, 1982.
42. Loveland BE, Hogarth PM, Ceredig Rh, McKenzie IFC: Cells mediating graft rejection in the mouse: I. Lyt-1 cells mediate skin graft rejection. J Exp Med 153:1044–1057, 1981.
43. Loveland BE, McKenzie IFC: Which cells cause graft rejection? Transplantation 33:217–221, 1982.
44. Dallman MJ, Mason DW: Role of thymus-derived and thymus-independent cells in murine skin allograft rejection. Transplantation 33:221–223, 1982.
45. Cantrell DA, Robins RA, Baldwin RW: Rat lymphocyte subset: Cellular requirements for the generation of T-cell growth factor. Cell Immunol 70:367–372, 1982.
46. Sonnenfeld G, Mandel AD, Merigan TC: *In vitro* production and cellular origin of murine type II interferon. Immunology 36:883–890, 1979.
47. O'Malley JA, Nussbaum-Blumenson A, Sheedy D, Crossmayer BJ, Ozer H: Identification of the T cell subset that produces human γ interferon. J Immunol 128:2522–2526, 1982.
48. Klein E: Lymphocyte mediated lysis of tumor cells *in vitro*: Antigen restricted clonal and unrestricted polyclonal effects. Springer Seminars in Immunopathol 512:147–159, 1982.
49. Kripke ML: Antigenicity of murine skin tumors induced by ultraviolet light. J Natl Cancer Inst 53:1333–1336, 1976.
50. Berendt MJ, North RJ: T cell mediated suppression of antitumor immunity: An explanation for progressive growth of an immunogenic tumor. J Exp Med 151:69–80, 1980.
51. Dye ES, North RJ: T cell mediated suppression as an obstacle to adoptive immunotherapy of the P815 mastocytoma and its metastases. J Exp Med 154:1033–1042, 1981.
52. Price MR, Preston VE, Robins RA, Zöller M, Baldwin RW: Induction of immunity to chemically induced tumours be cellular or soluble antigens. Cancer Immunol Immunother 3:247–252, 1978.
53. Price MR, Baldwin RW: Shedding of tumor cell surface antigens. In: Dynamic aspects of cell surface organisation, Poste G, Nicolson GL (eds). Amsterdam: Elsevier, North Holland, 1977, pp 423–471.
54. Baldwin RW, Byers VS, Robins RA: Circulating imune complexes in cancer: Characterization and potential as tumour markers. Behring Inst Mitt 64:63–77, 1979.
55. Baldwin RW: Immunotherapy of tumors. In: Cancer chemotherapy annual 3, Pinedo HM (ed). Amsterdam: Excerpta Medica, 1981, pp 178–202.
56. Baldwin RW: Specific and non-specific cellular interactions modulating host resistance to tumors. In: Fundamental mechanisms in human cancer immunology, Saunders JP, Daniels JC, Serrou B, Rosenfeld C, Denney CB (eds). Amsterdam: Elsevier, North Holland, 1981, pp 167–180.
57. Baldwin RW, Pimm MV: BCG immunotherapy of a rat sarcoma. Br J Cancer 28:281–287, 1973.
58. Hellström KE, Hellström I, Nepom JT: Specific blocking factors – Are the important? BBA Rev on Cancer 473:121–148, 1978.
59. Beyer J-H, Borberg H, Fuchs Ch, and Nagel GA: Plasmapheresis in immunology and oncology, Basel: S Karger, 1982, pp 1–266.
60. North RJ: Cyclophosphamide facilitated adoptive immunotherapy of an established tumor depends on elimination of tumor-induced suppressor cells. J Exp Med 55:1063–1074, 1982.
61. Hengst JCD, Mokyr MB, Dray S: Cooperation between cyclophosphamide tumoricidal activity and host antitumor immunity in the cure of mice bearing large MOPC-315 tumors. Cancer Res 41:2163–2167, 1981.

62. Mokyr MB, Hengst JCD, Dray S: Role of antitumor immunity in cyclophosphamide-induced rejection of subcutaneous nonpalpable MOP.315 tumors. Cancer Res 42:974–979, 1982.
63. Kahan BD, Pellis NR, Lefrue SJ, Tanaka T: Immunotherapeutic effects of tumor-specific transplantation antigens released by 1-butanol. Cancer 49:1168–1173, 1982.
64. Liew FY: Regulation of delayed-type hypersensitivity to pathogens and alloantigens. Immunol Today 3:18–23, 1982.
65. Greene MI, Dorf ME, Pierres A, Benacerraf B: Reduction of syngeneic tumor growth by an anti-I-J alloantiserum. Proc Nqt Acad Sci USA 74:5118–5121, 1977.
66. Perry LL, Kripke ML, Benacerraf B, Dorf ME, Greene MI: Regulation of the immune response to tumor antigen: VIII. The effects of host specific anti-I-J antibodies on the immune response to tumors of different origin. Cell Immunol 51:349–359, 1980.
67. Fox IJ, Perry LL, Sy M-S, Benacerraf G, Greene MI: The influence of ultraviolet irradiation on the system. Clin Imm and Immunopath 16:141–145, 1980.
68. Kanno M, Kobayashi S, Tokuhisa T, Takei I, Shinohana N, Taniguchi M: Monoclonal antibodies that recognise the product controlled by a gene of the I-J sub region of the mouse H-2 complex. J EXP Med 54:1290–1304, 1981.
69. Waltenbaugh C: Regulation of immune response by I-J products: I. Production and characterization of anti I-J monoclinal antibodies. J Exp Med 154:1570–1583, 1981.
70. Hiramatsu K, Ochi A, Miyatani S, Segawa A, Tada T: Monoclonal antibodies against unique I-region gene products expressed only on mature functional T cells. Nature 296:600–668, 1982.
71. Hanna MG, Peters LC: Morphological and functional aspects of active specific immunotherapy of established pulmonary metastases in guinea pigs. Cancer Res 41:4001–4009, 1981.
72. Yarkoni E, Hunter JT, Sukumar S, Rapp HJ: Active specific immunotherapy of guinea pigs with visceral tumor implants. Cancer Immunol Immunother 12:273–275, 1982.
73. Milas L, Scott MT: Antitumor activity of Corynebacterium parvum. Adv Cancer Res 26:257–260, 1977.
74. Baldwin RW, Pimm MV: BCG in tumor immunotherapy. Adv Cancer Res 28:91–147, 1978.
75. Pimm MV, Baldwin RW: Immunology and immunotherapy of experimental and clinic metastases. In: Secondary spread cancer, Baldwin RW (ed). London: Academic Press, 1978, pp 163–209.
76. Neri A, Welch D, Kawaguchi T, Nicolson GL: Development and biologic properties of malignant cell sublines and clones of a spontaneously metastasizing rat mammary adenocarcinoma. J Natl Cancer Inst 68:507–517, 1982.
77. Pimm MV, Baldwin RW: Antigenic heterogeneity of primary and metastatic tumors and its implications for immunotherapy. In: Metastatic tumor growth: Cancer campaign 4:305–312. Stuttgart: Gustav Fischer, 1980.
78. Sone S, Poste G, Fidler IJ: Rat alveolar macrophage are susceptible to free and liposome-encapsulated lymphokines. J Immunol 124: 2197–2202, 1980.
79. Fidler IJ, Sone S. Fogler WE, Barnes ZL: Eradication of spontaneous metastases and activation of alveolar macrophages by intravenous injection of liposomes containing muramyl dipeptide. Proc Natl Acad Sci USA 78:1680–1684, 1900.
80. Fidler IJ, Poste G: Macrophage-mediated destruction of malignant tumor cells and new strategies for the therapy of metastatic disease. Seminars in Immunopath 512:161–174, 1982.
81. Baldwin RW, Pimm MV: BCG-mediated responses in tumor immunotherapy. In: Bacteria

and cancer, J. Jeljaszewicz, G. Pulverer, W. Roskowski. London: Academic Press, 205–217, 1982.

82. Baldwin RW, Embleton MJ, Price MR: Monoclonal antibodies specifying tumour-associated antigens and their potential for therapy. Molec Aspects Med 4:329–368, 1981.
83. Pimm, MV, Embleton MJ, Perkins AC, Price MR, Robins RA, Robinson GR, Baldwin RW: *In vivo* localization of anti-osteogenic sarcoma 791T monoclonal antibody in osteogenic sarcoma xenograft. Int J Cancer 30:75–85, 1982.
84. Baldwin RW, Flannery GR, Pelham JM, Gray JD: Immunomodulation by IFN-conjugated monoclonal antibody to human osteogenic sarcoma. Proc Am Ass Cancer Res 23:254, 1982.
85. Hellström KE, Hellström I, Brown JP: Human tumor-associated antigens identified by monoclonal antibodies. Seminars in Immunopathol 512:127–146, 1982.
86. Farrands PA, Perkins AC, Pimm MV, Embleton MJ, Hardy JG, Baldwin RW, Hardcastle JD: Radioimmunodetection of human colorectal cancers using and anti-tumour monoclonal antibody. Lancet II:397–400, 1982.
87. Chatal JF, Saccavini JC, Fumoleau P, Bardy A, Douillard JY, Aubry J, Le Mevel B: Photoscanning localization of human tumors using radioiodinated monoclonal antibodies to colorectal carcinoma. J Nucl Med 23:8, 1982.
88. Sears HF, Mattis J, Herlyn D, Hayry P, Atkinson B, Ernst C, Steplewski Z, Koprowski Hb: Phase-I clinical trial of monoclonal antibody in treatment of gastrointestinal tumours. Lancet 1:762–765, 1982.

4. Cultured Human Antitumour T Cells and their Potential for Therapy

B.M. VOSE and A. HOWELL

INTRODUCTION

Over the past two decades, the availability of increasingly effective chemotherapeutic regimens has led to the cure of a large proportion of patients with Hodgkins disease, some non-Hodgkins lymphomas, childhood leukaemias and solid tumours, choriocarcinomas and teratocarcinomas. Progress has been much slower with the common solid tumour e.g. those of the lung, breast and gastrointestinal tract. In breast cancer for instance remissions occur in 50–70% of patients given chemotherapy and in 10–20% of these the remissions are apparently complete in that no residual tumour can be detected by conventional techniques. However, in the majority of patients in complete remission the tumour eventually regrows and kills the host. Thus in these common solid tumours chemotherapy may reduce the bulk of the disease but not eradicate it. The development of new cytotoxic agents and more effective scheduling may improve the cure rate but the addition of an alternative modality of treatment such as immunotherapy at the time the total tumour burden is low, possibly immediately after surgery, has long been an attractive approach.

Immunotherapy in the clinical setting may take two forms, (i) the administration of agents which modify the biological response of the host to the tumour, or (ii) the passive administration of specific antibodies or cellular reagents with antitumour activity. The former approach is considered in other sections of this volume but has met with limited therapeutic benefit to date. In this chapter, we will consider the possibility that passive cellular immunotherapy may have a role in cancer treatment.

Such cellular immunotherapy of human cancer could be achieved only if certain widely held views are proved to be correct. (1) It is implicit that human tumour cells express at the cell surface molecules which the host is capable of recognising as foreign and which are targets for host defence

R.B. Herberman (ed), Basic and Clinical Tumor Immunology. ISBN-13: 978-1-4613-3875-8
© 1983. Martinus Nijhoff Publishers, Boston.

mechanisms. (2) The fact that malignancy is evident points to a specific or general compromise or suppression of 'host defence' which should be corrected or reversed by administration of reactive cells. (3) 'Host defence' may be mediated through a number of effector mechanisms and concentration on any single component might be inappropriate to the goal of the immunotherapist. It is true to say that although several attempts at passive immunotherapy have been attempted there is to date little evidence of sustained clinical benefit.

1. PREVIOUS STUDIES USING PASSIVE CELLULAR IMMUNOTHERAPY

Passive immunotherapy of experimental and human cancer using both sera and cells has been reviewed by Rosenberg and Terry [1]. Studies over the last two decades have utilised both normal, non-immunised, allogeneic lymphocytes in an attempt to provide immunocompetent lymphocytes which might overcome the generalised immunosuppressions of advanced malignant disease and immunised allogeneic and even xenogeneic lymphocytes in the hope of inducing specific anti-tumour effectors. Among the first to use the former were Woodruff and Nolan [2]. Some therapeutic effect was claimed following administration of up to 5×10^{10} allogeneic AB matched or 0 group spleen cells to cancer patients in conjunction with chemotherapy and radiotherapy. Eight patients with melanoma or cancer of the ovary, breast or penis received normal lymphocytes and all had been previously treated. Nitrogen mustard, Busulphan, Prednisolone or Thiotepa were given to prevent rapid rejection of allogeneic cells. In every case the authors describe changes in patients' symptoms, physical signs or changes in biopsy. Since, in this study, other treatments were given it is difficult to assess the contribution of the allogeneic cells. Studies by other authors [3–5] using up to 1.2×10^{12} allogeneic lymphocytes from non cancer patients have shown brief responses in a small number of cases, but trials have been unsatisfactory because of concomitant treatment with other agents or because of the small number of patients examined. In addition, since rapid elimination of injected cells was likely to occur the failure to achieve long term benefit may not be surprising. However, one trial used HLA-matched normal thoracic duct or blood lymphocytes in an attempt to obtain more prolonged effects [6]. This study is important because it describes the persistence of injected lymphocytes for several months based on karyotyping of peripheral blood in instances where there was a genetic marker of the opposite sex. In spite of careful typing for ABO and HLA including mixed lymphocyte culture, 4 of 14 patients had symptoms of graft versus host disease with skin rash, lymphadenopathy and fever. Objective responses were ap-

parent in two of these (disappearance of ascites for 6 months in one patient with ovarian cancer and a 50% regression of perineal and inguinal masses from carcinoma of the rectum). In total, some 50 patients have been the subject of cellular immunotherapy using normal unimmunized lymphocytes in the different trials reviewed by Rosenberg and Terry. Objective responses attributable to this form of treatment were seen in 12 individuals, but in each, the effects were short-lived.

Many groups have taken the view that it might be advantageous to administer cells with some direct antitumour effect i.e. from immunised donors. The protocols involved have required immunisation with tumour material: a course which presents considerable management and ethical problems in man when it is considered that growth of tumor at the implantation site occurred in 2/118 patients in trial of Nadler and Moore [7] and resulted in the death of a normal volunteer in another study [8]. In most reports patients have been cross-immunised with tumour and then transfused with blood or leukocytes. In the first such study, Nadler and Moore [9] reported 23 objective responses lasting at least 6 weeks, in 118 patients most of whom had malignant melanoma and who were not receiving other therapy. The use of cross-immunization in these experiments may lead to graft versus host reactions at non tumour sites since donors would be sensitised to recipients histocompatibility antigens rather than be tumour-specific and recipients having received donor tumour were similarly sensitised with possible rapid elimination of injected cells. Nadler and Moore, in a subsequent study, exposed blood leukocytes to autologous melanoma cells *in vitro* for 10 days before intraperitoneal infusion of surviving lymphocytes to overcome these objections but no responses were noted in three patients so treated. Other authors have exchanged effectors raised by injection of tumour material to 'third party' patients. Under this protocol patients receiving lymphocytes were not sensitised to donor HLA. With this approach eight patients of 38 had objective responses in the trial of Humphrey et al. [10] using tumour homogenate as immunogen and 4 of 35 patients responded in the trial reported by Krementz et al. [11].

A large number of animal studies have been performed using cellular immunotherapy. In evaluating the studies described above it is instructive to examine the parameters necessary for success in these model systems (reviewed by Rosenberg and Terry [1] and Fefer (this volume)). These include: –

(1) the use of large numbers of effector cells with at least 10^8 cells necessary for therapeutic effect against a 1 gram murine tumour [12, 13]; (2) syngeneic lymphocytes preferably from highly immunised donors [12, 13]. Some success has been achieved with allogeneic or even zenogeneic lymphocytes but non-immune cells or cells of inappropriate specificity were without effect in

most studies [13]; (3) small tumour burden [12, 13]. Effects were limited by time after tumour infection even with large numbers of transferred effectors; (4) route of administration with intravenous route best [12]; (5) combination with chemotherapy [14, 15]. Several studies have established the effectiveness of immunotherapy when given after chemotherapy: a finding which may be attributed either to a reduction of tumour burden or the elimination of pre-existing immunosuppression in the tumour-bearing host.

It is clear that, if these same factors were to be important for success of immunotherapy in human disease, the studies in man described here do not critically address the problem. Indeed, in assessing passive cellular immunotheapy Rosenberg and Terry [1] drew the conclusion that 'until techniques are developed for culturing large quantities of specific lymphocytes capable of reacting against the antigens of the tumour, there is little rationale for continued efforts with this approach'. Techniques have now been described by which sensitised T lymphocytes can be maintained and expanded in continuous culture using T cell growth factors (TCGF or IL-2) [16, 17]. In this chapter we will consider the derivation of specific anti-tumour T cell lines, their use in defining human tumour immunogenicity and their potential in immunotherapy.

2. CELLULAR IMMUNE RESPONSE TO HUMAN TUMOURS

The concept that human tumours might express antigens against which the host was capable of mounting an immunological response derived much support from extensive studies in chemically and virally induced animal tumours in the 1950s and 60s. Experiments showed that in inbred animals and even in the authochthonous host, protection against subsequent tumour challenge could be achieved by immunization with tumour material [18–24], thereby laying the foundation for the hope that similar immunological manipulations could be beneficial in man. Because of ethical constraints the experimental approach in defining human tumour immunogenicity has been largely confined to *in vitro* assays of immunological function. The definition of tumour antigenicity in animal systems was quickly followed by the description of disease-related cytotoxic, proliferative and lymphokine-mediated immunological reactions in human tumours (reviewed in [25] and [26]). Interestingly, the pattern of reactivity in these *in vitro* assays showed that, unlike the animal systems where antigenicity was individually specific in the case of chemically induced tumours or virus specific for virally induced tumours, human tumours showed organ-related reactivities with reactions confined by tissue boundaries. Lymphocytes from patients with breast mal-

ignancies responded to allogeneic breast tumours but less frequently to, for example, colon tumours. It was against this background that the early studies of passive cellular immunotherapy described above and trials with non-specific immunological manipulation using agents such as C. Parvum BCG or Levamisole were initiated.

This period of high optimism was not sustained. As increasing numbers of patients and controls were included in test protocols it became clear that the pattern of *in vitro* reactivity in the cancer groups were less precise than originally described. In studies of cytotoxic activity lymphocyte preparations from healthy donors often showed reactivity which was as high if not higher than that manifested by cancer patients [27, 28] and the concept that human tumours were antigenic in the host were severely challenged with the erosion of the experimental base. At the same time Hewitt observed that in a large series of spontaneously arising murine tumours there was little evidence for an immune reaction inducing transplantation resistance [29] and the results of many large immunotherapy trials were disappointing [25]. It became widespread to consider human tumours as non antigenic and interest in the immunotherapy of cancer by manipulation of specific T cell-mediated immunity declined. Other effector mechanisms including natural killer (NK) cells [30] and macrophages were considered to play the major role in defence against malignancy (see Herberman and Fidler, this volume). Recent advances in cellular immunology require that the role of T cells in human malignancy be re-examined. In particular the recognition of the requirement of histocompatibility between target and effector of the demonstration of T cell-mediated cytotoxicity [31, 32] has an important bearing. Since T cells recognise neither antigen nor MHC product alone, but a complex or association between them, it is essential for the demonstration of T cell mediated lysis and proliferative response to antigen to work in autologous or HLA-matched combinations. It was for this reason that we have concentrated upon the cytotoxic potential and stimulation response of lymphocytes from different sites against autologous tumour cells. In considering tumour rejection and the role of the many possible effector mechanisms a close parallel can be drawn with rejection of histoincompatible grafts. In this process it appears that recognition of MHC antigens by helper T cells (LyT_1^+) is the primary event and the ability to reject allografts or tumour can be transferred with this subset. Cytotoxic T cells are not effective in transferring immunity to immunosuppressed recipients [33-36]. In the rat non-specific effectors with an NK-like phenotype can be detected at the graft rejection site [34]. These data suggest that lymphokine-mediated activation of host defence at the graft site is important in rejection rather than direct T cell-mediated cytotoxic events. A similar situation has been described in UV-induced murine tumours where mutational events associated

with the change from regressor to progressor status appear to be a loss of T cell activating determinants rather than changes of for example, susceptibility to lysis by natural killer cells [37]. With these data in mind, we have used assays for helper activity such as the mixed lymphocyte tumour culture [38] and primed lymphocyte tests [39] in addition to cytotoxicity to more fully define the nature of the antitumour T cell response. We take the view that under conditions where multiple effector types can induce target cell damage it is reasonable to consider the role of each in the elimination of tumour deposits. In an attempt to develop experimental systems in which the contribution of T cells can be assessed we have used as targets or stimulators freshly isolated tumour cells in preference to cultured cells. By stepwise application of separation procedures based upon differences of size, density and adherence to enzymatically dispersed human material, it has been possible to obtain tumour cell suspensions of high viability which can be used as stimulators in proliferation assays or labelled with ^{51}Cr. These cells show a spontaneous release of chromium that is comparable to that of many established cell lines in short term cytotoxicity assays [40, 41]. Freshly isolated tumour cell preparations have the advantage that they have not been exposed to prolonged periods of culture which can lead to the acquisition or loss of cell surface antigens or the selection of subpopulations of cell types. It has been possible using this methodology to obtain tumour cells from approximately 60% of specimens received: a number that is considerably higher than our success rate in establishing characterised short-term cultures of tumour cells. A further advantage was that these cells were refractory to killing by effectors from healthy donors in direct assays [42, 43] unless the large granular lymphocytes which in peripheral blood mediate NK [44, 45] were used. Animal studies have shown the rapid increase of NK susceptibility of tumour cells upon culture [46].

In examining a large group of cancer patients, blood lymphocytes from 35% (87 of 248) showed specific cytotoxic activity against autologous tumour cells [41, 47, 48]. Killing of allogeneic tumour control cells matched for site and histology and which themselves were lysed by autologous effectors was found in only 7% of cases. Further specificity studies have established that targets prepared from normal lung tissue were not lysed by autologous effectors whereas the tumour cells from the same patient were damaged [50]. In cold inhibition assays only autologous tumour cells blocked lysis [47, 49]. Taken together these data indicate that, in at least some patients, there is immunological recognition of tumour cells and the specificity of the reaction indicates either that human tumours express individually specific antigens or that in common with other antigens in humans against which T cell mediated cytotoxicity is demonstrable [51, 52], there is indeed a requirement for histocompatibility between target and

effectors for T cell-mediated lysis. It is markedly different from early studies of organ-restricted reactivity. Cytotoxicity was concentrated into lymphocyte subsets forming rosettes with sheep erythrocytes and passing through nylon wool [50]. There was no correlation between cytotoxicity against autologous tumour and the K562 cell line which is exquisitely sensitive to lysis by NK cells [49] and killed by PBL from the majority of cancer patients. The distinction between autologous cytotoxicity and NK was further emphasised when effectors were prepared from extravascular sites. Tumour draining lymph node and tumour infiltrating lymphocytes regularly killed autologous tumour cells under conditions where they induced minimal lysis of K562 cells. When autologous cytotoxicity was found in blood lymphocytes, lymph node and tumour infiltrating lymphocytes were also positive whereas NK cells were confined to blood and spleen [49, 53, 54]. Thus a proportion of cancer patients have lymphocytes which specifically lyse autologous tumour without *in vitro* stimulation. However, we have not noted any prognostic significance of the presence of such cytotoxic activity against autologous tumour in over 100 lung cancer patients followed for over 3 years. In studies with 50 colon cancer patients there was no change in the frequency or level of cytotoxic activity with disease progression [49].

In an attempt to define immune recognition of autologous tumour and to generate killer cells *in vitro*, several groups have used the mixed lymphocyte-tumour culture [38]. In these experiments responder lymphocytes were cocultivated with irradiated autologous tumour for 6 days and surviving cells monitored for incorporation of ^{3}H-thymidine, for the presence of lymphoblasts in the culture and for cytotoxicity against a range of target cells. The optimisation of the MLTC for stimulation has been reviewed by Vanky and Stjernsward [38]. Of critical importance were (1) the removal of adherent cells from both the responder and stimulator populations. Several studies have identified the suppressive activity of blood monocytes from cancer patients on stimulation by mitogens through prostaglandin release [55–58]. (2) the use of viable stimulator tumour cells which have been incubated overnight at 37 °C to allow regeneration of cell surface components essential to lymphocyte activation [59]. (3) removal of tumour-infiltrating lymphocytes from the stimulator pool since these could also suppress lymphocyte activation in both the MLTC and PHA stimulation [60]. Under optimal conditions significant stimulation of lymphocytes is induced in approximately 70% of patients with a variety of malignant diseases [38]. These data indicate that there are tumour-recognitive lymphocytes demonstrable in stimulation assays in a large number of patients which did not manifest cytolytic activity. The induction of blastogenesis in MLTC is often, but not invariably accompanied by the appearance of cytotoxic activity in surviving cells with specificity for the autologous tumour [47, 48, 61]. In the absence

of blastogenesis no induction of cytotoxicity was apparent [62]. It is important in assessing these data to distinguish the reactivity against tumour cells from that induced in the autologous mixed lymphocyte reaction (AMLR). Stimulation of autologous T cells is not restricted to tumour cells but can be induced by irradiated B lymphoblastoid cells and non-T cells (particularly B cells) with levels of stimulation frequently approaching those of the allogeneic mixed lymphocyte reaction [63]. Extensive studies have mapped the stimulatory determinant to the Ia region [64] and, in particular, to the invariant α chain. There is continuing controversy as to whether this reaction is indeed recognition of self Ia which may be important in antigen presentation and in the control of development of the immune system [65] or is a response to foreign antigens such as bovine proteins acquired during isolation procedure. The mapping of stimulatory activity to the Ia region under these circumstances would then represent the restriction element of antigen presentation for stimulation [66]. In our own studies it is apparent that extensive isolation of responder T cells is not required for MLTC reactivity in approximately 50% of the positive cases. In addition, stimulation by tumour cells can be detected when autologous monocytes, tumour associated macrophages or tumour infiltrating lymphocytes do not induce blastogenesis [67]. However, these data must be interpreted with caution since with monocytes and macrophages there was frequently an inhibition of spontaneous (^{3}H)-thymidine incorporation and these cells inhibit PHA responsiveness in cancer patients [58]. It can be considered that although these cells may express stimulatory determinants for the AMLR they suppress that stimulation by release of, for example, prostaglandin as demonstrated in recent reports [55, 58]. The ready demonstration of cytotoxic activity MLTC cultures is also at variance with the experience of some but not all groups working with the AMLR [68, 69].

Cytotoxic activity generated in the MLTC largely corresponds in specificity to that of the directly cytotoxic cells i.e. is restricted to the autologous tumour. In comparing a number of different stimulating protocols Vanky et al. [61] were able to conclude that stimulation with autologous tumour was the most consistent inducer of specific autorecognitive cytotoxic activity presumably as a result of clonal expansion of relevant T lymphocytes. The high degree of tumour specificity was also apparent in tests with autologous targets. We have seen only infrequent lytic activity against normal lung cells, lymphoblasts or monocytes in short term ^{51}Cr release assays [67]. The generation of autologous tumour killing is also induced by stimulation in mixed lymhpocyte cultures, particularly when pooled stimulators from up to six donors are used or by stimulation with lectins. Under these conditions induced killers show reactivity against a range of targets including autologous and allogeneic freshly isolated tumour cells. This observation was first

reported in leukaemias [70, 71] but now extended to studies of solid tumours [61, 72, 73]. The exact identity of the killer cells in this situation is not clear and several possibilities have been put forward including transtimulation by IL-2 of autorecognitive T cell killer clones activated *in vivo* to express the IL-2 receptor activation of NK-like 'anomalous' killers by IL-2 and IFN (K562 killing is high in MLR stimulated cultures) or by the expression of 'inappropriate' histocompatibility antigens on transformed cells. The characterisation of this phenomenon is in progress in several centres and the availability of T cell clones should enable rapid progress in this area.

It has been the experience of this laboratory that although pool stimulation of patients lymphocytes does induce cytotoxicity against autologous tumour, cultured lymphocytes cannot be restimulated by cells of that tumour or by autologous lymphocytes. They are restimulated by each individual pool member and by a proportion of allogeneic tumours presumably sharing histocompatibility antigens with the pool. Pool stimulated cultures thus show very different patterns of secondary response to those induced in MLTC which are restimulated by autologous tumour. The autologous tumour killers induced in MLC may be lymphokine activated killers [74] or expanded/activated NK-like cells [75], rather than the specific effectors induced by MLTC.

In this section we have reviewed recent work which suggest that human tumours evoke a T cell mediated response against a yet undefined antigen expressed on their cell surface. In both cytotoxic and proliferative assays significant reactivity was apparent in a proportion of patients (up to 70% in MLTC). These data offer some grounds for optimism for therapeutic exploitation provided that the specificity recognised is indeed tumour associated rather than some normal organ/epithelial differentiation antigen and that large numbers of cells were available. The production of large numbers of T lymphocytes is now possible following the description of a lymphokine (IL-2) with the capacity to maintain lymphoid cells in proliferation [16].

This soluble factor is released by a subpopulation of activated T lymphocytes (LyT_1^+ in the mouse OKT_4^+ in man) which has the capacity to maintain the long term growth of normal T lymphocytes with retention of functional characteristics and phenotype [17, 76-78]. There is no doubt that the description of this factor has had a major influence on the understanding of T cell activation and properties. IL-2 acts as the second signal for T cells inducing proliferation of cells upon which the first signal (antigen or mitogen) have induced a receptor for it [79]. Its' activity is restricted to 'activated' T lymphocytes. As such it offers the possibility of rapid selective expansion of antigen reactive cells for further specificity testing and investigation of efficacy *in vivo*. A major concern in this work has been the

characterisation of cells, in addition to T lymphocytes, which might be induced to proliferate in conditined media containing IL-2.

3. CULTURE OF CYTOTOXIC AND PROLIFERATIVE LYMPHOID CELLS

In preliminary studies it was considered that the cancer patient might, as a result of antigens on the tumour, have T cells *in vivo* which expressed IL-2 receptor and which could therefore be selectively isolated by growth in lectin-free lymphocyte conditioned media. Conditioned media (CM) were produced under a number of different stimulation protocols using PHA and B-lymphoblastoid cell lines and freed of lectin by ammonium sulphate precipitation, passage through rabbit anti-PHA immunoabsorbent columns and ion exchange chromatography ([80], Strong et al., manuscript in preparation). Lymphocytes from peripheral blood, lymph node and tumour all proliferated in these CM and short-term lines were maintained for up to 50 days. In common with several groups we were able to show that these cultures invariably lysed autologous tumour in short-term ^{51}Cr release assays [81–84]. The implication of these findings was that readily detectable tumour-reactive lymphocytes were present in the circulation and at extravascular sites and that addition of IL-2 was sufficient to allow expansion of cytolytic clones or expression of their cytolytic function. However, cultured IL-2 dependent lymphoid cell lines show heterogeneous effector function and may express specific T cell-mediated lysis as well as natural, antibody dependent and lectin dependent cellular cytotoxicity [85, 86]. The possibility exists that IL-2 may act as a proliferative signal for more than one cell type. There is abundant evidence that NK-like cells can be cloned in IL-2 dependent culture [86–89], continue to express lytic function and in the mouse, but not in man, maintain the phenotype of fresh NK cells. In order to clarify the type of cells expanded in IL-2 under different culture conditions, lines have been established from different lymphocyte subpopulations.

Considerable advances have been made in the understanding of natural killing in man since the realisation that the effector in PBL had characteristic morphology (the large granular lymphocytes (LGL)) and could be isolated (to 98% by morphology) from non-lytic elements by separation of lower density cells on discontinuous Percoll gradients [90]. The response of these cells to IL-2 was assessed in limiting dilution analysis. In limiting dilution assays decreasing numbers of responder cells are dispersed into multiple wells together with autologous mononuclear cells as feeders and lectin free conditioned media containing IL-2. Proliferation or cytotoxicity of well contents is scored after 7–10 days culture and the number of positive

wells counted. Positive is defined by a difference of at least 3 standard deviations from the mean reactivity of feeder cells alone. The technique relies on the ability to establish culture conditions which allow the expansion of a single cell to a level where an event (proliferation or cytotoxicity) in the progeny can be detected. Statistical analysis of the data yields the limiting frequency of the number of cells responding. Under these conditions it has been possible to establish that in the absence of lectin the frequency of proliferative precursors in LGL enriched populations responding to IL-2 was 30-fold greater than that of small T cells, (1/150 cells proliferating in LGL and 1/5000 in small T cells). More significantly, all cells proliferating showed NK-like activity since, when these same microcultures were tested for lytic activity against K562, it was found that the proliferative and K562 cytotoxic precursor frequency in LGL was the same. No cytotoxic precursors against K562 (frequency $1/10^4$) were identified among the small T cells even when these were induced to proliferate with PHA to give frequencies of 1/6–1/10. Cultured cells with NK-like activity retained the granular morphology characteristic of fresh NK effectors but growth in IL-2 rapidly induced changes in phenotype with a change towards a more mature T cell [73, 84, 85]. (LGL react for example, with monoclonals OKM1 (monocyte granulocytes), OKT10 (immature thymocytes) but not OKT3 (mature T cells) whereas cultured cells from LGL populations express OKT3 but not OKM1 and OKT10 [75, 86]). A subpopulation of low density blood lymphocytes thus spontaneously express the IL-2 receptors and can respond to growth factor, but it is difficult to firmly establish their derivation. Since proliferative frequency was low, it cannot be excluded that these cultured cells represent the progeny of the small number (<2%) of cells in LGL preparations expressing OKT3 (see Herberman, this volume). It has been possible to derive cultured lymphoid lines from LGL which show high lytic activity for NK sensitive cultured cell lines as well as insensitive freshly isolated tumour cells [75, 91]. Cultured small T cells do not kill NK sensitive lines, but can lyse alloblasts and some fresh tumour cells. Neither population kills mouse lymphoma/leukaemias. In the present context the importance of these findings in that conditions which favour IL-2 release are likely to expand NK-like cells as well as more specific effectors [74]. Under lectin-free conditions preferential outgrowth of these NK-like cells is likely. The broad pattern of cytotoxicity shown by short term cultured lymphoid cells against tumour cells has encouraged their consideration as a potential tool in immunotherapy [73, 74, 92]. The ready lysis of dividing bone marrow cells and immature thymocytes by NK cells [91, 92] and the lysis of normal tissue in longer term assays by cultured effectors [92] cautions against the ready acceptance of this approach.

Addition of T cell mitogens to the limiting dilution assays induces a dose

dependent rise in proliferative frequency in the small T cell pool. One of 6 cells produce IL-2 dependent microcultures in the presence of 2 μg/ml PHA, but the proliferative and cytotoxic frequency of LGL does not change markedly in the presence of lectins. Stimulation of T lymphocytes with consequent release of IL-2 and interferon can thus expand a subpopulation of NK-like cells as well as specific effectors. In mixed lymphocyte-tumour cultures this dual stimulation by tumour cells has been verified [61, 94] with expansion of both autologous killers and killers of K562. The number of lymphocytes responding to tumour stimulation in MLTC has also been quantified using limiting dilution analysis with peripheral blood lymphocytes and tumour infiltrating lymphocytes as responders.

It was anticipated that there might be a concentration of reactive lymphocytes within the tumour mass. This proved to be the case. The experimental protocol of these assays is presented in Figure 1. Lymphocytes from 16 patients were dispensed under limiting dilution conditions in the presence

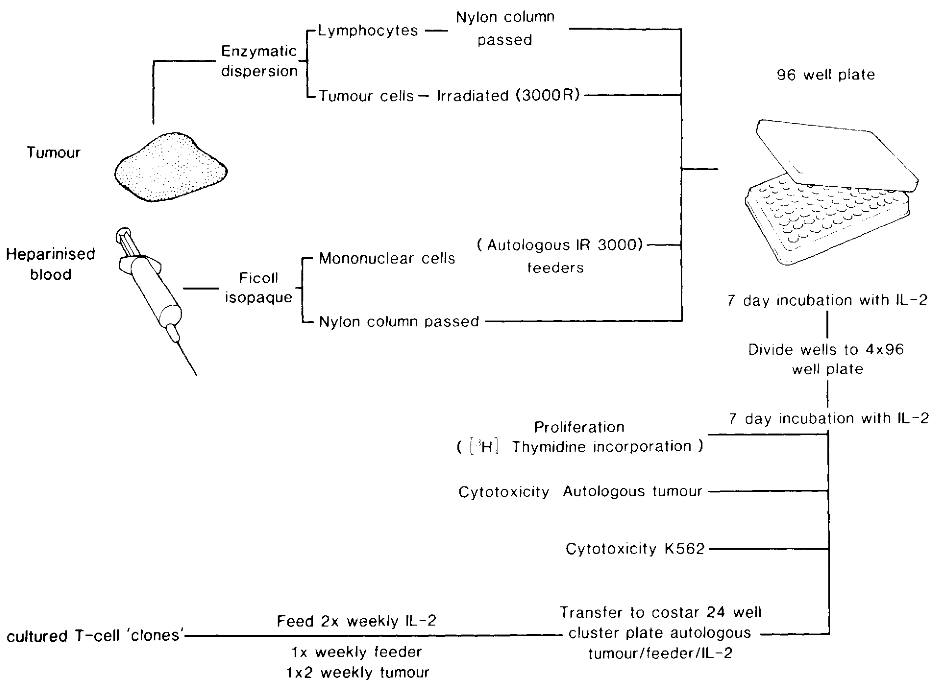

Figure 1. Experimental protocol for quantitation of tumour-reactive lymphocytes in blood and tumour. Peripheral blood and tumour infiltrating lymphocytes were separated on discontinuous ficoll-isopaque gradients and cocultivated with irradiated autologous tumour cells and blood mononuclear cells for 7 days in 96 well round bottomed plates with conditioned medium. Well contents were divided into four plates with fresh conditioned medium, incubated for a further 7 days and proliferative and cytotoxic frequencies assessed on day 14. 'Clones' of reactive cells could be taken from the fourth plate.

of irradiated autologous tumour cells, conditioned medium and peripheral blood mononuclear cells as feeders. After seven days in culture, well contents were split into four and growth continued in fresh medium and CM before enumeration of proliferative, autologous cytotoxic and NK precursors. In the absence of tumour stimulator cells, TIL showed a relatively high spontáneous response to lectin-free IL-2. In the peripheral blood of cancer patients the proliferative frequency was closely similar to that of a large series of healthy donors as stated before (approximately 1/5000 cells). Tumour-infiltrating lymphocytes responded with a proliferative frequency of 1/1000. Studies are in progress to determine if direct cloning of TIL without *in vitro* stimulation is a means by which highly active anti-tumour effectors may be obtained. The higher spontaneous proliferation frequency in TIL suggests that this population is 'activated' *in vivo* as suggested by other authors using different techniques [95]. Cocultivation of responder lymphocytes and autologous tumour increased the proliferative frequency in both PBL and TIL in the majority of cases studied and particularly in squamous cell carcinoma of the lung. In the whole series the proliferative frequency of PBL and TIL rose approximately 5-fold to 1/1000 and 1/200 respectively. The higher proliferative frequency of TIL was reflected in a higher frequency of autologous killers in assays where ^{51}Cr labelled targets were added to limiting dilution assays. However, the frequency of K562 killers was highest in PBL. It is of interest that K562 killer precursors were present in TIL when, at least in short term assays, direct killing of this target was not found [96]. Similar findings have suggested to others that the lymphokine-activated killers are not NK but represent a maturational stage of T lymphocytes [74]. Some support for this view comes from the observation that IL-2 dependent LGL-derived cultures show mature T lymphocyte phenotype rather than that characteristic of fresh NK cells. However, cells staining with Leu 7 (antihuman NK) are present in small numbers in tumour and tonsils (Hughes, unpublished finding). The identification of cells in the LGL fractions which proliferate in response to IL-2 is currently in progress using micromanipulation techniques to derive unambiguous clones from cells of defined phenotype.

The results of the frequency analysis of proliferative and cytotoxic precursors stimulated in MLTC is in accord with the studies of Vánky et al. [61]. They noted that MLTC was the most efficient inducer of specific autologous tumour killers although stimulation was accompanied by increased lytic activity against K562. Using MLTC, lysis of allogeneic tumour was only rarely seen. However, as detailed above, autologous tumour killers can also be induced by stimulation of patient PBL by mixed lymphocyte culture mitogens and even lectin free IL-2 preparations. It is possible that the IL-2 stimulation is the common feature of each of these [74] since our own stu-

dies have shown IL-2 production in MLTC particularly from TIL. Cold target inhibition assays in MLTC stimulated cultures confirm tumour specificity with blocking of lysis only apparent when fresh autologous tumour cells but not lymphocytes are used as inhibitors [97].

The studies described above detail the methods which have been found useful for the generation of cells which recognise autologous tumour in proliferative and cytotoxic assays. We have taken advantage of the selective capacity of IL-2 to expand activated cells i.e. those upon which interaction with the stimulating antigen has induced the IL-2 receptor. Following stimulation in MLTC, blasts were isolated on discontinuous Percoll gradients using methods which have improved specificity in cultured T cells (CTC) derived from MLC [98–100]. These MLTC blasts were then expanded in CM to given MLTC-blast-CTC. The cytotoxic activity of these lines as well as their restimulation upon exposure to cells of the autologous tumour has been measured after at least 10 days in culture during which time cell numbers increased over 10-fold. Cytotoxic reactivity of MLTC-blast-CTC showed the same pattern of reactivity as fresh effectors or those generated following *in vitro* stimulation i.e. was restricted to cells of the autologous tumour. In this respect the lines obtained are similar to those of other groups raised against solid tumours [97] and autologous EBV-infected B lymphoblastoid cell lines [101–102]. In the latter system cytotoxicity was restricted by HLA and extensive mapping studies have been carried out to show restriction to EBV antigens associated with HLA A B and C specificities [102]. Similar studies are envisaged in the solid tumour systems although the availability of sufficient tumour cells to allow maintenance of cell lines and repeated specificity assays presents some problems. Specificity of MLTC-blast-CTC has been established using a panel of tumour targets both matched and mismatched with the autologous tumour with respect to site and histology as well as autologous and allogeneic monocytes and lymphoblasts. The lysability of targets used was confirmed, using autologous MLTC-blast-CTC or MLC-blast-CTC. More recently, we have been able to test against cell preparations from autologous normal lung in patients with pulmonary neoplasia. In 6 cases normal lung was not killed when tumour preparations were lysed by MLTC-blast-CTC. These data must be interpreted with caution since in no case would the normal lung preparation correspond in cytology to that of, for example, squamous cell carcinoma. Normal lung preparatins show highly variable cytology with many cell types represented. MLTC-blast-CTC showed relatively low cytotoxicity against K562 compared with stimulated cultures which were not blast isolated [84]. However, in some cultured lines anti-K562 killing persisted together with the autologous killers. The exact significance of this finding is not clear, but our present attempts to clone both effector types (specific and NK) which

have met with some initial success, should help in this area. To date we do not have clones with specific cytotoxic reactivity against autologous tumour but in agreement with others we have been able to clone K562 killers from both MLC activated populations [86, 87, 103] and LGL fractions [91]. Continuing effort is being made in this area.

In addition to their cytotoxic activity, MLTC-blast-CTC also proliferate upon re-exposure to cells of the autologous tumour. In identifying this reactivity the primed lymphocyte test introduced by Reinsmoen et al. [104] to the study of leukaemia associated antigens has been used. MLTC-blast-CTC were washed free of conditioned medium and plated in the presence of autologous tumour, lymphocytes, monocytes or allogeneic control cells for two days and uptake of (^{3}H)-thymidine measured over the last 6 hours of culture. In all cases MLTC-blast-CTC were restimulated by cells of the autologous tumour but not by autologous or allogeneic monocytes or lymphocytes. In contrast to the restriction of cytotoxicity to autologous tumour PLT responses appeared to be confined to cells of a particular organ site and histology. In three tumour types where sufficient numbers have been examined, we have recorded cross-reactivities among colon, breast and lung tumours, but in no case did lung stimulate colon or breast. Among the lung tumours where different histological types of tumour are found, squamous cell carcinoma MLTC-blast-CTC were stimulated by autologous and allogeneic squamous cell tumours, but not by adenocarcinoma cells. It was anticipated that PLT responses like those to soluble antigens [105, 106] would be restricted by Ia region determinants. Experiments are currently in progress with anti Ia antisera to try to clarify this response, but it is of interest that a similar cross reactivity between leukaemias has been described using PLT responses of cloned T cells [107] and preliminary evidence with our own cloned lines showed cross reactivity among the squamous cell carcinomas of lung (Vose, unpublished findings).

The studies detailed in this section have been directed towards the establishment of cultured T cell lines with specific reactivity towards autologous tumour. The lines and clones which are now available should enable us to more thoroughly identify the nature of the antigens involved in these reactions. Experience to date appears to exclude reaction against self D/DR (failure to lyse or undergo secondary stimulation with autologous lymphocytes or monocytes) but it has yet to be shown that other normal tissue components (differentiation or organ related antigens) are involved. To a large extent the studies are limited by availability of suitable controls. However, in pursuing these goals the use of these lines in passive cellular immunotherapy has been considered and a trial is currently envisaged.

4. CELLULAR IMMUNOTHERAPY WITH CULTURED T LYMPHOCYTES

The first attempt to alter clinical course in cancer patients by injection of autologous cultured cells were made by Moore and Gerner in 1970 [108]. Autologous lymphocytes were taken into culture in RPMI 1640 supplemented with foetal calf serum under conditions where 20–50% of cells were immunoglobulin producers and contained herpetic-like virus particles similar to those found in cultures from Burkitt lymphoma. Three patients with advanced malignancy received injections of between 1 and 100 grams of cultured cells without clinical benefit. Three further patients were given multiple injections of autologous cultured cells receiving in total between 362 g and 672 g lymphocytes. In two of the three patients there was short lived subjective response. Another group of patients were infused into different sites with allogeneic lymphocytes cultured for 3 days with irradiated tumour cells from the recipient. No clinical response was noted even in five patients receiving between 115 and 236 grams of lymphocytes.

More recently, autologous lymphocytes from three patients with pulmonary metastases from sarcomas were expanded in conditioned medium containing IL-2 [109]. Conditioned media were prepared by stimulation of pooled leukocytes from 4–6 donors with phytohaemagglutinin. No attempt to remove lectin was reported. Infusion of cultured cells ($1.5-5 \times 10^9$) did not induce reduction in size or number of pulmonary metastases.

The importance of these studies is that they establish that infusion of large numbers of autologous cultured lymphocytes are not associated with gross toxicity. Moore and Gerner [108] describe the adverse effects of cell infusion as fever and chills during treatment and for several hours afterwards with infrequent nausea. Lotze et al. [109] report no toxicity or changes in blood chemistry or haematological profile. Similar studies in chimpanzees in which 10^9 MLC primed autologous lymphocytes were reinfused also found little toxicity [110]. No significant changes of total white count, absolute lymphocyte count, or renal function occurred. Transient increase of SGOT and in one chimp of SGPT were noted in the first 24 hours but liver biopsy was normal. A weak antibody response to PHA (used in preparation of CM) was found but no activity was detected against the cultured T cells used for infusion. In spite of the lack of gross toxicity upon reinfusion of cultured lymphocytes, there are clearly a number of other problems to resolve before cellular immunotheapy of human cancer becomes a reality.

4.1 *The introduction of transformed cells*

A major concern with this form of treatment is the introduction of cells transformed in culture. As a minimum estimate, it should require 4–6

weeks in culture to obtain the large number of specifically activated cells required for trials. In our own studies we have grown 5×10^8 cells (approximately 10–20 doublings) from culture initiation with 5×10^5 MLTC blasts in 4 weeks. Moore and Gerner [108] found no evidence that cultured cells induced a leukaemic state and this has not been found in animal studies. Indeed, the strict dependence of cultured T cell maintenance on addition of IL-2 as well as the stability of karyotype of cells maintained in the presence of antigen and feeder cells over prolonged periods in both animal [111] and man (Vose, unpublished findings) argue against this possibility. However, murine CTC maintained on IL-2 alone have shown karyotypic abnormalities [112] and initial clinical studies must therefore be necessarily restricted to patients with advanced disease in the absence of firm *in vivo* data. Expectation of clinical benefit with lymphocytotherapy may, however be limited because cultured T cells are so critically dependent upon exogenous IL-2 for continued proliferation. Without IL-2 cells complete the cell cycle, collect in G_1 and die as blast cells if further growth factor is not added. It is therefore possible that unlike the situation in which normal autologous [113] or HLA compatible [6] fresh cells are injected, persistence of CTC *in vivo* will be short unless there is contemporaneous administration of IL-2 as has been shown to be effective in murine passive cellular immunotherapy [114] or unless cultured T cells could be induced to secrete IL-2 *in vivo* upon stimulation with the antigen. It is for this reason that we view the capacity of our CTC lines and clones to be restimulated in PLT assays by tumour in the absence of added IL-2 as critical. The administration of CM containing IL-2 in man has been reported by Hersey et al. [115]. Intravenous injections of CM showed that the time for clearance of half the IL-2 activity was 22.5 minutes and was associated with neutrophilia, lymphocytopenia and pyrexia as well as glucocorticoid release and hypoglycaemia. Interestingly, following this *in vivo* administration lymphocytotoxicity against cultured autologous melanoma cells was detectable which was not evident in the untreated patient. Again the nature of the effector cell is unknown. In crude stimulated lymphocyte supernates it is not possible to identify the role fo IL-2 or other lymphokine/monokines in the preparations in the clinical effects seen. However, administration of purified IL-2 and alloantigen, significantly increases the level of both allospecific kill and NK in murine studies [78]. The injection of highly purified lymphokine preparations may offer an alternative or adjunct to cellular immunotherapy.

4.2 *Lymphocyte traffic*

Methods have recently become available by which human lymphocyte traffic can be studied by reinjection of radiolabelled autologous blood lymphocytes. Isotopes used have included ^{51}Cr chromium, ^{67}Ga Gallium and

indium [109, 113]. Labelling with indium-oxine has been found to be most useful in studies in man because of its high labelling efficiency, low elution and produced gamma emissions ideal for external imaging on gamma camera equipment [109, 113, 116]. However, experience in murine systems demonstrating compromise of physiological behaviour of small lymphocytes by intercellular radiation damage [117] means that labelling conditions must be carefully controlled for radiation dose for maintainance of normal recirculation. In the study of Wagstaffe et al. [113] in man, damaged lymphocytes were rapidly removed from the blood by uptake to the lung and liver with subsequent elimination to the liver. Splenic uptake was minimal. By contrast, 20% of injected viable lymphocytes could be detected in the blood over a 48 hour period and there was marked uptake to the spleen at 4 hours. Lung and liver uptake was minimal. Spleen passage time has been calculated as 6–8 hours and there was gradual accumulation of labelled lymphocytes in peripheral lymph nodes. Interestingly, Wagstaffe et al. [118] have extended their observation to patients with malignancy and have been able to show alterations of traffic in leukaemia/lymphoma. Labelled peripheral blood lymphocytes accumulate in lymph nodes involved with Hodgkins disease and at the site of tumour in a patient with abdominal metastases of osteogenic sarcoma.

The capacity of blood lymphocytes to traffic to secondary tumour deposits offers hope that should similar redistribution patterns be maintained in anti-tumour cultured T cell lines, their restimulation by putative tumour associated antigens may produce IL-2 and lead to persistence at sites of disease. However, studies in animal systems have shown that CTC have abnormal homing patterns. Alloantigen specific or MSV reactive cytotoxic T cell clones fail to eliminate ^{131}IUDR-labelled tumour cells of appropriate specificity in the peritoneal cavity when injected intravenously, although they are reactive when injected at the tumour site [119]. Significantly, uncloned secondary MLC cells cultured for 19 days were effective when injected IV suggesting that impairment of homing was not a result of culture. Traffic experiments with labelled cloned lines showed accumulation in the lungs. These data confirm those of Lotze et al. [109] who used non-clonal cultured murine T cell lines which were taken up by lungs, liver and showed minimal accumulation in spleen (4%). As discussed above, lymphocyte traffic data must be interpreted with the awareness that labelling conditions can markedly influence lymphocyte behaviour. The study of Lotze et al. [109] reported only 25% of normal non-cultured lymphocytes in spleen at 4 hours and 25% in liver using ^{51}Cr labelled cells. Using the same isotope or Indium III-oxine, Sparshott et al. [117] reported 44% of cells in spleen, 3% in liver and 3% in lungs with subsequent redistribution to peripheral lymph nodes of rats under optimal conditions. Lung/liver accumulation of

normal lymphocytes was found only with damaged cells emphasising that patterns should be carefully scrutinised before deciding between physiological migration and artefact. It is possible that cells maintained in IL-2 for prolonged periods may be more susceptible to radiation or manipulation damage although recent reports suggest a more fundamental deficit in cultured T cell clone: the lack of receptors of lymphocytes for high walled endothelium as evidenced by their failure to migrate to peripheral lymphoid tissue or to bind to frozen sections of lymph node [120].

In examining the distribution pattern of Indium III-oxine or ^{67}gallium-labelled cultured human T lymphocytes, Lotze et al. [109] noted preferential uptake to liver at 3 hours with lesser counts in lung and spleen. Over 48 hours radioactivity was concentrated in the liver, although 25% of total cells remained in the spleen. No evidence of localisation to peripheral lymphoid organs was presented. The failure of CTC to conform to normal recirculation patterns was disappointing and suggested removal of large blast cells at the lungs as the first capillary bed. Whether this is attributable to technical difficulties in labelling, growth of cells in the presence of lectin or to real properties of cultured cells remains to be established. Tumour infiltrating lymphocytes appear to represent a population rich in cytotoxic and proliferative precursors reactive with the autologous tumour and a relatively high proportion of them spontaneously express the receptor for IL-2 in that they proliferate without requirement for *in vitro* stimulation i.e. they are activated [94]. There is also evidence that TIL produce large amounts of IL-2 upon stimulation *in vitro* with autologous tumour cells. These observations together with the phenotypic analysis showing elevated numbers of OKT_8 $^+$ cytotoxic/suppressor cells and activated T cells (Tac$^+$) suggests that trapping of reactive lymphocytes at primary tumour sites does occur with concentration of T cells with anti-tumour potential. It is for these reasons that we wish to re-examine the distribution pattern of CTC and are currently attempting to raise CTC from *in vitro* activated TIL since these may show a more specific homing pattern than those derived from PBL.

In spite of problems of recirculation of CTC there is growing evidence in murine systems that innoculation of sensitised lines can have real therapeutic potential (reviewed by Fefer, this volume). Cultured T lymphocytes have been used in passive immunotherapy of virus induced leukaemia [121] in protecting against otherwise lethal intranasal influenza virus challenge [122] and in inducing accelerated skin graft rejection [123]. Again it is not clear if, in bringing about their *in vivo* effects, traffic of injected cells to appropriate sites is necessary or if they can be mediated by factor production and activation of host defence mechanisms.

4.3 *Specificity*

Different authors have taken markedly different views on the type of cultured T lymphoid cells which might be effective in passive cellular immunotherapy. For example, it has been suggested that patients' lymphocytes activated by lectin [73] or by growth in IL-2 [92, 74, 83] could be used because they lyse autologous tumour. Our view has been that reinnoculation should only be attempted with highly specific anti-tumour T cells and possibly with clones [67, 84, 97]. The reasons for this are manifold but prime among them is the consideration that injected non-specific effectors may have deleterious effects on the host. Kedar et al. [92] have shown that in long-term (18 hour) ^{51}Cr release assays, autologous and allogeneic normal lymphoblasts are lysed by cultured lymphoid effectors and there is to date no definition of the effector cell type expanded in IL-2 which lyse a range of freshly isolated tumour targets [91]. NK cells are also known to lyse bone marrow cells and thymocytes [93] and injected normal lymphocytes do traffic to bone marrow [118]. It was because of these considerations that we have made strenuous efforts to derive anti-tumour T cells of low activity against NK sensitive cell lines and normal tissues. It is also of note that the lines we have obtained show both proliferative and cytotoxic activities against cells of the autologous tumour [67]. A growing body of evidence exists suggesting that in both allograft and tumour rejection in immunocompromised animals passive transfer can be achieved only with helper T cell subpopulations and not by lines of high specific cytotoxic activity [33-36]. This does not rule out a contribution by cytotoxic T cells but does suggest that activation of other effector mechanisms *in vivo* may be of prime importance. It may thus be considered that the successful elimination of radiolabelled tumour cells injected intraperitoneally by secondary MLC blasts under conditions where cytotoxic T cell clones of the same specificity were ineffective reflects not the failure of the clones to recirculate, but their inability to secrete the lymphokines necessary for elimination *in vivo* [119]. It is clear that in human disease, blood monocytes and tumour-associated macrophages can have high cytotoxic potential against autologous tumour cells but not normal control cells [124]. In addition, recent evidence suggests that isolated large granular lymphocytes which mediate NK also kill autologous fresh tumour cells [45]. Thus the concentration of tumour immunologists on the cytotoxic activity of activated populations has enormous impact on the view of the nature of tumour-associated antigens, but may not be appropriate to experiments of immunotherapy.

4.4 *Suppression*

Human malignant disease is associated with depressed cell-mediated immunity, particularly in patients with advanced neoplasia. The primary me-

diators of this suppression are cells of the monocyte/macrophage series which exert their effect, at least in part, by secretion of prostaglandin [55–58]. In addition, studies from this laboratory have shown that at extravascular sites (tumour-draining lymph nodes and tumour-infiltrating lymphocytes as well as in the blood of some patients) T cells may also depress mitogen and MLC driven proliferation [58, 60, 125].

Other sources of suppression have also been described in the serum of cancer patients [125, 126]. Roth et al. [127] were able to extract carbohydrate structures from different fresh tumours which non specifically inhibited proliferation of blood lymphocytes in response to mitogen and attogeneic cells. Any attempt to induce tumour rejection by administration of active effectors must consider the role of this pre-existing state of suppression on the outcome of immunotherapy. The barrier of T cell-mediated immunosuppression to immunotherapy with sensitised T cells has been demonstrated in murine systems [15]. The reversal of this state of immunosuppression induced by the Meth. A fibrosarcoma has been achieved by combination therapy using both cyclophosphamide and immune T cells. Neither alone were effective in eliminating established malignancy as previously shown for the MSV induced sarcoma and Friend virus leukaemia [14, 119]. These studies suggest that combination therapy might also be important in the treatment of human disease.

4.5 *Tumour heterogeneity*

It is becoming increasingly apparent that tumours consist of 'a mixed bag of cells' as a result of the evolution of phenotypic variants during tumour growth [128, 129]. One manifestation of this is the establishment of metastases at different sites which home preferentially to that site [131]. Other variants may show antigenic differences with regression or progression depending upon the expression of certain membrane components capable of inducing T cell-mediated responses [37] or susceptibility to NK [132]. The degree of heterogeneity of human disease is apparent from their response to chemotherapeutic agents or secretion of marker substances. It is not possible to say at this time if this heterogeneity will present a barrier to successful immunotherapy, although it is clearly a possibility. The immunogenicity of tumour metastases has been studied infrequently, but some positive reactions have been found [41]. The heterogeneous nature of tumours may be an argument against the use of cloned cytotoxic of helper T cell lines in initial studies since MLTC-blast-CTC may more fully express functions against a range of different determinants.

5. CONCLUSIONS

In this report we have attempted to review briefly the possible role of anti-tumour cultured T cells in immunotherapy. Studies are incomplete and a number of problems remain before any real answer to the questions raised can be made.

A number of technical and fundamental problems need to be answered. Does the rapid elimination of labelled infused cells in the lungs and the liver in patients studied to date, result from isotopically damaged cells or is this pattern of elimination inherent in cultured blasts? If expanded cell populations 'home' to the tumour how great is tumour mediated immunosuppression and can it be overcome? A more fundamental problem is that the effector cell may be a helper rather than a cytotoxic T lymphocyte. However, the relative safety of infusions of large numbers of cultured autologous lymphocytes suggests that it is now appropriate to begin to address these questions in patients with advanced malignant disease. It is probable, however, that if such treatments are shown to have some effect in advanced disease that their major application would be in patients with small tumour volumes as in the adjuvant setting or after reduction of bulky disease with radiotherapy or chemotherapy.

ACKNOWLEDGEMENTS

This work was supported by grants from the Cancer Research Campaign of Great Britain. The authors are grateful to Dr M. Moore and Professor D.G. Crowther for encouragement, and to Ms E. Mercer for preparation of the manuscript.

REFERENCES

1. Rosenberg SA, Terry WD: Passive immunotherapy of cancer in animals and man. Adv Cancer Research 25:323–388, 1977.
2. Woodruff MFA, Nolan B: Preliminary observations on treatment of advanced cancer by injection of allogeneic spleen cells. Lancet 2:426–429, 1963.
3. Andrews GA, Congdon CC, Edwards CL: Preliminary trials of clinical immunotherapy. Cancer Res. 27:2535–2541, 1967.
4. Symes MO, Riddell, AG, Immelman EJ, Terblanche J: Immu ologically competent cells in the treatment of malignant disease. Lancet 1:1054–1056, 1968.
5. Sch arzenberg L, Mathe G, Schneider M, Amiel JL, Cattan A, Schlumberger JRb: Attempted adoptive immunotherapy of acute leukaemia by leucocyte transfusions. Lancet 2:365–368, 1966.
6. Yonemoto RH, Terasaki PI: Cancer immunotherapy with HLA-compatible thoracic duct lymphocyte transplantation. Cancer 30:1438–1443, 1972.

7. Nadler SH, Moore GE. Arch Surg (Chicago) 99:376–381, 1969.
8. Scanlon, EF, Hawkins RA, Fox WW, Smith WS: Fatal homotransplanted melanoma: A case report. Cancer 18:782–789, 1965.
9. Nadler SH, Moore GE: Clinical immunologic study of malignant disease: Response to tumour transplants and transfer of leucocytes. Ann Surg 164: 482-490, 1966.
10. Humphrey LJ, Jewell WR, Murray DR, Griffen WO: Immunotherpay for the patient with cancer. Ann Surg 173:47–54, 1971.
11. Krementz ET, Mansell PWA, Hornung MO, Samuels MS, Sutherland CA, Benes EN: Immunotherapy of malignant disease: The use of viable sensitized lymphocytes or transfer factor prepared from sensitised lymphocytes. Cancer 33:394–401, 1974.
12. Alexander P, Delorme EJ: The use of irradiated immune lymphoid cells for immunotherapy of primary tumours in rats. In: Immunological parameters of host-tumour relationships, Weiss DW (ed.). New York: Academic Press, 1971, pp 239–245.
13. Borberg H, Oettgen HF, Choudy K, Beatty EJ Jrb: Inhibition of established transplants of chemically induced sarcomas in syngeneic mice by lymphocytes from immunised donors. Int. J Cancer 10:539–547, 1972.
14. Fefer A: Immunotherapy and chemotherapy of Molony sarcoma virus-induced tumors in mice. Cancer Res. 29:2177-2183, 1969.
15. North RJ: Cyclophosphamide-facilitated adoptive immunotherapy of an established tumor depends on elimination of tumour-induced suppressor T cells. J Exp Med 55:1063–1074, 1982.
16. Morgan DA, Ruscetti FW, Gallo R: Selective *in vitro* growth of T lymphocytes from normal human marrow. Science 193:1007–1008, 1976.
17. Ruscetti FW, Morgan DA, Gallo RC: Functional and morphologic characterization of human T cells continuously grown *in vitro*. J. Immunol 119:131–138, 1977.
18. Foley EJ: Antigenic properties of methylcholanthrene-induced tumours in mice of the strain of origin. Cancer Res 13:835–837, 1953.
19. Baldwin RW: Immunity to methylcholanthrene-induced tumoours in induced rats following atrophy and regression of the implanted tumours. Brit J Cancer 9:652–657, 1955.
20. Prehn RT, Main JH: Immunith to methylcholanthrene-induced sarcomas. J. Nat. Cancer Inst. 18: 769-779, 1957.
21. Klein G. Sjogren HO, Klein E, Hellstrom KE: Demonstration of resistance against methylcholanthrene-induced sarcomas in the primary autochtonous host. Cancer Res. 20: 1561–1572, 1960.
22. Klein G, Sjogren HO, Klein E: Demonstration of host resistance against isotransplantation of lymphomas induced by the Gross agent. Cancer Res 22: 955–961, 1962.
23. Pasternak G, Graffi A: Induction of resistznce against isotransplantation of virus-induced myeloid leukaemias. Brit J Cancer 17: 532–539, 1963.
24. Sjogren HO: Studies on specific-transplantation resistance to polyoma-virus-induced tumours: III. Transplantation resistance to genetically compatible polyoma tumours induced by polyoma tumour homografts. J. Nat. Cancer Inst. 32: 645-659, 1964.
25. Cochran AJ: Man, cancer and immunity. New York: Academic Press, 1978, pp 206.
26. Moore M: Human tumour-associated antigens; Methods of *in vitro* detection. In: Immunological aspects of cancer, Castro JE (ed.) Lancaster: MTP Press, 1978, pp 51–87.
27. Takasugi M, Mickey MR, Takasugi PI: Reactivity of lymphocytes from normal persons on cultured tumour cells. Cancer Res 33:2898–2902, 1973.
28. Rosenberg EB, McCoy JL, Green SS, Donelly FC, Savorsky DF, Levine PH, Herberman RB: Destruction of human tissue-culture cell lines by human peripheral blood lymphocytes in ^{51}Cr-release cellular cytotoxicity assays. J. Natl Cancer Inst 52:345–352, 1974.

29. Hewitt HB, Blake ER, Walder AS: A critique of the evidence for active host defense against cancer based on personal studies of 27 murine tumours of spontaneous origin. Brit J Cancer 33: 241–259, 1976.
30. Herberman RB (ed): Natural cell-mediated immunity against tumours. New York: Academic Press, 1980.
31. Zinkernagel RM, Doherty PC: Restriction of *in vitro* T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semi-allogeneic system. Nature 248:701–702, 1974.
32. Shearer GM, Rehn TG, Garbarino CA: Cell-mediated lympholysis of trinitrophenyl modified autologous lymphocytes. J. Exp. Med. 141:1348–1363, 1975.
33. Loveland BE, McKenzie IFCb: Which T cells cause graft rejection. Transplantation 33:217–221, 1982.
34. Dallman MJ, Mason DW: Role of thymus-derived and thymus-independent cells in murine skin allograft rejection. Transplantation 33:221–224, 1982.
35. Fernandez-Cruz E, Gilman SC, Feldman JD: Immunotherapy of a chemically-induced sarcoma in ratsb: Characterization of effector T cell subset and nature of suppression. J. Immiunol. 128:1112–1117, 1982.
36. Greenberg PD, Cheever MA, Fefer A: Eradication of disseminated murine leukaemia by chemoimmunotherapy with cyclophosphamide and adaptively transferred immune syngeneic Lyt $1^{+}2^{-}$ lymphocytes. J Exp Med 154:952–963, 1981.
37. Urban JL, Burton RC, Holland JM, Kripke ML, Schreiber H: Mechanism of syngeneic tumour rejection: Susceptibility of host-selected progressor varients to various immunological effector cells. J Exp Med 155: 557–573, 1982.
38. Vánky FT, Stjernswärd J: Lymphocyte stimulation (by autologous tumour biopsy cells). In: Immunodiagnosis of cancer, Part 2, Herberman RB, McIntire KR (eds). New York: Marcel Dekker Inc., 1979, pp 998–1032.
39. Sheehy MJ, Sondel PM, Bach ML, Wank R, Bach FH: HL-A LD (lymphocyte defined) typing: A rapid assay with primed lymphocytes. Science 188:1308–1310, 1975.
40. Vose BM: Separation of tumour and host cell populations from human neoplasms. In: methodological surveys, Vol. 2, Reid E (ed). Chichester, U.K.: Ellis Harwood, 1982, pp 45–56.
41. Vose BM, Vánky F, Klein E: Lymphocyte cytotoxicity against autologous tumour biopsy cells in humans. Int J Cancer 20:512–519, 1977.
42. Vose BM, Moore M: Natural cytotoxicity in humans: Susceptibility of freshly isolated tumour cells to lysis. J Natl Cancer Inst 65:257–263, 1980a.
43. Vánky FT, Argov S, Einhorn S, Klein E: The role of alloantigens in natural killing: Allogeneic but not autologous tumour biopsy cells are senstive for interferon induced cytotoxicity of human blood lymphocytes. J Exp Med 151:1151–1165, 1980.
44. Timonen T, Saksela E, Ranki A, Hayry P: Fractionation, morphological and functional characterization of effector cells responsible vor human natural killer activity against cell-line targets. Cell Immunol. 48:133–148, 1979.
45. Serrate SA, Vose BM, Timonen T, Ortaldo JR, Herberman RB: Association of human natural killer cell activity against human primary tumours with large granular lymphocytes. In: Natural cell-mediated immunity, Vol. 2, Herberman RB (ed). New York: Academic Press. In press, 1982.
46. Becker S, Kiessling R, Lee N, Klein G: Modulation of sensitivity to natural killer cell lysis zfter *in vitro* explanation of a mouse lymphoma. J Natl Cancer Inst 61:1495–1498, 1978.
47. Vánky FT, Vose BM, Fopp M, Klein E: Human tumor-lymphocyte interaction *in vitro*b: VI Specificity of primary and secondary autologous lymphocyte-mediated cytotoxicity. J. Natl. Cancer Inst. 62:1407–1413, 1979.

48. Vose BM, Vánky F, Fopp M, Klein E: Restricted autologous lymphocytotoxicity in lung neoplasia. Brit J Cancer 38:375–381, 1978.
49. Vose BM, Gallagher P, Moore M, Schofield PF: Specific and non-specific lymphocyte cytotoxic function in colon cancer. Brit J Cancer 44:846–855, 1981.
50. Vose BM: Specific T-cell mediated killing of autologous lung tumoour cells. Cell Immunol 55:12–19, 1980.
51. Dickmeiss E, Soeberg B, Svejgaard A: Human cell-mediated cytotoxicity against modified target cells is restricted by HLA. Nature '»%:?'«-528, 1977.
52. McMichael AJ, Ting H, Zweerink HJ, Askonas BA: HLA restriction of cell-mediated lysis of influenza virus-infected human cells. Nature 270:524–526, 1977.
53. Vose BM, Vánky F, Klein E: Human-tumour lymphocyte interaction *in vitro*: V. Comparison of the reactivity of tumour infiltrating blood and lymph node lymphocytes with autologous tumour cells. Int. J. Cancer 20:895–902, 1977a.
54. Klein E, Vánky F, Galili U, Vose BM, Fopp M: Separation and characteristics of tumour-infiltrating lymphocytes in man. Cont. Topics in Immunobiol 10:79–107, 1980. Witz IP, Hanna Jr MG (eds). New York: Plenum Press.
55. Goodwin JS, Messner RP, Bankhurst AD, Peake GT, Saiki JH, Williams RC: Prostaglandin-producing suppressor cells in Hodgkins disease. N. Engl. J. Med. 297: 963–968, 1977.
56. Quan PC, Burtin P: Demonstration of non-specific suppressor cells in the peripheral lymphocytes of cancer patients. Cancer Res 38:288–296, 1978.
57. Jerrells TR, Dean JH, Richardson GL, McGoy JL, Herberman RB: Role of suppressor cells in depression of *in vitro* lymphoproliferative responses of lung cancer and breast cancer patients. J. Natl. Cancer Inst 61:1001–1009, 1978.
58. Vose BM, Moore M: Heterogeneity of suppressors of mitogen responsiveness in human malignancy. Cancer Immunol. Immu other. 2:163–171, 1980b.
59. Vánky F, Klein E, Stjernsward J, Nilsonne V, Rodriguez L, Peterffy A: Human tumour-lymphocyte interaction *in vitro*: II Conditions which improve the capacity of biopsy cells to stimulate autologous lymphocytes. Cancer Immunol Immunother 5:63–69, 1978.
60. Vose BM, Moore M: Suppressor cell activity of lymphocytes infiltrating human lung cancer. Int J Cancer 24:579–585, 1979.
61. Vánky F, Argov S, Klein E: Tumor biopsy cells participating in systems in which cytotoxicity of lymphocytes is generated: Autologous and allogeneic studies. Int. J. Cancer 27: 273–280, 1981.
62. Vánky F, Vose BM, Fopp M, Klein E, Sjernward J: Human tumour lymphocyte interaction *in vitro*: Comparison of the results with autologous tumour stimulation (ATS) and lymphocytotoxicity (ALC). In: Immunodiagnosis and immunotherapy of malignant tumors, Flad H-D, Herforth CL, Betzler M (ed). Berlin: Springer Verlag, 1979, pp 143–152.
63. Kuntz MM, Innes JB, Weksler ME: Lymphocyte transformation induced by autologous cells: IV. Human T lymphocyte proliferation induced by autologous or allogeneic non-T lymphocytes. J Exp Med 143b: 1042–1054, 1976.
64. Huber CH, Fink U, Leibold W, Schmalzl AF, Peterson PA, Klareskag L, Braunsteiner H: The role of adherent HLA-DR$^+$ mononuclear cells in autologous and allogeneic MLR. J. Immunol. 127: 726–731, 1981.
65. Weksler ME, Kuntz MM, Birnbaum G, Innes JB: Lymphocyte transformation induced by autologous cells. Fed. Proc. 37: 2370–2373, 1978.
66. Huber C, Merkenschlager M, Gottringer C, Royston I, Fink U, Braunsteiner H: Human autologous mixed lymphocyte reactivity is primarily specific for xenoprotein determinants adsorbed to antigen-presenting cells during rosette formation with sheep erythrocytes. J Exp Med 155:1222–1227, 1982.

67. Vose BM, Bonnard GD: Specific cytotoxicity against autologous tumour and proliferative responses of human lymphocytes grown in Interleukin 2. Int J Cancer 29:33–39, 1982.
68. Vande Stouwe RH, Kunkel HG, Halper JP, Weksler ME: Autologous mixed lymphocyte culture reactions and generation of cytotoxic T cells. J Exp Med 146: 1809-1814, 1977.
69. Miller RA, Kaplan HS: Generation of cytotoxic lymphocytes in the autologous mixed lymphocyte culture. J Immunol 121:2165–2167, 1978.
70. Bach ML, Bach FH, Zarling JM: Pool priming: A means of generating T lymphocytes cytotoxic to tumour or virus-infected cells. Lancet: 20–21, 1978.
71. Zarling JM, Robins HI, Raich PC, Bach FH, Bach ML: Generation of cytotoxic T lymphocytes to autologous human leukaemic cells by sensitization to pooled allogeneic normal cells. Nature 274: 269–271, 1978.
72. Strausser JL, Mazumder A, Grimm EA, Lotze MT, Rosenberg SA: Lysis of human solid tumors by autologous cells sensitized *in vitro* to alloantigens. J Immunol 127:266–271, 1981.
73. Mazumder A, Grimm EA, Zhang HZ, Rosenberg SA: Lysis of fresh human solid tumours by autologous lymphocytes activated *in vitro* with lectins. Cancer Res 42:913–918, 1982.
74. Grimm EA, Mazumder A, Zhang HZ, Rosenberg SA: Lymphokine-activated killer cell phenomenon. J Exp Med 155:1823–1841, 1982.
75. Ortaldo JR, Timonen TT, Vose BM, Alvarez JA: Human natural killer cells as well as T cells maintained in continuous culture with IL-2. In: The potential role of T cell subpopulations in cancer therapy. Fefer A (ed). New York: Raven Press. In press.
76. Bonnard GD, Schendel DJ, West WH, Alvarez JM, Maca RD, Yasaka K, Fine RL, Herberman RB, de Landazuri MO, Morgan DA: Continued growth of normal human T lymphocytes in culture with retention of important functions. In: Human lymphocyte differentiation: Its application to cancer, Serrou B, Rosenfeld C (eds). Amsterdam: Elsevier/North Holland Biomedical Press, 1978, pp 319–326.
77. Farrar JJ, Benjamin WR, Hilfiker ML, Howard M, Farrar WL, Fuller-Farrar J: The biochemistry, biology and role of interleukin 2 in the induction of cytotoxic T cell and antibody-forming B cell responses. Imm. Rev. 63: 129-166, 1982.
78. Gillis S, Mochizuki DY, Conlon PJ, Hefeneicher SH, Ramthon CA, Gillis AE, Frank MB, Henney CS, Watson JD: Molecular characterisation of interleukin 2. Imm. Rev. 63:167–209, 1982.
79. Bonnard GD, Yasaka K, Jacobson D: Ligand-activated T cell growth factor induced proliferation: Absorption of T cell growth factor by activated T cells. J Immunol 123:2703–2708, 1979.
80. Bonnard GD, Yasaka K, Maca RR: Continued growth of functional T lymphocytes: Production of human T cell growth factor. Cell Immunol 51:390–401, 1980.
81. Vose BM, Moore M: Cultured human T cell lines kill autologous solid tumours. Imm Letters 3: 237–241, 1981.
82. Hersey P, Bindon C, Edwards A, Murray E, Phillips G, McCarthy WH: Induction of cytotoxic activity in human lymphocytes against autologous and allogeneic melanoma cells *in vitro* by culture with interleukin 2. Int J Cancer 28: 695–703, 1981.
83. Lotze MT, Grimm EA, Mazumder A, Strausser JL, Rosenberg SA: Lysis of fresh and cultured autologous tumour by lymphocytes cultured in T cell growth factor TCGF. Cancer Res 41:4420–4425, 1981.
84. Vose BM, Bonnard GD: Human tumour antigens defined by cytotoxicity and proliferztive responses of cultural lymphoid cells. Nature 296:359–362, 1982.
85. Alvarez JM, de Landazuri MO, Bonnard GD, Herberman RB: Cytotoxic activities of normal cultured human T cells. J Immunol 121:1270–1275, 1978.

86. Pawelec GP, Hadam MR, Ziegler A, Lohmeyer J, Rehbein A, Kumbier I, Wernet P: Long term culture, cloning and surface markers of mixed leucocyte culture derived human T lymphocytes with natural killer-like cytotoxicity. J. Immunol 128: 1892-1896, 1982.
87. Sugamura K, Tanaka Y, Himura Y: Two distinct human cloned T cell lines that exhibit natural killer-like and anti-human effector activities. J Immunol 128:1749–1752, 1982.
88. Dennert G, Yogeeswaron G, Yamagata S: Cloned cell lines with natural killer activity. J Exp Med 153:545–566, 1981.
89. Nabel G, Bucalo LR, Allard J, Wigzell H, Cantor H: Multiple activities of a cloned cell mediating natural killer cell function. J Exp Med 153:1582–1591, 1981.
90. Timonen T, Saksela E: Isolation of human natural killer cells by density centrifugation. J Imm Meth 36: 285–290, 1980.
91. Vose BM, Bonnard GD: Limiting dilution analysis of the precursor cells capable of specific cytotoxic or proliferative responses to human tumour cells and their expansion with lectin-free interleukin-2. In: Modern trends in human tumor immunology, Serrou B, Rosenfeld (eds). Amsterdam: Elsevier, 1982, pp 385–393.
92. Kedar E, Herberman RB, Gorelik E, Sredni B, Bonnard GD, Navarro N: Antitumour reactivity *in vitro* and *in vivo* of mouse and numan cells cultured with T cell growth factor. In: The potential role of T cell subpopulations in cancer therapy, Fefer A (ed). New York: Raven Press. In press, 1981.
93. Hansson M, Kiessling R, Anderson B, Kärre K, Roder J: NK cell-sensitive subpopulations in thymus: Inverse correlation to host NK activity. Nature 278: 174–176, 1979.
94. Vose BM: Quantitation of proliferative and cytotoxic precursor cells against numan tumours: Limiting dilution analysis in peripheral blood and at the tumour site. Int. J. Cancer. In press, 1982.
95. Galili U, Vánky F, Rodriguez L, Klein E: Activated T lymphocytes within human solid tumours. Cancer Immunol. Immunother 6: 129–133, 1979.
96. Vose BM, Vánky F, Argov S, Klein E: Natural cytotoxicity in man: Activity of lymph node and tumour-infiltrating lymphocytes. Eur J Imm 7:753–757, 1977b.
97. Vánky F, Gorsky T, Gorsky Y, Masucci M-G, Klein E: Lysis of tumor biopsy cells by autologous T lymphocytes activzted in mixed cultures and propagated with T cell growth factor. J. Exp. Med. 155: 83–95, 1982.
98. Bach FH, Inouye H, Hank JA, Alter B: Human T lymphocyte clones reactive in primed lymphocyte typing and cytotoxicity. Nature 281:307–309, 1979.
99. Pappas F, Bonnard GD, Hartzman RJ, Strong DM: Human BL cells grown as continued T cell cultures. Transplant Proc 11:1986–1989, 1979.
100. Schendel DJ, Wank R, Bonnard GD: Genetic specificity of primary and secondary proliferative and cytotoxic responses of human lymphocytes grown in combined culture. Scand J Immunol 11:99–107, 1980.
101. Sugamura K, Tanaka Y, Hinuma Y: Establishment of human cytotoxic T-cell lines specific for Epstein-Barr virus-transformed autologous cells. Int J Cancer 28:137–142, 1981.
102. Rickson AB, Moss DJ, Allen DJ, Wallace LE, Rowe M, Epstein MA: Reactivation of Epstein–Barr virus-specific cytotoxic T cells by *in vitro* stimulation with the autologous lymphoblastoid cell line. Int J Cancer 27:593–601, 1981.
103. Vose BM, Riccardi C, Marchmont RJ, Bonnard GD: Clonal analysis of human natural killer cells. In: Natural cell-mediated immunity, Vol. 2, RB Herberman (ed). New York: Academic Press. In press, 1982.
104. Reinsmoen NL, Kersey JH, Yunis EJ: Antigens associated with acute leukaemia detected in the primed lymphocyte test. J Natl Cancer Inst 60:537–543, 1978.
105. Infante AJ, Atassi MZ, Fathman CG: T cell clones reactive with sperm whale myoglobin: Isolation of clones with specificity for individual determinants on myoglobin. J Exp Med 154: 1342–1356, 1981.

106. Sredni B, Tse HY, Chen C, Schwartz RH: Antigen-specific clones of proliferating T lymphocyte: I. Methodology, specificity and MHC restriction. J Immunol 126b: 341–347, 1980.
107. Csako G, Binder RA, Kales AN, Neefe JR: Cloning of human lymphocytes reactive with autologous leukaemia cells. Cancer Research 40:3218–3221, 1980.
108. Moore GE, Gerner RE:Cancer immunity - Hypothesis and clinical trial of lymphocytotherapy for malignant disease. Anrgn Surg 172:733–739, 1970.
109. Lotze MT, Line BR, Mathisen DJ, Rosenberg SA: The *in vitro* distribution of autologous jhuman and murine lymphoid cells grown in T cell growth factor (TCGF): Implications for the adoptive immunotherapy of tumors. J. Immunol 125:1487–1493, 1980.
110. Slease RB, Strong DM, Gowith KE, Bonnard GD: Clinical effects of infusions into chimpanzees of primed autologous cultured T cells. J Natl Cancer Inst 67:489–493, 1981.
111. Glasebrook AL, Sarmiento M. Loken MR, Dialynas DP, Quintens J, Eisenberg L, Lutz CT, Wilde D, Fitch FW: Murine T lymphocyte clones with distinct immunological function. Imm. Rev. 54:225–266, 1981.
112. Engers HD, Collovo D, North M, von Boehmer H, Hoas W, Hengartner H, Nabholz M: Characterization of cloned murine cytolytic T cell lines. J. Immubol. 125: 1481–1486, 1980.
113. Wagstaffe J, Gibson C, Thatcher N, Ford WL, Scharma H, Benson W, Crowther D: A method for following human lymphocyte traffic using indium-III oxine labelling. Clin. Exp Immunol 43:435–442, 1981.
114. Cheever MA, Greenberg PD, Fefer A, Gillis S: Augmentation of the anti-tumour therapeutic efficacy of long-term cultured T lymphocytes by *in vitro* administration of purified interleukin-2. J Exp Med 155:968–980, 1982.
115. Bindon C, Czerniecki M, Ruell P, Edwards A, McCarthy WH, Harris R, Hersey P: Clearance rates and systemic effects of intravenously administered interleukin-2 (IL-2) containing preparations in human subjects. Brit J Cancer, in press, 1983.
116. Lavender JP, Goldman JM, Arnot RB, Thakur ML: Kinetics of Indium-III labelled lymphocytes in normal subjects and patients with Hodgkins disease. Br Med J ii:797–799, 1977.
117. Sparshott SM, Sharma H, Kelly D, Ford WL: Factors influencing the fate of Indium-III labelled lymphocytes after transfer to syngeneic rats. J Imm Meth 1981.
118. Wagstaffe J, Gibson C, Thatcher N, Ford WL, Sharma H, Crowther D: Human lymphocyte traffic assessed by indium-III oxine labelling: clinical observations. Clin Exp Immunol 43:443–449, 1981.
119. MacDonald HR, Sekaly RP, Kanogawa O, Thiernesse N, Taswell C, Cerottini J-C, Weiss A, Gliselvook AL, Engers HD, Kelso A, Brunner KT, Bron C: Cytolytic T lymphocyte clones. Immunobiol 161:84–106, 1982.
120. Dailey MO, Falkman CG, Butcher EC, Pillemer E, Weissman I: Abnormal migration of T lymphocyte clones. J Immunol 128: 2134–2136, 1982.
121. Cheever MA, Greenberg PD, Fefer A: Specific adoptive therapy of established leukaemia with syngeneic lymphocytes sequentially immunized *in vitro* and *in vitro* and non-specifically expanded by culture with interleukin-2. J Immunol 126:1318–1322, 1981.
122. Lin Y-L, Askonas BA: Biological properties of an influenza A virus-specific killdr T cell clone. J Exp Med 154:225–234, 1981.
123. Rosenstein M, Eberlein T, Kemeny MM, Sugarboker PH, Rosenberg SA: *In vitro* growth of murine T cells: VI Accelerated skin graft rejection caused by adoptively transferred cells expanded in T cell growth factor. J Immunol 127:566–571, 1981.
124. Vose BM: Cytotoxicity of adherent cells associated with some human tumours and lung tissues. Cancer Immunol Immunother 5: 173–179, 1978.

125. Vose BM, Ferguson R, Moore M: Mitogen responsiveness and inhibitory activity of mesenteric lymph node cells: Conditioned medium containing T cell growth vactor reverses suppressor function. Cancer Immunol Immunother., in press, 1982.
126. Sample WF, Gerlner HR, Chretien PB: Inhibition of phytohaemag-glutinin induced *in vitro* lymphocyte transformation by serum from patients with carcinoma. J Natl Czncer Inst 46: 1291-1297, 1971.
127. Roth JA, Grimm EA, Gupta RK, Ames RS: Immunoregulatory factors derived from human tumours: I. Immu ologic and biochemical characterisation of factors that suppress lymphocyte proliferative and cytotoxic responses *in vitro*. J Immunol 128:1955-1962, 1982.
128. Nowell PC: The clonal evaluation of tumour cell populations: Acquired genetic lability permits stepwise selection of varient sublines and underlies tumour progression. Science 194: 23-28, 1976.
129. Marx JL: Tumours: A mixed bag of cells. Science 215: 275-277, 1982.
130. Poste G, Fidler IJ: The pathogenesis of cancer metastasis. Nature 283: 139-146, 198.
131. Collins JL, Patek PE, Cohn M: Tumorigenicity and lysis by natural killers. J Exp Med 153:89-106, 1981.

5. Human Tumor Antigens: Detection and Characterization with Monoclonal Antibodies

KENNETH O. LLOYD

1. INTRODUCTION

The development of a general procedure for the routine production of monoclonal antibodies (moAbs[1]) of defined specificity [1] opened up a new era in tumor immunology. It raised the possibility that answers to such basic questions as the existence of tumor-specific antigens in human cancer and whether patients are capable of mounting an immune response to their tumors would finally be answered. Moreover, the application of monoclonal antibodies in the diagnosis and therapy of cancer promises to give the clinician a powerful new approach to the treatment of malignancy. Although none of these goals has yet been achieved it is appropriate at this time to review progress in this area.

The power of monoclonal antibodies was realized as soon as the hybridoma technique was introduced in 1975. In fact, their advantages had been appreciated before that time and had led to earlier efforts to produce monoclonal antibodies (reviewed in ref. [2]). The most important property of moAbs is their precise specificity, that is their ability to recognize a single antigenic determinant (epitope) on a complex antigen. It follows from this property that any 'cross-reactions' observed must be due to the presence of the same antigenic determinant in the cross-reactive component. It is

[1] Abbreviations used. MoAb: monoclonal antibody; Ig: immunoglobulin; MHA: mixed hemagglutination assay; gp: glycoprotein; PAP: peroxidase-anti-peroxidase; PA: protein A; RBC: red blood cell; cALL: common acute lymphocytic leukemia; AML: acute myelocytic leukemia; T-ALL: T-cell acute lymphocytic leukemia; CML-BC: chronic myelocytic leukemia in blast crisis; CLL: chronic lymphocytic leukemia; ATL: adult T-cell leukemia; CALLA: common acute lymphocytic leukemia antigen; PBL: peripheral blood lymphocytes; HTLV: human T-lymphoma virus; DMSO: dimethyl sulfoxide; con A: concanavalin A; β_2m: β_2-microglobulin; SDS-PAGE: sodium dodecyl sulfate - polyacrylamide gel electrophoresis; CEA: carcinoembryonic antigen; SSEA: stage specific embryonic antigen.

R.B. Herberman (ed), Basic and Clinical Tumor Immunology. ISBN-13: 978-1-4613-3875-8
© 1983. Martinus Nijhoff Publishers, Boston.

important to realize, however, that the component (protein, glycoprotein, glycolipid, etc.) does not have to be biochemically identical to the original antigen but merely that it carry the same or very closely related determinant. This in contrast to the situation with even the best absorbed antiserum which may contain antibodies of unknown specificity in addition to the desired antibody. In practical terms, the ability to produce an antibody to a single antigen in a complex mixture, by selecting for the appropriate hybridoma clones, and the continued availability of the antibody-secreting cell line are important advantages. Monoclonal antibodies also have disadvantages and problems. A minor drawback is their inability to form immunoprecipitates, in immunodiffusion gels or in solution, with antigens having only one determinant for the antibody. A more serious limitation is sometimes the precise specificity of the antibody – the very property that is so prized in most applications. An example of this is the inability of many moAbs prepared to cell surface antigens to recognize denatured, precursor, or *in vitro* translated forms of the antigen. Polyclonal antisera would usually have at least a small proportion of antibody reacting with all forms of the antigen.

The format of this review will be to discuss first the results with different tumor types and this will be followed by a more speculative section on the nature and classification of the antigens detected. Certain areas such as the application of moAbs to diagnosis and therapy, a currently evolving field, will not be discussed.

2. MALIGNANT MELANOMA

Melanoma, together with leukemias-lymphomas, are the human tumors that have been most studied using mouse monoclonal antibodies. The reason for the emphasis on melanoma would seem to be the availability of many cell lines derived from this tumor and because of previous interest in the immunology of melanoma stemming mainly from the analysis of antibodies in the sera of melanoma patients reacting with their own or allogeneic tumors (reviewed in ref. [3]). Specificity studies have been somewhat hampered by the difficulty of analyzing the normal cell counterpart, i.e. melanocytes. This problem has, however, been largely resolved by the recent development of methods for the short term culture of melanocytes [4], nevus cells [5] and by the use of tissue sections of normal skin.

Melanomas and cell lines derived from them exhibit great heterogeneity in cell morphology, pigmentation and other characteristics [6–8]. These differences may reflect the malignant transformation of melanoblasts-melanocytes in different differentiation stages, although this view is far from being confirmed (see Section 2.13). The picture is further confused by uncertain-

ties in the precise origin of the various types of melanoma; some investigators believing that some melanomas originate from nevi and are therefore distinctly different from those originating from melanocytes [9]. Whatever the origin of the heterogeneity in melanomas and melanoma cell lines, it results in an additional complication in the analysis of monoclonal antibodies prepared to this tumor. Since the initial study of Koprowski et al. [10] in 1978, over 30 papers have been published on the development and analysis of mouse monoclonal antibodies to malignant melanoma. These investigations have resulted in the description of approximately 60 different moAbs and it was therefore very gratifying that a workshop arranged by the National Cancer Institute in April of 1982 could agree on the grouping of these reagents into only 10–12 different antigenic systems [11]. At the time of writing no antibody defining an antigen strictly confined to melanoma cells has been described, nevertheless, a number of these antibodies have sufficiently restricted specificities to be worthy of further study. Table 1 summarizes the data available on antibodies to malignant melanoma with the reagents being arranged according to the molecular weight of the antigen detected.

2.1 *High molecular weight antigen complex*

A number of groups have described moAbs detecting a group of high molecular weight antigens [12–14]. These antibodies (e.g. 225.28, 653.25/40, 9.2.27 and Mel-14) are highly reactive with almost all melanoma cell lines and tumor tissues tested. Some astrocytomas and skin carcinomas also react with these moAbs [11]. Although this group of moAbs is unreactive with most other tumors and normal cells and tissues tested, there is definite reactivity with blood vessel walls and with nevi. Normal adult and fetal fibroblasts and hair follicles may also be weakly reactive [11]. Melanocytes in sections of normal skin were reported [15] to be unreactive with 225.28 (using indirect immunofluorescence) whereas cultured melanocytes were weakly positive using the anti-mouse Ig-MHA assay [11]. Cryostat sections of both melanomas and nevi exhibited a fluorescent membrane pattern when stained with moAbs 225.28. All types of melanoma lesions tested were positive (with moAb 653.25) and there was no relationship to the clinical stage of the tumor, with the degree of invasiveness of primary tumors or with the melanotic nature of the tumor [16]. Considerable heterogeneity of staining within a given tumor was, however, noticed, the significance of this finding, which has been observed with other antibodies and in tumors other than melanoma, is at present unclear. In summary, the antigen(s) expressed by this group of moAbs is strongly expressed on melanomas with most other cells and tissues, both normal and malignant, being negative or weakly positive. The antigen is not strongly expressed on skin melanocytes but can

Table 1. Monoclonal antibodies to malignant melanoma.

Antibody designation	Ig class	Molecular weight of antigen(s) detected	Chemical nature of antigen	Distribution of antigen	Ref.
653.25	IgG1	280,000 and >500,000	Glycoprotein (280 kd) and proteoglycan	Most melanomas (both culture cell lines and tumors) were positive. Antigen is also expressed on some brain tumors and cultured normal melanocytes and skin nevi. Other cells and tissues (including skin melanocytes) were negative. Basal epidermal cells, skin carcinomas, and hair follicles also positive.	13
763.74		280,000 and >500,000			11
225.28	IgG2a	280,000 and >500,000			15
Mel-14	IgG1	280,000 and >500,000			12
9.2.27	IgG2a				14
691-19-19	IgG1	250,000	Glycoprotein	Most melanomas, neuroblastomas and astrocytomas positive. Nevi also positive. Not on adult skin melanocytes, lymphocytes or carcinomas.	10
5.1	IgG1	210,000	Protein	Approx. half of melanomas and carcinoma cell lines tested are positive. Normal brain and kidney and some fetal tissues are also positive.	18
6.1	IgM	155,000	Protein	Approx. 50% of melanomas and 80% of kidney carcinomas tested are positive. Low or no expression with other cell types.	18
R_{23}	IgG1	130,000	Glycoprotein	Most melanomas and astrocytomas cell lines positive. Cultured normal kidney cells, blood vessel endothelium and smooth muscle also positive.	19
N_9	IgG1	130,000	Glycoprotein		19
Q_{24}	IgG1	130,000	Glycoprotein		19
436.G10	IgG1	122,000	Glycoprotein		20
69115 Nu-4 B	IgG2a	116,000; 95,000; 29,000 & 26,000	Glycoproteins	Most melanomas and astrocytomas (cell lines and tumors), positive also fetal epithelial cells. Skin melanocytes and intradermal nevi negative.	10, 22

4.1	IgG1	97,000	Glycoprotein	Antigen present in high amounts in most cultured melanoma cells, in melanoma tumors, and in some other tumors. Sweat gland epithelia also positive. Present in small amounts in normal cells. Melanocytes negative.	25
96.5	IgG2a	97,000			43, 27
118.1	IgG2a	97,000			28
8.2					28
I_{12}	IgG2b	95,000 (pI = 5.0)	Glycoprotein	Same as 4.1 group	19
L_{10}	IgH1	95,000 (pI = 5.0)			19
K_5	IgG1	95,000 (pI = 5.0)			19
376.96	IgG2a	94,000	Glycoprotein	All melanoma (cell lines and tissues) and most carcinomas positive. Weakly expressed on epithelia of gastrointestinal tract. Fibroblasts positive. Nevi positive but skin melanocytes negative	30
705.F6	IgG2b	100,000			20, 11
465.12	IgG2a	94,000; 75,000; 70,000 & 30,000	Glycoproteins	Common cytoplasmic antigen.	31
15.75	IgG2a	74,000	Glycoprotein	Most melanomas and carcinomas positive. Lymphoid and red blood cells negative.	34
15.95	IgG3	49,000	Glycoprotein	Similar to 15.75	34
R_{24}	IgG3	N.A.[1]	Glycolipid (G_{D3})	Most (90%) melanoma cell lines and tumors positive. Astrocytomas, melanocytes (cultured and skin) and MOLT-4 also positive. Other cells and tumors weak or negative.	19, 38
4.2	IgM	N.A.	(G_{D3})		39
3.1	IgG1	N.D.[2]	N.D.	Expressed strongly only on immunizing melanoma cell line.	35
Mel-5	N.D.	N.D.	N.D.	Most melanoma cell lines positive; other cells tested were negative.	12
Mel-7	N.D.	N.D.	N.D.	Similar to Mel-5	12

[1] N.A. – Not applicable.
[2] N.D. – Not determined.

be detected on skin nevi and cultured melanocytes; this result indicates that synthesis of this antigen is stimulated or activated in pigmented cells undergoing proliferation. There is some difference in the pattern of reactivity between the various moAbs of this group. These variations may be due to the reactivity of the antibodies with different determinants on the same antigen(s). There is some indication that the high molecular weight complex is antigenically heterogenous since some moAbs precipitated only a subpopulation of the molecules [11].

This group of antibodies immunoprecipitates a complex series of high molecular weight components from melanoma cells. From the cell lysates labeled with [^{3}H] valine [16, 14], a component with a molecular weight of 240–280 kd was precipitated together with a higher molecular weight component (100–1000 kd) which barely entered a 5% acrylamide SDS-PAGE gels. The mobilities of the components are unchanged under reducing conditions indicating that they are not linked by interchain disulfide bonds. Although the molecular weight of the antigen recognized by these moAbs resembles fibronectin, it seems clear that it is a distinct entity. Further studies [17] have shown that the antigen recognized by moAb 9.2.27 (and presumably other members of the group) is a proteoglycan complex. The lower molecular weight (240–280 kd) component is a sialoglycoprotein with *N*-linked carbohydrate chains, whereas the higher molecular component contains sulfate groups, has carbohydrate chains susceptible to alkaline β-elimination and is digested by chondroitinase. Evidence to date suggests that the antigen detected by these moAbs is a chondroitin sulfate-type proteoglycan and that the 240–280 kD chain is the 'core' glycoprotein of such a complex. MoAb 9.2.27 seems directed against determinants in the core glycoprotein; whether this is the case with the other antibodies has yet to be determined.

In keeping with its proteoglycan nature, the antigen detected by this group of antibodies does not behave as an integral membrane protein. It can be extracted from melanoma cells under mild dissociating conditions such as 0.1 M urea and even by phosphate buffer [14, 16]. In fact moAb 9.2.27 was produced by immunizing a mouse with a 4 M urea extract of M14 melanoma cells. Although the antigen detected by moAb 9.2.27 is shed into the culture medium of cells growing in a chemically-defined medium [14], this does not seem to be the case when the cells are grown in RPMI medium containing fetal bovine serum [14].

Antibody 691-19-19 [10] also precipitates a high molecular weight antigen (240,000 daltons) but since the antigen is not associated with a very large component it would seem that this antibody recognizes an antigen different from the proteoglycan complex type [11]. Although the specificity of the antibody is very similar to the first group in that it react mainly with mela-

nomas, astrocytomas and nevus cells, it is reactive with a lower proportion of melanomas than are the other moAbs.

2.2 *Gp210 and gp155*

Antibodies 5.1 and 6.1, produced by immunization with cultured melanoma cells [18], were not as restricted in their patterns of reactivity as the antibodies described above. MoAb 5.1, which precipitates a protein of 210,000 daltons from melanoma cells, reacted with approximately half of all melanoma and carcinoma cell lines tested. The antigen is also present in normal adult brain and on fetal brain and heart. MoAb 5.1 was reported not to react with normal melanocytes. The other antibody described in this study (moAb 6.1) reacts most strongly with melanomas and renal cancer cells. An interesting aspect of the specificity of this antibody is that although it precipitates a 150,000 dalton protein from melanoma cells, two components of approximately 250,000 and 60,000 daltons were identified in renal cancer cells.

2.3 *Gp130*

A number of moAbs have been derived which recognize a glycoprotein with a molecular weight in the 120,000–130,000 range [19, 20]. These antibodies react with most melanoma and astrocytomas. A few carcinomas and cultured normal kidney epithelia cells also carry the antigen recognized by these antibodies. Immunofluorescent antibody studies have shown that this antigen is strongly expressed on blood vessel endothelia and normal smooth muscle cells. Gp130 (originally referred to as gp150 in reference [19]) was originally detected in a study in which melanoma cell line SK-MEL-28 was used as the immunogen; four different antibodies (N_9, R_{23}, Q_{16}, and Q_{24}) were derived. More recently another group of antibodies (moAbs 846, 829 and 239) have become available which also identify gp130 [21]. Another laboratory has described a moAb (436 G10) that identifies an antigen with an almost identical weight [20]. Although moAb R_{23} and 436.G10 do not block each other in binding assays, it would seem that they react with the same glycoprotein. This group of moAbs recognizes at least four different epitopes on gp130 [21].

Gp130 is a very acidic glycoprotein with an isoelectric point of 4.2; this characteristic seems to be related to the high sialic acid content of the antigen [19]. The glycoprotein binds to wheat germ agglutinin, a property that indicates the existence of regions of clustered sialylated carbohydrate chains in the molecule. Biosynthetic studies using pulselabeling experiments and tunicamycin have shown that the peptide chain of gp130 has an apparent molecular weight of only 80,000 [21]. This form is converted in the cell into

the mature form via an intermediate form with a molecular weight of 100,000.

2.4 *Gp116/95/29/26 complex*

MoAb 69-115-Nu-4-B reacted preferentially with cultured melanoma and astrocytoma cells in binding assays [10]. The specificity was later confirmed by examining sections of melanomas, nevi and normal skin [22]; only melanomas (17/17) stained with moAb Nu 4B. Other normal tissues have not yet been examined.

This antigen has an unusual four chain structure [23, 24]. The molecular weight of the native antigen is about 250,000; it consists of four subunits of apparent molecular weights of 116,000, 95,000, 29,000 and 26,000 daltons. The 116 kD component is linked to the 29 and 26 kD by disulfide bonds and the 95 kD component seems to be associated with the complex by non-covalent interactions. It is not known whether only one of the four chains carries the Nu 4B antigenic determinant or whether the determinant is present on more than one of the chains.

2.5 *Gp95*

A glycoprotein of approximately 95,000 daltons is the best studied melanoma cell surface antigen. Ten different moAbs (I_{12}, K_5, L_1, L_{10}, R_{19}, M_{17}, 96.5, 4.1, 8.2 and 118.2) reacting with this antigen, which is referred to as gp95 by Dippold et al. [19] and p97 by Woodbury et al. [25], have been described.

Approximately half of all melanoma cell lines tested express high levels of gp95. The antigen can be detected using immunofluorescence or PAP techniques on a similar proportion of melanoma tumors. The antigen is also present on astrocytomas and a few epithelial cancers [19, 25]; other cell types express a very low amount of gp95. Recent studies on tissue sections using immunofluorescence or PAP techniques have shown that some antibodies in this group react with sweat gland epithelia in normal skin [26, 11].

The quantitative expression of gp95 in a large variety of cells and tissues has been determined using a double-determinant immunoassay (DDIA). This assay uses two moAbs reacting with two different antigenic determinants on the same antigen and is a highly specific and sensitive assay for p97 [27]. Of the cultured cells studied using this procedure, SK-MEL-28, the immunizing cell line, contained most p97 and bound 270 ng of moAb 96.5/mg membrane protein. Other melanoma cell lines had reactivities in the 58–170 ng/mg range whereas fibroblasts reacted in the 0.3–4.8 ng/mg range. Application of the assay to tumor samples showed a wide variation in the expression of p97; of the melanomas examined, 4 had binding levels of

Table 2. Epitopes of melanoma gp95 antigen recognized by monoclonal antibodies (cf. ref. [28]).

Epitope	Antibodies recognizing epitope	Expressed on 40 kd fragment
a	96.5, 4.1, M_{17}	Yes
b	118.1	Yes
c	8.2, L_1, L_{10}, R_{19}	Yes
d	I_{12}	No
e	K_5	No

150–610 ng/mg, 6 had intermediate binding levels of 13–75 ng/mg and 6 had very low levels (0.1–6.8 ng/mg). These results are in good agreement with the reactivity of anti-p97 antibodies with tumor sections using immunohistological procedures. When normal adult tissues were examined using the DDIA method it was found that all tissues contain detectable amounts of p97; levels ranged from 0.4 ng/mg in skin to 10 ng/mg in uterus. Some fetal tissues, particularly colon, expressed substantial amounts of the antigen.

A considerable amount of information is also available on the biochemical properties of the antigen detected by this group of antibodies. The antigen can be labeled metabolically with both ^{35}S-methionine and ^{3}H-glucosamine, indicating that it is a glycoprotein [19]. Its isoelectric point is 5.0 and it binds to concanavalin A but not to wheat germ agglutinin [19]. The molecule does not exist as a disulfide-linked dimer or multimer [25]. This is in contrast to another cell surface antigen with a similar subunit molecular weight detected by OKT-9 and other moAbs (see Section 12). It has been shown that some of the moAbs reacting with gp95 identify different epitopes on the molecule. This was originally indicated by small differences in the ability of the antibodies to react with certain cell lines [19]. Later, binding studies [28] using ^{125}I-labeled moAbs showed that the 10 different antibodies recognized 5 different epitopes (Table 2). Information is also available on the localization of the determinants in the gp95 molecule. Digestion of gp95 with either trypsin or papain results in the formation of a protease-resistant fragment of 40 kd [19, 28]. This fragment carries the antigenic determinants for 96.3, 4.1, M_{17}, 118.1, 8.2, L_1, L_{10} and R_{19} moAbs but not for the other two moAbs. This peptide can also be released by proteolysis of intact cells and it carries at least some of the carbohydrate chains of the antigen [28].

Recent studies have also suggested a biological function for this glycoprotein. Brown et al. [29] purified gp95 by affinity chromatography and determined its N-terminal amino acid sequence using a new, highly sensitive

p97				Gly	Met	Glu	Val	Arg	Trp	Cys	Ala	Thr	Ser	Asp	?	Glu
Transferrin			Val	Pro	Asp	Lys	Thr	Val	Arg	Trp	Cys	Ala	Val	Ser	Glu	His	Glu
Lactoferrin	Gly	Arg	Arg	Arg	Arg	Ser	Val	Gln	Trp	Cys	Ala	Val	Ser	Gln	Pro	Glu

Figure 1. N-terminal amino acid sequence of melanoma p97 antigen compared with that of transferrin and lactoferrin. Shared amino acids residues are boxed (ref. [29]).

protein sequenator. By comparison with the amino acid sequence of other proteins, gp95 was found to have sequence homology with transferrin and lactoferrin (Figure 1). Although moAbs to gp95 do not react with transferrin, a mouse antiserum to denatured gp95 cross-reacted with denatured transferrin thus indicating a structural relationship between these proteins. Like transferrin and lactoferrin, gp95 binds iron. When melanoma cells were incubated with ^{59}Fe and lysed in nonionic detergent, approximately 4% of the added ^{59}Fe could be bound by moAb to gp95. Other unrelated antibodies bound less than 0.05% of the added iron. Although these studies need to be confirmed by direct functional studies, they suggest a role for gp95 in iron binding and possibly transport in melanoma cells.

2.6 *Gp94*

MoAb 376.96 reacts with an antigen having a molecular weight (94 kD) very similar to that of the antigen detected by the above group of antibodies [30]. Preclearing experiments with moAb I_{12} showed, however, that the antigens were not identical. Moreover, gp94 has a different tissue distribution than gp95. Thus, the former antigen is widely distributed on carcinomas as well as on melanoma cell lines whereas gp95 is mainly confined to melanomas. In indirect immunofluorescent antibody tests, most normal tissues were negative for gp94 although weak staining was given by stomach epithelium and some basal epidermal cells. Primary (15/18) and metastatic (13/20) melanomas gave positive staining reactions, as did 6/12 skin basal and squamous cell carcinomas. Interestingly, the fluorescence was less bright in metastatic melanomas than in primary melanomas. Among benign skin lesions, moAb 376.96 stained compound nevi (5/8) but not blue nevi (0/6) or other pigmented skin lesions (0/6).

The antigen detected by moAb 376.96 was shown to be a glycoprotein by metabolic labeling with ^{3}H-glucosamine and surface labeling with ^{125}I-Iodine. Gp94 does not exist as disulfide-linked polymers. As it is easily released from the cells, e.g. by extraction with 10 mM phosphate buffer, 3M KCl or 0.1 M urea, it is probably not an integral membrane component. A glycoprotein of 94 kD was also precipitated by moAb 376.96 from bladder carcinoma cell lines (MANO and T-25) and from a prostate carcinoma cell line

(H 494). MoAb 705F6 [20, 11] seems to detect the same antigen as 376.96.

2.7 *gp94/75/70/30 complex*

MoAb 465.12 identifies an antigen which has been localized as a cytoplasmic component as well as being released into culture supernatants [15, 31]. Indirect immunofluorescent staining of tissue sections showed that this antigen was detectable in most melanoma cell lines and tumors but not in skin melanocytes. The expression on other normal adult tissues was variable, with colon and rectal epithelial cells being strongly positive, some tissue, such as breast and kidney, showing weak expression and others, such as brain and liver, being negative. Interestingly, tumors deriving from tissues normally unreactive with moAb 465.12 were positive for the antigen. Most intradermal and compound nevi also stained with this antibody. The pattern of immunofluorescent staining in melanomas and other cells was of granular nature and located in the cytoplasm of the cell; no plasma membrane staining was noticed, although since melanoma cells show low binding to moAb 465.12 as detected with ^{125}I-protein A, small amounts of the antigen may be expressed on the cell surface [31].

Structurally, 465.12 antigen extracted from melanoma cells consists of four glycosylated proteins of 94,000, 75,000, 70,000 and 25,000 daltons; the 75 and 70 kd components are most strongly labeled with ^{3}H-glucosamine [31]. This antigen is also shed into the culture medium of melanoma cultures; in this form, the 94 kD component predominates. Treatment of cell cultures with tunicamycin reduces the amount of 46.512 antigen that is secreted. The relationship of the secreted to the cytoplasmic forms of this antigen is unclear, as is the interrelationship of the four chains of the cellular forms to each other. The 75 kd component incorporates only [^{3}H]mannose and [^{3}H]glucosamine and may therefore have high mannose-side chains characteristic of a biosynthetic precursor of one of the other chains.

By immunization of mice with a partially purified spent medium antigen from melanoma cells, Chee et al. [32, 33] derived a moAb (F11) that reacted with melanomas and carcinomas and their spent medium components. Although they have not as yet been directly compared, it seems possible that moAbs F11 and 465.12 react with the same antigen. MoAb F11 precipitated three components (100, 77 and 75 kD) from extracts of ^{3}H-glucosamine labeled cells, however pulse-chase experiments strongly indicated that the two smaller glycoproteins were biosynthetic precursors of the 100 kd antigen. Interestingly, neuroblastoma cells, which also react with moAb F11, secrete an antigen with a smaller molecular weight of 90,000 [33].

2.8 *Gp74 and Gp49*

Two antibodies (15.75 and 15.95), derived from the same fusion, were found to react with melanomas (8/9 cell lines) and with various carcinomas [34]. Lymphocytes, red blood cells and fibroblasts were found to be unreactive. Fresh melanoma tumor cells were also positive for the antigens detected by these antibodies. MoAb 15.95 precipitated a 74 kD component from both a melanoma (Ju So) and a breast carcinoma (SK-BR-3) cell line; the molecular weight is unchanged by the addition of reducing agent. MoAb 15.75 immunoprecipitates a monomeric 49 kd glycoprotein from the same two cell lines.

2.9 *Antigen 3.1*

Three monoclonal antibodies (3.1, 3.2 and 3.3) derived from a mouse immunized with human melanoma cell line M1804 were found to react strongly only with the immunizing cell line [35, 36]. The antigen was expressed in smaller amounts in M1801 and M1477 melanoma lines but not in detectable amounts on any of the other cell lines tested. The antibodies did not precipitate any components from labeled cells and the biochemical nature of the antigens is unknown. Antibody 3.2, which is a IgG2a immunoglobulin, is cytotoxic to melanoma cells in the presence of complement (CDC) and can mediate antibody-dependent cellular cytotoxicity (ADCC). This is in contrast to moAb 3.1, a IgG1 antibody, which is only weakly active in these assays [37].

2.10 *Antigens Mel-5 and Mel-7*

These two moAbs were derived from the same fusion as moAb Mel-14 that identifies the high molecular weight melanoma antigen complex [12]. In binding assays, both Mel-5 and Mel-7 bind to melanoma cell lines but not with any other tumors or normal cells tested. The antibodies are directed against different antigenic determinants but it is not known if they react with the same antigen since no biochemical data are available.

2.11 *Ganglioside (G_{D3}) antigen*

Two moAbs (R_{24} and 4.2) prepared against melanoma cells have been found to identify a ganglioside antigen [19, 38, 39]. Ab R_{24}, a IgG3 antibody, was found to react with all melanoma cell tested and with 2/5 astrocytoma cell lines; no other cell line tested using direct serological assays or absorption tests was positive. Cultured normal melanocytes were, however, found to react with moAbR_{24} and normal brain tissue gave variable results in absorption tests [19]. In later studies [40], using immunofluorescent assays on tissue sections, moAbR_{24} was found to react with 33/35 fresh melanoma tumor samples but not with a variety of carcinomas examined. Skin

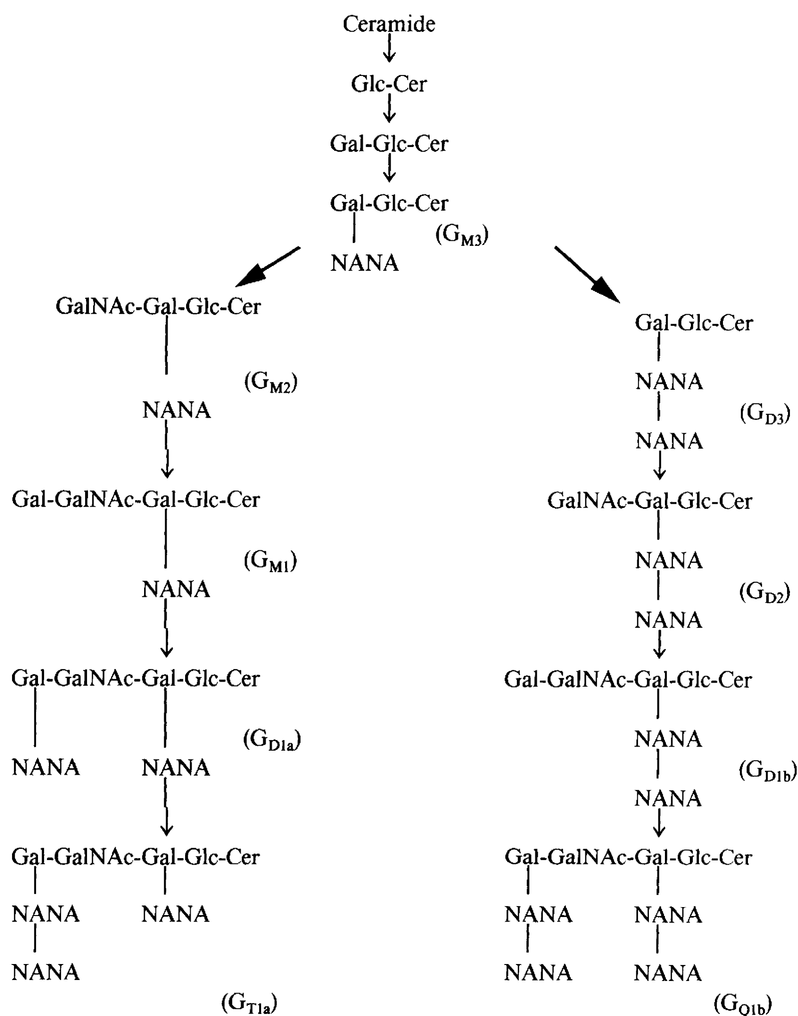

Figure 2. Partial structures and biosynthetic inter-relationships of some common gangliosides. Modified after Yu and Ando [170]. The inter-residue linkages in the other structures are identical to those in G_{Q1b}: αNANA(2→8)αNANA(2→3)βGal(1→4)βGalNAc(1→4)βGal[3←2NANAα(8←2)NANAα](1→4)Glc-Cer. Abbreviations: NANA: *N*-acetylneuraminic acid; Gal: D-galactopyranose; GalNAc: *N*-acetyl-D-galactosamine; Glc: D-glucopyranose.

melanocytes and occasional, unidentified cells in the dermis were also positive but the antigen could not be detected, within the limit of the technique, on any of the other normal tissues examined. MoAb 4.2 (IgM) showed a specificity similar to moAbR$_{24}$, except that a small number of melanoma cell lines tested were essentially unreactive [39]. A few other tumor cell lines (from ovarian, brain cancer and liposarcoma) were also positive, as was 8402 T-cell line. Binding to normal kidney and lung tissue was also observed.

Early studies showed that moAbR_{24} detected a heat- and protease-resistant antigen with the properties of a glycolipid [19]. Further investigation [38] identified the antigen as the G_{D3}[NANA(2→8)NANA(2→3) βGal(1→4)Glc-Cer], which had been earlier isolated from brain. This identification was based on carbohydrate composition, partial structural analysis and co-migration of the antigen with brain G_{D3} in thinlayer chromatography [38]. Furthermore, it was shown that brain G_{D3} reacts with moAbR_{24} but that other gangliosides (G_{M1}, G_{M3}, G_{D1a}, and G_{T1b}) were unreactive (Figure 2). MoAb 4.2 was also shown to identify G_{D3} ganglioside [41]. In this study, the inter-sugar linkages were fully identified and shown to precisely correspond to brain G_{D3}. One difference from brain G_{D3} was however found: the ceramide of melanoma ganglioside had a predominance of longer chain fatty acids (C22:0 and 24:0) whereas brain ganglioside had C18:0 as the major fatty acid.

It had previously been noted by Portoukalian et al. [42] that human melanomas have G_{M3}, G_{M2} and G_{D3} as their predominant gangliosides. This characteristic ganglioside pattern in melanomas was partly confirmed by Pukel et al. [38] who showed that most melanomas have a very simplified pattern with only G_{M3} and G_{D3} as predominant gangliosides. Only MOLT-4 T-cell line of the other cells studied showed a similar pattern; this finding is consistent with the demonstration by Yeh et al. [39] that moAb 4.2 binds to 8402 T-cell line. When the content of G_{D3} in extracts of a large variety of cells and tissues was estimated using moAbR_{24} with inhibition assays, the distribution of the antigen was found to closely resemble the distribution determined on cultured targed cells in the original survey using the PA-MHA assay [38]. A surprising result was, however, obtained when a more sensitive assay (detection of antigen separated by thin layer chromatography using moAbR_{24} and ^{125}I-protein A) was used: the antigen was found in all samples tested. Two factors probably account for the apparent discrepancy between the restricted specificity of moAbR_{24} when tested against intact cells and tissue sections and the ubiquitous presence of G_{D3} in cell and tissue extracts: (i) melanomas have higher levels of gangliosides and G_{D3} in particular than most other cells and (ii) GD_3 is localized to the cell surface in melanomas whereas in most other cell types it may be an intracellular biosynthetic precursor to other gangliosides.

In immunochemical terms, the high degree of specificity of moAbs R_{24} and 4.2 for G_{D3} ganglioside is remarkable. No detectable cross-reactivity of these moAbs with any of the other glycolipids tested was observed [38, 41]; these include G_{T1a} and G_{Q1b} which share the terminal αNANA(2→8) αNANA(2→3)βGal - structure with G_{D3} (Figure 2).

2.12 *HLA-DR (Ia-like) antigens in melanoma*

Although originally detected as a B-lymphocyte marker, HLA-DR antigen is also expressed on other cell types, including melanoma [44, 45, 8]. Different cultured melanoma cell lines vary widely in the amount of DR antigen expressed; some cell lines have levels equal to or approaching those of B-lymphocytes whereas others have no detectable levels. The majority of cell lines express intermediate levels of DR antigen; these cultures are highly heterogeneous consisting of mixed populations of cells with high and low levels of DR antigen [44]. As will be discussed later, the melanoma cell lines lacking in DR antigen are those that are highly pigmented; cultured melanocytes are also DR-negative. These original observations were made with polyclonal antisera but the results have been confirmed using anti-HLA-DR monoclonal antibodies [46, 10, 47, 48]. With the availability of these reagents it became possible to examine melanoma tumor specimens, as well as normal skin, nevi, and other tissues for the presence of DR-antigen. All or most melanoma lesions expressed DR-antigen although there was considerable heterogeneity within a single tumor and in the intensity of staining between different tumors [40, 22]. Normal melanocytes in the skin appeared to be DR-antigen negative, as were nevi. Within the context of pigmented cells therefore, DR-antigen is a marker for malignancy although, in fact, it is probably a differentiation antigen for early cells in this lineage (see Section 2.13).

Melanoma HLA-DR antigen resembles lymphocyte DR-antigen in being a glycoprotein complex consisting of two non-disulfide bonded sub-units with molecular weights of approximately 32,000 and 28,000, respectively [23, 44]; both chains are glycosylated and are expressed on the cell surface [8, 46]. Two-dimensional electrophoresis revealed a more complex pattern of chains with most cells producing 1 or 2 heavy chains and 4 light chains [46]. A portion of the complexity, at least in SK-MEL-37 cells, is due to differences in the degree of sialylation of the chains as neuraminidase treatment simplified the pattern to 1 heavy chain and 2 light chains [46]. Of the two anti-melanoma DR monoclonal antibodies that have been studied in detail, one (691-13-17) detects common determinants and reacts preferentially with the biosynthetically complete, dimeric form of the antigen [23, 46]; the other (16.23) is specific for DRw3 and detects a determinant on the light chain of this species [48].

2.13 *Melanocyte differentiation and melanoma subsets*

With the development of monoclonal antibodies to melanoma antigens it became possible to define subsets of melanomas on the basis of their expression of surface antigens. Recently a set of moAbs to cultured melanocytes has been produced and the use of these reagents, together with other

moAbs, has permitted an analysis of melanocyte differentiation and has formed the basis of a classification of melanomas based on the expression of melanocyte markers [49].

Melanocyte antigens detected by this panel of reagents were grouped into four categories: (1) not detectable on newborn or adult melanocytes: HLA-DR, M-1, M-2 and M-3, (2) detected on newborn but not adult melanocytes: M-4 to M-8, (3) detected on adult melanocytes but not on most newborn melanocytes: M-9 and M-10, and (4) detected on both newborn and adult melanocytes: M-11 to M-34. Antigens M-2, M-3, HLA-DR, M-4, M-5, M-6, M-9 and M-10 were selected for further analysis on the basis of their ability to define melanoma subsets and their expression on fetal/newborn or adult melanocytes. HLA-DR, M-2 and M-3 are expressed on some melanoma cell lines but not on melanocytes and these specificities were considered to represent an early stage of melanocyte development, not represented among newborn melanocytes. Antigens M-4, M-5 and M-6 appear on fetal and newborn melanocytes but not on adult melanocytes and therefore represent intermediate stage markers. Antigens M-9 and M-10 were designated as late-stage antigens as they were more strongly expressed on adult melanocytes. These results are summarized in Figure 3. Also shown is a classification of melanomas corresponding to early, intermediate and late melanocyte forms. This classification on the basis of antigen expression also correlates with other indicators of differentiation such as morphology and pigmentation. Thus, most melanomas belonging to the early group are epithelioid in shape and lack pigmentation and tyrosinase activity; those of the intermediate group have a bipolar, dendritic morphology, while melanomas expressing late markers have a polydendritic morphology, resembling adult melanocytes, and are highly pigmented.

Although these results are valuable in suggesting a classification of melanomas, they do not explain the basis for the heterogeneity in melanoma morphology, pigmentation and antigen expression. Further work is necessary to determine whether this heterogeneity results from transformation of melanocytes in different stages of differentiation with the corresponding phenotype becoming 'frozen' in the tumor or whether transformation occurs in early melanocytes and this is followed by limited and variable differentiation of the malignant clone [49].

3. BRAIN CANCER

A number of mouse monoclonal antibodies reacting with brain tumors have been produced by immunizations with neuroblastomas [50], astrocytomas [51, 52], and fetal brain [53]; their properties are summarized in

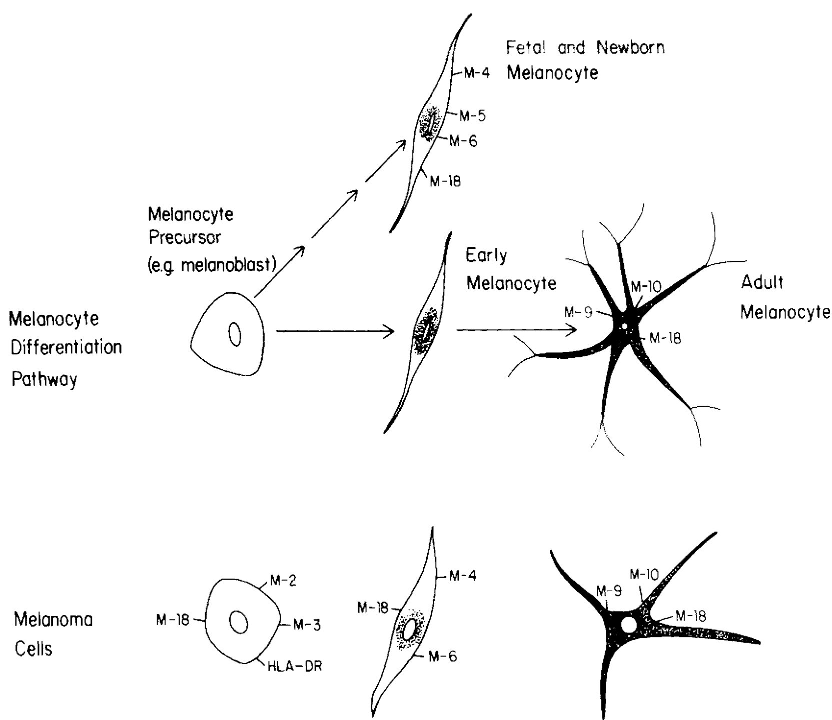

Figure 3. Proposed pathway of melanocyte differentiation based on surface antigenic phenotype and morphology (ref. [49]). The phenotype of melanomas corresponding to early, intermediate and late stages in the proposed melanocyte pathway is also illustrated. M-2, -3, -4, -5, -6, -9, and -10 are antigens described in the text. M-18 is G_{D3}. Reproduced with the permission of the authors and the *Journal of Experimental Medicine.*

Table 3. In addition, many of the moAbs raised to melanomas show reactivity with astrocytomas and/or neuroblastomas, presumably because of the common embryological origin (neuroectodermal) of the tissues from which these tumors arise.

Kennett and Gilbert [50] produced an IgM moAb (PI 153/3) to a neuroblastoma cell line that reacted preferentially with neuroblastomas, astrocytomas and fetal brain. This antibody identified a 20,000 dalton glycoprotein in the neuroblastoma cell line 1MR-5 that bound to wheat germ agglutinin but not to concanavalin A. Interestingly, the determinant recognized by moAb PI 153/3 seems to be carbohydrate in nature [54].

Monoclonal antibodies BF7 and GE2 showed selective binding to astrocytoma (malignant glioma) cell lines but very little or no binding to a variety of other cell types, including 3 melanomas and 1 neuroblastoma [51]. The antigen detected by these antibodies could not be detected on normal adult or fetal brain by absorption tests. MoAbs BF7 and GE2 therefore show a high degree of specificity for astrocytoma although it still remains to

Table 3. Monoclonal antibodies to brain tumors.

Antibody designation	Ig class	Molecular weight of antigen detected	Chemical nature of antigen	Distribution of antigen	Ref.
Pi153/3	IgM	20,000	Glycoprotein	Neuroblastoma (6/6), retinoblastoma (1/2), glioblastoma (1/1) and fetal brain positive. Other cell lines tested and adult brain negative.	50
BF7	N.D.	N.D.	N.D.	Astrocytoma cell lines (13/18) positive. Other cells tested including adult and fetal brain negative.	51
CG12	N.D.	N.D.	N.D.	Astrocytoma (10/18), melanomas (5/5) cell lines and adult and fetal brain positive. Other cells tested negative.	51
390	IgG	26,000	Glycoprotein (Thy-1)	Neuroblastomas, astrocytomas, leimyosarcoma, rhabdomyosarcoma and teratoma positive. Also brain, fibroblasts and 12% of thymocytes positive.	53
AJ225	IgM	145,000	Protein	Astrocytoma (16/16), melanoma (1/10) and epithelial cancer (1/17) cell lines positive. Other cells and tissues (including brain) negative or low expression.	52
AO10	IgG1	N.D.	N.D.	Astrocytoma (7/16), melanoma (3/10), neuroblastoma (2/2), MOLT-4 cell lines, adult and fetal brain positive [1]. Other cells and tissue negative or low expression.	52
AJ8	IgG1	N.D.	N.D.	Astrocytoma (9/10), melanoma (4/10) cell lines, fibroblasts and melanocytes positive [1]. Other cells and tissues negative or low expression.	52
AO122	IgG1	255,000; 150,000 135,000 and 115,000	Protein	Astrocytoma (9/16), melanoma (8/10) cell lines, melanocytes, fibroblasts and brain positive. Other cells and tissues negative or low expression.	52

[1] Cells that are $AO10^+$ are $AJ8^-$ and vice-versa.

be determined whether the antigen may be expressed by a small population of cells in normal brain or elsewhere. Another antibody (CG12) produced in this study had a wider pattern of reactivity and seems to detect an antigen common to cells of neuroectodermal origin, both normal and malignant.

Another antigen showing restriction to neuroectodermally-derived cells and a few other cell types, is the Thy-1 antigen. A monoclonal antibody has recently been raised to this antigen by immunization of mice with human fetal brain [53]. It was used to demonstrate the distribution of Thy-1 on neuroblastoma, glioma, rhabdomyosarcoma, leiomyosarcoma, teratoma cells as well as on normal brain, cultured fibroblasts and 12% of thymocytes. The antibody reacted with a 26,000 dalton component on neuroblastoma and glioma cell lines. Although this is very similar in size to the antigen detected by moAb PI 153/3, the different distribution of the antigen suggests that the latter antibody does not detect Thy-1 antigen.

As mentioned above, a high proportion of the monoclonal antibodies produced to melanoma cells also show reactivity with astrocytomas and/or neuroblastoma cells [10, 19, 55–57]. Some antibodies reacted with melanomas and neuroblastomas but not with astrocytomas but most reacted with a proportion of all three cell types. In the latter group, five antibodies analyzed by Carrell et al. [57] reacted with varying numbers of melanomas, gliomas and neuroblastomas but showed no reactivity with any of the other cells and tissues examined, including normal adult and fetal brain. In contrast, a moAb (G13-C6) raised to a glioma cell line, in addition to reacting with a proportion of melanomas, gliomas and neuroblastomas, also reacted with fetal brain. The authors suggest that, in comparison to the other five antibodies, moAb G13C6 identifies an antigen characteristic of more mature form neuroectodermal cells.

In view of their ability to divide brain tumor cell lines into subsets and to show varying patterns of reactivity with normal tissues, it would seem that most of the antibodies discussed are detecting antigens related to neuroectodermal differentiation and development. This phenomenon is most clearly seen in a recent study by Cairncross et al. [52] on monoclonal antibodies to astrocytoma. Four antibodies (AJ225, AO10, AJ8 and AO122) showed reactivity to astrocytomas and related cell types. Their pattern of reactivity is summarized in Table 4. Almost all possible permutations of reactivity are seen with this panel of antibodies and cells. Within the context of neural cells, AJ225 and AJ8 represent tumor-restricted antigens in that they are not detected on normal brain (within the limits of absorption tests). Nevertheless, they are not completely 'tumor-specific' as they are also weakly expressed on melanocytes and fibroblasts, respectively. The antigens detected by moABs AO10 and AJ8 are particularly interesting as they show almost mutually exclusive expression on the panel of cells tested. The obser-

Table 4. Summary of distribution of antigens detected by anti-astrocytoma moAbs on normal and malignant cells (ref. [52]).

Cells	Antigen			
	AJ225	AO10	AJ8	AO122
Astrocytomas	most + [1]	+ or −	+ or −	most +
Neuroblastomas	− or weak	+	−	−
Melanomas	most −	+ or −	+ or −	most +
Brain	−	+	−	+
Fibroblasts	−	−	weak	+
Melanocytes	weak	−	+	+
T-cell (MOLT-4)	+	+	−	−

[1] +: antigen expressed;
−: antigen not expressed.

vation that AO10 is present on glial fibrillary acidic protein (GFA) - positive tumors and that AJ-8 positive tumors are GFA-negative, suggests that these antigens reflect the state of differentiation of astrocytomas. On the basis of AJ8, AO10 and GFA expression the authors suggest that cultured astrocytomas can be divided into three groups on the basis of differentiation-related phenotypic characteristics. Cultured astrocytomas that are $AJ8^{+}/AO10^{+}/GFA^{+}$ represent more differentiated cell lines; those that are $AJ8^{+}/AP10^{-}/GFA^{-}$ represent less differentiated cell lines; and those that are $AJ8^{-}/AO10^{+}/GFA^{-}$ represent a group at an intermediate stage of differentiation (Figure 4). It will be important to determine whether this classification extends to fresh astrocytoma samples and to determine the distribution of these markers in normal brain diffentiation and development.

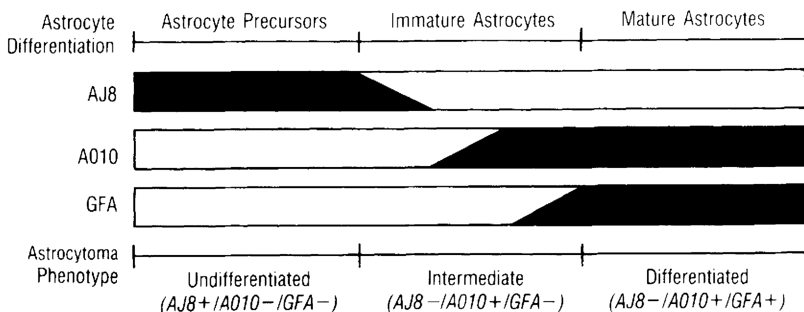

Figure 4. Schematic illustration of relationship between astrocyte differentiation and phenotypic characteristics of cultured astrocytomas based on serological typing for antigens AJ8, AO10 and GFA (glial fibrillary acid protein). Modified from ref. [52].

Table 5. Monoclonal antibodies to colorectal carcinoma.

Antibody designation	Ig class	Molecular weight of antigen	Chemical nature of antigen	Distribution of antigens	Ref.
1116 NS-3a	IgG1	N.D. [1]		Colorectal ca. (6/8), positive. Lung ca. (0/3), brest ca. (0/1), melanoma (0/10), astrocytomas (0/2), sarcomas (0/2), fibroblasts (0/2), negative.	60, 61
1116 NS-3b	IgG1	N.D.		Colorectal ca. (5/8) positive; other lines negative.	60, 61
1116 NS-15	IgM	N.D.		Colorectal ca. (4/8) positive; other lines negative.	60, 61
1116 NS-19	IgG1	N.A. [2]	Fugoganglioside	Colorectal ca. (5/8); positive; gastric ca. (1/1) pancreatic ca. (1/1), positive; other lines negative.	60, 61
1116 NS-22	IgG1	N.D.		Colorectal ca. (8/8) positive; other lines negative.	60, 61
1116 NS-29	IgG1	N.D.		Colorectal ca. (8/8) positive; other lines negative.	60, 61
1116 NS-33a	IgM	N.A.	Glycolipid (Le^b)	Colorectal ca. (8/8) positive; other lines negative.	60, 61
1116 NS-36	IgG1	N.D.		Colorectal ca. (5/8) positives; other lines negative.	60, 61
1116 NS-38a	IgM	N.A.	Glycolipid (Le^b)	Colorectal ca. (6/8) positive; other lines negative.	60, 61
1116 NS-38b	IgM	N.D.		Colorectal ca. (6/8) positive; other lines negative.	60, 61
1116 NS-38e	IgG1	N.D.		Colorectal ca. (7/8) positive; other lines negative.	60, 61
1116 NS-43	IgG1	N.A.	Glycolipid (Le^b)	Colorectal ca. (7/8) positive; other lines negative	60, 61
116 NS-52a	IgG1	N.A.	Fucoganglioside	Colorectal ca. (5/8), gastic ca. (1/1), pancreatic ca. (1/1) positive; other lines negative.	60, 61
1116 NS-56-2	IgG1	N.D.		Colorectal ca. (5/8) positive; other lines negative.	60, 61
1083-17-1A	IgG2a	39,000 and 32,000	Protein ?	Colorectal ca. (8/8) positive; other lines negative.	60, 61
1116 NS-10	IgM	N.A.	Glycolipid (Le^b)	Colorectal ca. (7/8), melanomas (1/10) positive; other lines negative.	60, 61
1116 NS-52b	IgG1	N.D.		Colorectal ca. (5/8), melanomas (1/20) positive; other lines negative.	60, 61
1116 NS-33b	IgM	N.D.		Colorectal ca. (8/8), melanomas (2/10) positive; other lines negative.	60, 61
1116 NS-20	IgG1	N.D.		Colorectal ca. (8/8), breast (1/1), melanomas (9/10), and astrocytomas (1/2) positive; other lines negative.	60, 61
$C_4 14$	IgG2a	N.D.		Colorectal ca. (4/6), gastric ca. (1/1) positive; other lines negative.	67

[1] N.D. – Not determined. [2] N.A. – Not applicable.

Human monoclonal antibodies have also been produced to astrocytoma antigens. In an early study, Sikora and Phillips [58] produced a number of hybridomas by fusing glioma lymphocytes with mouse myeloma lines. Although these hybrids produced antibodies to glioma antigens, they were unstable and antibody production soon ceased. More recently Sikora et al. [59] have produced a number of human-human hybridomas using the human myeloma line LICR-LON-HMy2 as the fusion partner with intratumoral lymphocytes. Seven of the 71 stable hybrids produced by this procedure secreted antibody reacting with a glioma cell line. Two of the antibodies also bound to the HT29 colon cancer cell line but none of the antibodies bound to the human fibroblast cells. More detailed studies are needed to determine the precise specificity of these antibodies but the formation of such a high proportion of anti-glioma antibody-producing hybrids is significant.

4. COLORECTAL CANCER

A number of studies have developed mouse moAbs to colon carcinoma [60, 61]. One of the antibodies raised is of unusual interest in that it is directed towards a previously unrecognized ganglioside. Anti-colorectal monoclonal antibodies are summarized in Table 5. Although they have been tested against a rather limited panel of target cells, a number show distinct promise as reagents for detecting colon cancer. The moAbs listed in the Table 5 reacted with at least 5/8 colorectal cancer lines tested; the majority did not react with the other cell lines tested and some reacted with a few other cell lines (mainly melanoma).

Four of the antibodies (1116 NS-33a, −38a, −43 and −10) were shown to be directed against blood group Le^b antigen [62]. In this study, neutral glycolipid fractions from adenocarcinoma cell line SW 1116 were found to contain a component reacting with moAb 1116 NS-10. The antigen was also shown to be present in extracts of meconium from $Le(a^-b^+)$ individuals but not in extracts of meconium from $Le(a^+b^-)$ individuals. The specificity of moAb 1116 NS-10 for le^b antigen was confirmed by showing that purified Le^b ceramide hexasaccharide reacted with NS-10 and that the antibody was inhibited by lacto-N-difucopentaose I (Figure 5) but not by any of the structurally-related oligosaccharides tested at concentrations of 10 mM (lacto-N-fucopentaose I, lacto-N-fucopentaose II and lacto-N-tetraose). The antibody was also unreactive with A Le^b ceramide heptasaccharide. Thus, moAbs of this group resemble anti-G_{D3} antibodies in having a high degree of specificity for a certain sequence of sugars and in showing no detectable cross-reactivity with related structures. The results of this study illustrate

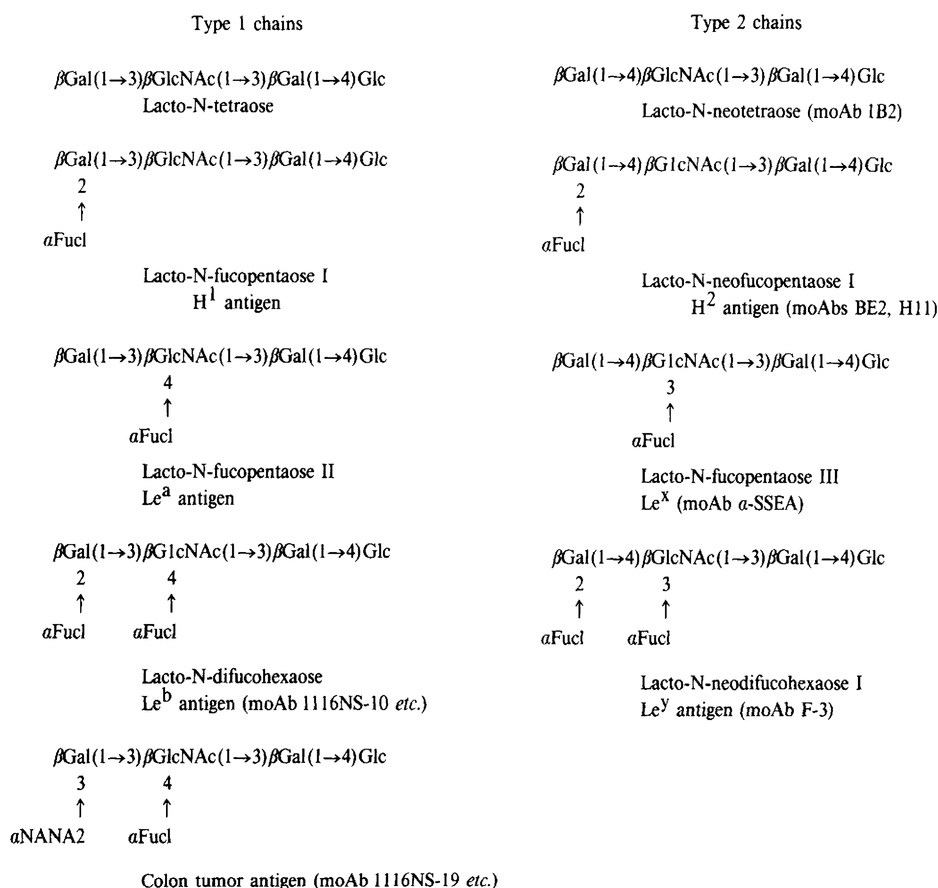

Figure 5. Structures of oligosaccharides related to blood group antigens. Nomenclature for the blood group determinants is not yet standardized; H^1 and H^2 are used to designate H(O) structures based on type 1 and type 2 chains respectively; Le^x and Le^y are used to designate type 2 structures corresponding to Le^a and Le^b, respectively, in the type 1 series. The mouse monoclonal antibodies detecting the various structures are discussed in the text except for 1 B2 which is described in ref. [77].

the difficulty of determining the specificity of a moAb by determining its reactivity against a panel of targets using a serological assay such as the ^{125}I-anti-mouse Ig binding assay. In the initial survey, moAb 1116 NS-10 appeared to have a specificity directed against colon cancer whereas in fact Le^b antigen is a relatively common antigen in tissues of individuals of the appropriate blood type. This is undoubtedly a quantitative effect, again resembling G_{D3}, with the added factor that some colon adenocarcinomas can express Le^b regardless of the Lewis blood group of the patient [63].

Two of the moAbs listed in Table 5, (1116 NS-19-9 and -52a) identified a novel carbohydrate structure not previously identified in either glycopro-

teins or glycolipids [64, 65]. This structure is a sialyl derivative of the Lewis[a] blood group determinant (Figure 5). In colon carcinoma cell line SW 1116 the antigenic specificity is carried by a glycolipid (fuco-ganglioside) although there is some indication that the same determinant can be found in glycoproteins in the serum of patients and in some tumors [65]. The antigen detected by these antibodies (gastrointestinal-cancer antigen, GICA) is released into the medium of cultured colon carcinoma cell lines and is found in the serum and urine of some colorectal carcinoma patients [66]. Using an assay based on the inhibition of binding of [^{3}H]glucosamine-labeled glycolipid antigen to 1116 NS-19-9, it was shown that the glycolipid fraction from the sera of 11/22 colon carcinoma patients showed greater than 20% inhibition whereas samples from 26 non-colon carcinoma patients were non-inhibitory. Of 22 samples of urine collected from colon carcinoma patients, 9 showed inhibition in the range of 10 to 78%. Some patients with serum antigen did not have detectable levels of antigen in their urine. In a recent more extensive study, using a different assay, it was shown that 163 of 255 sera from patients with colorectal carcinoma, 45 of 49 sera from patients with pancreatic cancer and 8 of 11 sera from patients with gastric cancer were able to inhibit moAb 1116 NS-52a; only 2 of 108 sera from normal individuals were inhibitory [67]. As GICA has a structure based on the Le[a] determinant, Koprowski et al. [68] have suggested that Le(a^{-}b^{-}) individuals would be unable to synthesize GICA and that they may have a different susceptibility to gastrointestinal cancer.

Another blood group-related antigen is detected by other moAbs produced to colon carcinomas [69]. These antibodies are not as specific for colon carcinoma as are 1116 NS-19-9 and -52, as they also bind to glycolipids of erythrocytes, granulocytes and various normal and malignant tissues. The antigenic determinant recognized is lacto-N-fucopentaose III or Le[x] which has a structure similar to the Le[a] antigen except that position of substitutions of α-L-fucose and β-D-galactose on the penultimate N-acetyl-D-glucosamine are reversed (Figure 5). Antibodies to stage-specific embryonic antigen (SSEA-1), an antigen of 8-cell mouse embryos, have been reported to have the same or related specificity [70, 71]. Again the remarkable degree of specificity of monoclonal antibodies is evident since anti-SSEA is inhibited only by fucosylated type 2 structures and does not cross-react with the type 1-based Le[a] structures [72].

5. LUNG CANCER

Lung cancer is one of the most common human cancers and it is therefore not surprising that a number of studies have used the hybridoma approach to search for antigens characteristic of this tumor.

In a study on small cell lung cancer (SCLC), Cuttitta et al. [73] produced three moAbs after immunizing mice with NCI-H69 cell line (Table 6). Clones were originally selected on the basis of the ability of their antibodies to bind SCLC but not to the autologous B-cell line. The 3 moAbs reacted with small cell, adenocarcinoma and squamous carcinoma lung cancer cell lines and tumor tissues. These antibodies also reacted with 3/3 neuroblastomas and 2/3 breast carcinomas, but most other tumors (including melanoma) and normal tissues tested were unreactive although normal kidney tissue showed some binding. No data were provided on the molecular nature of the antigens detected.

By immunization with squamous cell tumor cell line UCLA-SO-P3, Kasai et al. [74] produced three monoclonal antibodies (Table 6). One of these moAbs (16D4) had a very restricted specificity and reacted only with the immunizing cell line and HT-29 (a colon carcinoma cell line). Later work [75], showed that moAb 16D4 also reacted with normal lymphocytes from 2% of the population. Typing of these reactive lymphocytes for blood group antigens revealed that they were of the rare A_1 Le^d phenotype i.e. A_1 Le a^-b^- secretors; an erythrocyte sample of the same blood group also absorbed this antibody. Since the tumor cell line but not the autologous lymphoblastoid cell line carried the A Le^d, it would be interesting to know the RBC blood type of the patient. Another moAb in this group, (172 D5) bound to a proportion of lung cancer, colon cancer and breast cancer cell lines but not to a limited panel of other tumors and normal cells tested. MoAb 179E3 reacted with almost all tumor cell lines tested but not to a small panel of other cells tested. No biochemical data were presented.

In a separate study on lung cancer, Anger et al. [76] produced a moAb reacting preferentially with 0 red blood cells. Further work (Lloyd, K.O., unpublished data) showed that this antibody identified the difucosyl type 2 blood group determinant (Figure 5). Two other studies have produced anti-H moAbs that react with monofucosyl type 2 determinants [77, 78].

Mazauric et al. [79] produced four moAbs having varying degrees of specificity for lung cancer. MoAb 900-1-7 bound very strongly to the immunizing cell line, and with melanoma cell lines (5/5) but not with other cells and tissues tested. MoAb 1680-25-35 reacted strongly with two lung cancer cell lines (including a cell line derived from the squamous cell tumor used for immunization) but not with other cells and tissues tested. This moAb precipitated components of 149,000 and 119,000 daltons from ^{125}I-iodinated proteins from lung cancer cell line WL 1680 but the 149,000 dalton component predominated in immunoprecipitates from melanoma cell line WM9. Two other moAbs (9812-16B-13 and 427-2-F) reacted with antigens present on lymphocytes as well as tumor cell lines.

Table 6. Monoclonal antibodies to lung cancer.

Antibody Designation	Ig class	Molecular weight of antigen	Chemical nature of antigen	Distribution of antigen	Ref.
525A5	IgM	N.D.[1]	N.D.	Small cell lung cancers (cell lines and tumors), neuroblastomas positive; nm-SCLC and breast ca. less positive. Other tissues (except normal kidney) negative.	73
534F8	IgM	N.D.	N.D.		73
538F12	IgM	N.D.	N.D.		73
16D4	IgM	N.D.	A_1 Le^d blood group (glycolipid?)	Only immunizing lung ca. line, A_1 Le^d lymphocytes and red cells and colon cancer cell line (HT-29) positive.	74, 75
172D5	IgG1	N.D.	N.D.	Lung ca. (3/6), colon ca. (2/2) and 3/3 breast ca. cell lines positive. Other tumor cell lines and normal lymphocytes negative.	74
179E3	IgG1	N.D.	N.D.	All tumors (except sarcoma) tested positive; limited panel of normal cells tested negative.	74
F-3	IgM	N.D.	H blood group (glycolipid?)	Red cells (0), many tumor and normal cells positive.	76
9812-16B-13	IgG2a	37,000 and 19,000	Protein	Lung ca. (6/6), colon ca. (5/5), breast ca. (3/3), melanoma (1/5) cell lines and RBC and lymphocytes positive.	79
427-2-f	IgG1	145,000; 127,000 and 113,000 (not S-S linked)	Protein	Lung ca. (6/6), colon ca. (6/6), breast ca. (2/3), melanoma cell lines (3/5) and lymphocytes positive.	79
900-1-7	IgG1	126,000	Protein	Lung ca. (3/6), breast ca. (1/3), melanoma cell lines (5/5) positive. RBC and lymphocytes negative.	79
1680-25-35	IgG1	149,000 and 119,000	Protein	Lung cancer (4/6) positive; low binding to colon ca. (1/5), breast ca. (1/3), melanoma (1/5) WI38. RBC and lymphocytes negative.	79

[1] N.D. – Not determined.

6. OVARIAN CANCER

The detection of ovarian tumors is often fatally delayed because of their deep location and lack of diagnostic methods for early cancer. As the prognosis for patients with Stage I disease (when the tumor is confined to the ovary) is unusually good, early diagnosis would have important clinical benefits. For this reason, numerous attempts have been made to detect markers suitable for the diagnosis and monitoring of epithelial ovarian tumors (reviewed in references [80, 81]). Despite these efforts, however, no effective marker emerged and recent investigations have used monoclonal antibodies in attempts to detect new antigens.

By immunization of mice with an ovarian cancer line (OvCA 433) Bast et al. [82] derived one monoclonal antibody (OC 125) that showed preferential reactivity with ovarian tumors. The binding of the antibody to a range of tumor cells was determined by indirect immunofluorescence using a FACS-1. It was found that all 6 ovarian carcinoma cell lines studied bound antibody but that colon, cervical, pancreatic, renal cell, breast and oat cell carcinoma cell lines did not. Lymphoid cell lines, including the autologous B-cell line, were also unreactive. The only non-ovarian cell to react was 1 of 4 melanoma cell lines tested. Tissue sections were also studied by indirect immunofluorescence but no reactivity with normal tissues could be detected within the sensitivity of this procedure. Most significantly,little immunofluorescence was associated with the surface epithelium or other structures in normal ovary. In contrast, 5 of 9 ovarian carcinomas showed strong reactivity. The brightest staining was on the luminal surface of the malignant epithelium. This study was later extended to the examination of tissue sections from 60 ovarian tumors by indirect immunofluorescence [83]. MoAb OC 125 stained benign and borderline serous ovarian tumors (7/7), serous adenocarcinomas (19/23), mixed serous and endometrioid carcinomas (2/2), endometrioid carcinomas (2/3), clear cell carcinomas (1/4) and undifferentiated carcinomas (2/2). No reactivity was found in 8 mucinous ovarian tumors or any of the other 11 epithelial, sex cord, germ cell tumors or lymphomas tested. Within reactive tumors, the pattern of reactivity was very hetergenous with areas of positive and negative staining being found. There was no relationship between OC 125 expression and clinical characteristics of the tumors. It is interesting that the antibody was unreactive with the mucinous cystadenocarcinomas tested. The authors suggest that this result may indicate a separate lineage for mucinous tumors as compared to serous tumors.

In contrast to moAb OC 125, an antibody developed by Bhattacharya et al. [84] reacted only with mucinous ovarian cystadenocarcinomas. In this study, mice were immunized with saline extracts of a poorly differentiated

ovarian cystadenocarcinoma. An antibody-secreting clone (1 D_3) from the fusion was selected on the basis of its reactivity with ovarian tumor extracts and lack of reactivity with pooled normal ovarian extracts and pooled normal human serum. Extracts for 12 of 14 mucinous cystadenocarcinomas reacted with this antibody whereas extracts of 58 serous adenocarcinomas and 12 non-epithelial ovarian cancers were unreactive. Benign mucinous ovarian cystadenomas were also negative. The antigen was undetectable in any normal adult tissue examined but could be detected in extracts of fetal intestine. The localization of the antigen detected by moAb 1 D_3 has not yet been examined by immunofluorescence techniques on tissue sections, however its solubility properties suggest that it may be a secreted product rather than a cell surface antigen.

Our group at the Sloan-Kettering Institute has developed a number of monoclonal antibodies reacting with ovarian cancer cell lines (Mattes, J. et al., unpublished results). Mice were immunized with either SK-OV-3, 2774 or SW 626 ovarian carcinoma cell lines or with the uterine carcinoma cell line SK-UT-1. After fusion with NS-1 mouse myeloma, antibody-secreting clones were screening against the immunizing cell line and a variety of other cell types including melanomas and astrocytomas. Hybridomas producing antibody reacting with the ovarian or endometrial carcinomas but not with melanoma and astrocytoma cell lines were selected for further analysis. By testing against approximately 140 cell lines it was shown that a number of interesting moAbs had been generated. These include one (moAb MD144) reacting only with the immunizing ovarian carcinoma cell line and another (moAb MF61) reacting only with the immunizing cell line and 6/16 renal cancer cell lines. Two moAbs with restricted specificities resulted from the immunization with SK-UT-1; these are moAb MH55 which reacts only with 4/8 ovarian carcinoma and the immunizing uterine cancer cell line and moAb MH94 which reacts with 3/8 ovarian carcinoma, SK-UT-1 and a number of other epithelial cancers including 3/7 colon cancer, and 2/18 lung cancer cell lines. None of these moAbs (MD144, MF61, MH55, MH94) reacts with normal fibroblasts, kidney epithelia or lymphocytes in direct tests. Three other moAbs (ME46, ME195 and MF116) reacted with cultured normal kidney epithelia as well as a number of ovarian, uterine, renal and bladder cell lines. Biochemical studies showed that these antibodies recognized a glycoprotein of 110,000 daltons which is shed from the cells into the culture supernatant. The reactivity of many of these moAbs with normal and/or malignant kidney epithelial cells is interesting in terms of the similar embryonic origin of kidney, ovarian and uterine epithelia.

Two approaches have been taken to studying breast cancer antigens: first, monoclonal antibodies to mammary tumors and cell lines have been produced [85, 86] and second, moAbs to normal milk fat globule membranes have been prepared [87-90]. Both groups of reagents will be useful in helping to understand the biology of normal breast tissue and the pathology of breast cancer.

By immunization with membrane fractions of metastatic mammary carcinomas, Colcher et al. [85] produced 11 moAbs with apparent selective reactivity with breast carcinomas (Table 7). These antibodies could be divided into five groups according to their reactivities with extracts of two metastases and three breast cancer cell lines. Some antibodies reacted with

Table 7. Monoclonal antibodies to breast cancer.

Antibody designation	Ig class	Distribution of antigens	Ref.
B 6.2 [1]	IgG1	Breast ca. cell lines (3/3), metastases (2/2) positive; other cells tested negative.	85
B 38.1	IhH1	Same as B6.2 except that some other epithelial ca. cell lines also positive.	85
B 50.4 and B 50.1	IgG1	Breast ca. cell lines (1/3), metastases (2/2) positive; other cells tested negative.	85
B 25.2	IgM	Metastasis (1/2) positive; other cells and tissues negative.	85
B 72.3	IgG1	Metastasis (1/2, different from B 25.2) positive; other cells and tissues negative.	85
H59	IgM	Breast ca. (3/6), brain ca. (1/2) endometrial ca. (1/3) cell lines positive; other cell lines tested negative. Also positive on sections of breast ca. (38/71), fibroadenomas (9/11) and fibrocystic (20/1) disease. Uninvolved breast tissue negative.	86
F 5.5	IgG1	Reacts with breast ca. CEA and less well with colon CEA.	95
1.10.F3 and 3.14.A3	N.D. [2]	Milk fat globule antigen present on normal eipithelial cells with secreting activity, breast ca's, lung ca's and ovarian ca's.	87
M3, M8, M18 and M24	N.D.	Milk fat globule antigen present on breast and other tissues.	89

[1] Other moAbs (B14.2, B29.1, F64.5, F25.2 and B84.1) of similar specificities were produced in the same study.

[2] N.D. — not determined.

all mammary carcinomas tested whereas others were selective and reacted with one of the metastatic tissues tested but not with the cell lines. The reactivity of these antibodies with tissue sections was studied using an immunoperoxidase procedure. All the moAbs reacted with a proportion of primary (infiltrating ductal and/or lobular) and metastatic mammary carcinomas tested. Uninvolved areas of the breast were unreactive with these antibodies. A more detailed study of the specificity of moAb B72.3 from this group has recently been reported which used the avidin-biotin complex immunoperoxidase technique on tissue sections [91]. The previously reported specificity of the antibody was confirmed: 19/41 primary breast tumors and 13/21 metastatic lesions were positive whereas no staining was observed with a large selection of normal tissues, including breast tissue. A variety of primary mammary tumors of different histological types were reactive with moAb B72.3 but none of the six medullary carcinomas tested were positive. Of the 15 benign breast lesions tested, two specimens showed positive staining. The only other tissues that reacted with moAb B72.3 were 4/4 colon carcinomas and 2/4 lung carcinomas. In this study the heterogeneity of staining observed in most sections of breast tumors was emphasized. Two types of heterogeneity were observed: (i) a 'patchwork' effect seen within a given tumor mass and (ii) variations between different areas of the same tumor.

The same laboratory has also produced a series of human monoclonal antibodies by fusion of the mouse myeloma NS/1 cell line with lymph node cells from breast cancer patients [92]. Several of these moAbs demonstrated preferential binding to mammary carcinoma cells. One antibody (MBE6, IgM) was studied in more detail and was shown to react with mammary carcinoma cells in tissue sections of mammary tumors from 55 of 59 patients using an immunoperoxidase technique. Normal mammary epithelial cells, stroma and lymphocytes, were unreactive with this antibody although some benign breast tumors showed weak staining, with moAb MBE6.

Monoclonal antibody H59 derived by Yuan et al. [86] from immunization with breast cancer cell lines ZR-75-1 reacted with a high proportion of breast carcinoma tissue sections as tested with a novel autoradiographic assay. The antibody also reacted with sections from benign breast disease including fibroadenomas and fibrocystic disease. MoAb H59 reacted preferentially with estrogen receptor-positive cell lines showing well differentiated glandular morphology.

A number of monoclonal antibodies have been produced to milk fat globule antigens [87–90]. Some of these detected antigens present on the epithelia of resting as well as lactating breast; others detected antigens preferentially expressed on lactating breast. These moAbs reacted with a high

proportion of breast carcinomas and sometimes with other epithelial cancers also. A notable feature of these studies is the demonstration of a wide range of reactivity, yet with each moAb showing a characteristic pattern of staining within and between different normal tissues and tumors.

Although carcinoembryonic antigen (CEA) was originally described as an antigen of colon carcinoma [93], subsequent studies have shown that it is also produced by cancers of the breast and other sites and even by normal tissues [94]. A recent study by Colcher et al. [95] described a number of moAbs to CEA, produced by immunization with breast tissue samples, one of which showed preferential reactivity with breast cancer as opposed to colon cancer and melanoma CEA. This result adds further support to the concept that different organs may produce CEAs having distinctive as well as shared determinants. Monoclonal antibodies to such restricted determinants may be more useful in the diagnosis of tumors than polyclonal antisera have been.

8. RENAL CANCER

A series of seventeen mouse monoclonal antibodies were produced to renal cancer cell lines by Ueda et al. [96]; these antibodies recognized nine different antigenic systems (Table 8). None of the antibodies was completely specific for renal cancer, although three (moAbs S_4, S_{25} and S_{23}) reacted only with renal cancer and normal kidney epithelial cells. MoAb S_4 reacted with 7/13 renal cancer cell lines and 9/9 normal kidney epithelial; this antibody precipitated a glycoprotein of 160,000 daltons. The antigen detected by moAb S_{25} could be detected on 3/13 renal cancer cell lines and 1/10 normal kidney epithelia by direct tests although absorption tests showed that it was present on four more renal cancer cell lines. MoAb S_{23} reacted with 9/13 renal cancer cell lines and 10/10 normal kidney epithelia; it precipitated a glycoprotein of 120,000 daltons. MoAb S_6 also precipitated a glycoprotein of 120,000 daltons; this antibody showed a wider spectrum of reactivity than moAb S_{23} since it reacted with all renal cancer cell lines and normal kidney cells tested as well as with four other epithelial cancers, with four astrocytoma cell lines and with normal fibroblasts. The antigens detected by moAbs S_{23} and S_6 were designated gp120r and gp120nr, respectively. Sequential immunoprecipitation experiments showed that moAbs S_6 and S_{23} reacted with the same glycoprotein. Differences in specificity between the two antibodies could be explained by (i) kidney cells having gp120 with both S_6 and S_{23} epitopes whereas other cells would have gp120 with only the S_6 epitopes or (ii) moAb S_6 having a higher affinity than does moAb S_{23} and therefore reacting only with those cells (normal and malignant kidney) bear-

Table 8. Monoclonal antibodies to renal cancer (ref. [96]).

Antibody designation	Ig class	Molecular weight of antigen	Chemical nature of antigen	Distribution of antigen
S_4	IgG2a	160,000	Glycoprotein	Renal cancer (7/13) and normal adult (9/9) and fetal (2/3) kidney epithelia positive. Other cells and tissues negative.
S_{25}	IgG1	N.D.	N.D. [1]	Renal cancer (7/13) and normal adult (4/9) and fetal (3/3) kidney epithelia positive. Other cells and tissues negative.
S_{23}	IgG1	120,000	Glycoprotein	Renal cancer (9/13) and normal adult kidney epithelia (10/10) positive. Other cells and tissues negative.
S_6	IgG1	120,000	Glycoprotein	Renal cancer (13/13), normal adult (10/10) and fetal (3/3) kidney epithelia, bladder cancer (2/4), colon cancer (1/2), lung cancer (1/2), astrocytoma (3/3), monkey kidney (1/1), and fibroblasts positive. Other cells and tissues negative.
S_{22}	IgG1	115,000	Glycoprotein	Renal cancer (13/13), normal adult (10/10) and fetal (3/3) kidney epithelia, bladder cancer (4/4), lung cancer (1/2), ovarian cancer (1/1), astrocytoma (1/1), fetal fibroblasts and brain positive. Other cells and tissues negative.
V_1	IgG1	N.D.	Heat stable	All human cells tested, except astrocytomas, adult fibroblasts and erythrocytes, positive.

[1] N.D. – Not determined.

ing larger amounts of gp120. Although preclearing experiments favor the first explanation, it is difficult to completely rule out the second possibility. The antigen detected by moAb S_{22} has an interesting distribution – although it is expressed on all renal cancer cell lines tested, on some other epithelial cancers and on normal kidney epithelia, the titer of the antibody against two of the cancer lines is 5,000 and 10,000 greater than with normal kidney epithelia. This result suggests that some kidney tumors may express substantially more S_{22} antigen than do normal cells. The other antibodies raised

in this study had a wide degree of reactivity. One (moAb S_{21}) reacted with HLA-A, B, C antigens, another (moAb M_2) reacted with blood group A antigen and a third (moAb S_8) reacted with blood group B antigen. MoAb V_1 also recognized a widely distributed antigen; this antigen was heat stable and may therefore be carbohydrate in nature. Although almost all cells carry V_1 antigen, an interesting exception were astrocytoma cell lines. Since melanoma cells were strongly reactive with moAb V_1, this antigen seems to be one of the few that distinguishes melanoma from astrocytoma cells (see Section 3).

Application of this set of monoclonal antibodies to an analysis of normal kidney sections using immunoperoxidase procedures has provided an antigenic profile of the normal nephron [97]. Whereas some antibodies, e.g. moAb S_{25} reacted with the epithelial layer in all parts of the kidney, others stained discrete regions, e.g. moAb S_{23} (detecting gp120r) reacted only with the proximal tubules and anti-blood group antibodies (moAbs F-3, M_2, or S_8) reacted only with the collecting and distal tubules. These results are summarized schematically in Figure 6. A similar analysis of tumor specimens showed that most renal cancers carry antigenic markers indicating that they originate from proximal tubule epithelia.

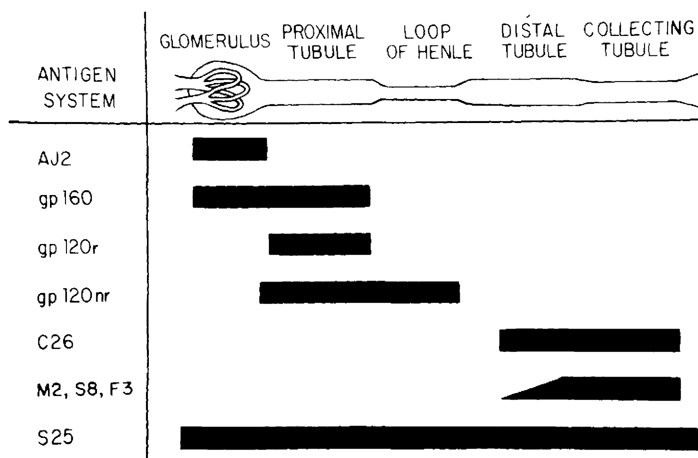

Figure 6. Schematic representation of the distribution of monoclonal antibody-detected antigens in epithelia of the normal nephron, (ref. [97]). AJ2 was originally described as an astrocytoma antigen [52]; gp160, gp120r and gp120nr and S_{25} are kidney antigens [96]; C26 is an anti-colon cancer monoclonal antibody (D.Morrissey et al., unpublished data), M_2, S_8 and F-3 are anti-A, -B and O(H) blood group antibodies, respectively [96, 76].

9. PANCREATIC CANCER

Metzgar et al. [98] have described five mouse monoclonal antibodies to pancreatic adenocarcinoma. These moAbs were produced by immunization with the pancreatic cancer cell line HPAF and were tested by immunoperoxidase and immunofluorescence procedures against a number of cancer cell lines, lymphoid cells and sections of tumors and normal tissues. MoAb DU-PAN-1 reacted with 4/5 pancreatic cell lines but not with any other cell line tested including other epithelial cancers, peripheral blood B- and T-cells, red cells or skin fibroblasts. The specificity of this moAb was confirmed by studies on tissue sections - both pancreatic cancers tested were positive but adult and fetal normal pancreas were unreactive. One transitional bladder cancer was also positive but no other type expressed this antigen. Although these results show that DU-PAN-1 has considerable promise as a pancreatic cancer antigen, the data need to be confirmed using more quantitative assays for antigen expression. Antibodies DU-PAN-2 and DU-PAN-3, from the same study, detect organ-specific antigens of pancreatic ductal epithelial cells. The antigens are not completely restricted to the pancreas however, as DU-PAN-2 is found on fetal small intestine cells and DU-PAN-3 is expressed on other epithelial cancers (colon, lung and renal) and on 2/4 melanoma cell lines.

10. PROSTATE CANCER

An interesting study has described the production of monoclonal antibodies to benign prostate hyperplasia membrane fractions [99]. Eight moAbs were selected for further study and were shown to identify three different types of antigen, i.e. prostate epithelium-specific, polyepithelial and stroma-specific. Ware et al. [100] also described three prostate epithelial-specific antibodies and recently a preliminary communication [101] has described an antibody (DU 83.21) reacting preferentially with prostatic cancer.

11. OSTEOGENIC SARCOMA

A mouse monoclonal antibody (791T/36) produced to human osteogenic sarcoma cell line 791T reacted with three of 10 osteogenic sarcoma cell lines tested by ^{125}I-protein A binding assays [102]. The antibody was unreactive with fibroblasts, including those of the patient from whom sarcoma 791T was derived. A large panel of other tumor cell lines as well as erythrocytes and white cells were negative. Strong reactivity of moAb 791/36 was, how-

ever, observed with HeLa (cervical carcinoma), and EB33 (prostate carcinoma) and lower, but significant reactivity with A549 (lung carcinoma) and HT-29 (colon carcinoma) cell lines.

By immunizing mice with freshly resected osteosarcoma cells, Hosoi et al. [103] produced three monoclonal antibodies (OST6, OST7 and OST15) having selective reactivity with osteosarcomas. These antibodies reacted in immunofluorescence assays with sections from all 5 osteosarcomas tested and with 1/1 chondrosarcoma. Fetal mesenchymal cells or cranial and vertebral bone tissue of two neonates were negative, as were other malignant and benign tumors and normal cells tested (with one exception). This exception was the weak staining given by chondrocytes near the subchondral bone in the two samples tested. The two osteosarcoma cell lines tested (TE85 and MG63) were also unreactive with these antibodies. Although inhibition tests showed that the three antibodies were directed towards different antigenic determinants, it was not determined whether they were on the same or different antigens. MoAbs OST6, OST7 and OST15 seem to detect an antigen expressed on a minor population of normal chondrocytes and on osteosarcomas but not on fibroblasts.

12. LEUKEMIAS AND LYMPHOMAS

Mouse monoclonal antibodies to normal lymphocyte differentiation antigens have been of considerable diagnostic and prognostic value in leukemias and lymphomas in that they aid in the classification of this heterogeneous group of malignancies. These studies will not be discussed in detail as they have recently been reviewed [104, 105]. This discussion will focus on antigens showing a more direct relationship to malignancy and will particularly emphasize those that are candidates for leukemia-specific antigens.

The development of antibodies reactive with antigens on leukemic cells that are not differentiation antigens has proven difficult, although a few candidates will be discussed below. Exhaustive specificity analyses must be performed before an antigen can be accepted as being leukemia specific.

12.1 *Blast antigens in leukemia*

Blast antigens are characterized by their expression on a broad range of cells, including activated normal lymphocytes, many leukemia cells and some non-hematopoietic tumor cell lines [106, 107]. These antigens do not exhibit tumor specificity so much as a correlation with proliferation.

The widely reactive monoclonal antibody B5 was produced by immunization of mice with the cultured common acute lymphoblastic leukemia (cALL) line Reh [108]. The antibody demonstrated cytotoxicity toward

69% non-B non-T ALL, 50% T-ALL, 18% AML, and 66% CML-BC lymphoid cells. Also positive were the immunizing cell line Reh as well as Daudi and Raji (B-cell lines), MOLT 4, 8402, HSB 2 (T-ALL lines), K562 (erythroleukemic line), Cole and HL60 (AML lines), IM normal B-cell line), normal PHA-stimulated blast cells and normal bone marrow myeloblasts. Negative cells included AMML, CML (chronic), CML-BC (myeloid), CLL, Sezary, and hairy cell leukemia cells as well as non-stimulated normal T- and B-lymphocytes, spleen cells, granulocytes and monocytes.

MoAb B5 appears to be distinct from other reported blast antigens, such as OKT9 [109], 5E9 [106] and B3/25 [107]. Billing et al. [108, 110] found B5 to be a different antigen from that detected by their previously described heteroantiserum 157, which is functionally similar to the OKT9 monoclonal antibody, also directed toward a blast antigen. Evidence that OKT9 recognizes a blast antigen comes from experiments with DMSO-induced differentiation of HL-60 cells. Expression of the antigen would be expected to decrease upon differentiation, and this was shown to be the case [106, 107]. Omary et al. [107] pointed out striking structural similarities between the antigen recognized by B3/25 and the abnormal, malignancy-related glycoprotein of Bramwell and Harris [116, 117].

OKT9, an IgG antibody, recognizes the most immature of three subsets of thymocytes according to the classification of Reinherz and Schlossman [111, 109, 112, 113]. It is also positive on most T-ALL cells, one non-T-ALL, Con A-stimulated T-lymphocytes, and fetal liver cells but negative on circulating lymphocytes. MoAb OKT9 reacts with the same antigen as monoclonal antibodies 5E9 [106] and B3/25 [107]. This antigen has been identified as the cell surface receptor for transferrin [114, 115]. The very frequent occurrence of this antigen on hematopoietic and non-hematopoietic cell lines is not surprising in view of the requirement of cultured cells for iron, which is bound by transferrin. OKT9 precipitated a ~180,000 dalton dimeric (unreduced) glycoprotein from detergent lysates of T-leukemia cell lines labeled with ^{125}I, ^{35}S-methionine, or by the galactose oxidase-[^3H]-$NaBH_4$ method [109, 115]. A recent structural study of the transferrin receptor, utilizing monoclonal antibody OKT9, indicated that the antigen is a phosphorylated transmembrane glycoprotein bearing both complex and high mannose oligosaccharide chains. It has an approximate pI of 5.2 and each 180 kD dimer appears to bind two moledules of transferrin [115].

12.2 *T-cell leukemias and lymphomas*

12.2.1 *TL-like antigens*

Thymus-leukemia (TL) antigens in the mouse are differentiation antigens which are expressed on immature lymphocytes of T lineage that have not yet exited the thymus; they can also be expressed on leukemia cells in mice

whose normal thymocytes were either positive or negative for the antigen. Like the H-2 antigens, to which they are genetically linked, TL antigens have a two-chain structure consisting of a 45,000 dalton heavy chain non-covalently associated with the 12,000 dalton β_2-microglobulin (β_2m) chain. Various monoclonal antibodies to human homologue of TL-antigens have been reported, including M241 [118], NA1/34 [118, 119], and OKT6 [109]. The latter two have been shown to be directed toward the same antigen by the identical migration in SDS-PAGE of the two immunoprecipitated antigens, by IEF, and by sequential immunoprecipitation of β_2m-associated material [109].

OKT6 detects a differentiation antigen of common or Stage II thymocytes, which comprise approximately 70% of the normal thymocyte population [112]. This antigen reflects a later stage of differentiation than OKT9, which, indeed, is absent from these thymocytes [112]. Twenty percent of T-ALL are OKT6 positive. OKT6 reacts with the T-ALL cell lines MOLT-4 and HPB-ALL but not with normal T-lymphocytes or activated lymphocytes [111, 112]. OKT6 immunoprecipitate a 49,000 dalton (reduced or non-reduced) glycoprotein from NP40 lysates of fresh human thymocytes labeled with either ^{125}I or sodium periodate [^{3}H]NaBH$_4$. The OKT6 antigen is associated with β_2m molecules in a heavy and light chain complex analogous to the murine TL antigens. Ziegler and Milstein [119] have reported a slightly larger light chain, designated β_2t, to be associated with NA1/34 antigen of MOLT-4 cells.

Another human TL-like antigen, defined by moAb M241, has been described by Knowles and Bodmer [118] as consisting of a 43,000 dalton heavy chain and the 12,000 dalton β_2m light chain. Heteroantiserum to human TL-like antigens on B cell lysates also recognizes a heavy chain of 43,000 in association with β_2m [121]. M241 has a tissue distribution similar to that of NA1/34.

12.2.3 *Candidate T-leukemia-specific antigen*

Deng et al. [122] recently reported an IgM monoclonal antibody, CALL2, reacting with 4/6 T-ALL cell lines, 1 T-lymphoma line, T-ALL cells from 7/8 patients, and T-CLL cells from 1/5 patients. The antibody was produced by immunizing with cultured T-ALL line 8402, and had high titers against the above positive cells when assayed by cytotoxicity. CALL2 was not detected on 2/6 T-ALL cell lines, 4/4 B-lymphoma lines, 2 cALL lines, 1 each of CML-BC, B-lymphocytic, histiocytic lymphoma, astrocytoma, and neuroblastoma lines. CALL2 was also negative on the following normal cells: 40/40 T-lymphocytes, 4/4 B-lymphocytes, 20/20 spleen cells, 10/10 monocytes, 8/8 granulocytes, 22/22 skin fibroblasts, 2/2 thymocytes, 2/2 platelets, 5/5 red cells (O, A, B), and as well as on the following malignant

blood cells: 14/14 cALL, 20/20 AML, 6/6 B-CLL, 2/2 CML-BC, and 3/3 Sezary. This antibody did not inhibit normal bone marrow CFU-C growth in the presence of complement.

12.3 *B-cell leukemias and lymphomas*

Monoclonal antibody FMC7 has been chosen for detailed consideration here [123, 124]. Cells from 17/17 prolymphocytic leukemias (B-PLL) and 8/9 hairy cell leukemia (HCL) patients, a fraction of peripheral blood B-lymphocytes, and 3/10 B-cell lines were positive by immunofluorescence. FMC7 did not react with cells from 32/38 chronic lymphocyte leukemia (B-CLL) patients or with ALL cells.

12.4 *Non-T cell, non-B cell acute lymphocytic leukemias*

12.4.1 *CALLA*

Immunization of rabbit with common acute lymphoblastic leukemia cells (cALL) generated polyclonal antibodies (reviewed in 104) detecting a membrane antigen designated CALLA. Initially it appeared that the expression of this antigen was confined to cALL and to CML in lymphoid blast crisis, but subsequent studies identified it on neoplasias and cell lines of both T- and B-cell origin [125] as well as on a number of normal cells, including some non-hematopoietic tissues [126–129].

Anti-CALLA monoclonal antibodies have also been produced. J5, an IgG2a antibody, was produced by immunization with cryopreserved ALL cells previously determined to be CALLA positive with heteroantisera [130]. Specificity was determined by indirect immunofluorescence and by microcytotoxicity [130, 131]. In these early reports J5 was shown to react with cryopreserved cells from 21/34 non-T-ALL patients and 3/5 CML-BC patients. J5 was negative on cryopreserved cells from 8/8 T-ALL, 10/10 CLL, 10/10 AML, and 3/3 CML stable phase patients; 5/5 T-ALL lines; normal peripheral mononuclear cells; T- and B-lymphocytes; Con A-stimulated T-cells; monocytes and splenic lymphocytes; fetal liver and thymus; and regenerating, normal adult, and fetal bone marrow.

Subsequent investigations have added to the list of CALLA positive cells: Normal fetal and adult renal proximal tubules and glomeruli, fetal small intestinal epithelium, myoepithelial cells of normal adult breast by immunoperoxidase staining [127]; 3/3 Burkitt's lymphoma cell lines, 3/8 T lymphoblast cell lines, 5/12 T lymphoblastic lymphoma, 3/33 T-ALL, 5/6 Burkitt's lymphomas, 2/15 diffuse histocytic B lymphomas, 2/16 diffuse poorly differentiated lymphocytic B lymphomas, and 4/4 nodular poorly differentiated lymphocytic B lymphomas as judged by indirect immunofluorescence and immunoperoxidase methods [125]. This latter study also reported slight staining above background of normal bone marrow and fetal liver by J5.

Polyclonal and monoclonal [130, 132] anti-CALLA antibodies precipitated a 95,000–100,000 dalton glycoprotein under reducing and non-reducing conditions from detergent lysates of [^{35}S]-methionine-, [^{3}H]-glucosamine- or galactose-oxidase-[^{3}H]-KBH_4-labeled ALL lines or tumor cells. CALLA of lower molecular weight (90,000) has been precipitated by J5 from extracts of ^{125}I-iodinated kidney membranes [127]. A portion of CALLA molecules bind to lentil lectin colums [130, 132]. Pesando et al. [133] propose that a family of 100 kD antigens detected on a cALL line, a leukemic T cell line and a transformed B cell line by partially and extensively absorbed heteroantisera to cALL cells are related surface glycoproteins, one of which is CALLA.

Monoclonal antibody BA-2 identifies a 24,000 dalton surface antigen present on lymphohemopoietic progenitor cells in normal bone marrow, cells from 54/70 unclassified ALL, 2/11 T-ALL, and 5/10 B-CLL patients [134]. Normal granulocytes and erythrocytes were negative for this antigen [134].

12.4.2 *Candidate non-T, non-B leukemia specific antigen*

Ueda et al. [135] have described an IgM monoclonal antibody, NL-22, that appears to be specific for non-T, non-B leukemia and yet distinct from CALLA. Because of the IgM nature of monoclonal antibody NL-22, it was not possible to immunoprecipitate the corresponding antigen, therby making a direct comparison with J5 difficult. In contrast to J5, however, NL-22 does not react with any normal cells tested. These were 7 sources of fetal thymocytes, 14 PBL, 8 spleens from patients with gastric cancer, 4 tonsils and 7 bone marrows from patients with idiopathic thrombocytopenia purpura (ITP). Fetal liver and regenerating bone marrow were not tested for NL-22 reactivity, although a minority of such cells has been found positive for J5. Activity against CFU-C was not determined. NL-22 reacted with cells from 12/16 null-ALL patients and 1/3 null-ALL cell lines, but did not recognize antigens on cells from 4 T-ALL, 7 ATL, 11 AML, 6 CML-BC and 7 B-CLL patients. Nor was NL-22 reactive with the following cell lines: 2 CML-BC, 5 T-ALL, 3 Burkitt's lymphoma, 2 B-ALL, 1 DHL, 1 myeloma, nor with various solid tumor lines. Reactivity was determined by immune adherence assays and absorption tests.

12.5 *Granulocytic and monocytic leukemias*

It is convenient to consider the production of monoclonal antibodies to neoplasias of monocytic lineage together with antibodies to granulocytic malignancies since virtually all of these reagents have some reactivity with both granulocytic and monocytic subtypes of AML. An overview of such

monoclonal antibodies is given in reference 104; it will be seen that all of the antibodies reactive with AML also have some reactivity with normal peripheral blood monocytes and/or granulocytes. Two antibodies will be described in detail here.

D5D6 is an IgM monoclonal antibody recently made by Linker-Israeli et al. [136] against AML cells and found to be cytotoxic to 100% of 44/50 AML. These cases represented myelocytic, myelomonocytic and monocytic forms of the disease. MoAb D5D6 was reactive with normal monocytes and the myeloid-monocyte stem cells (CFU-C) but not with normal granulocytes. In addition, this antigen was not expressed on various solid tumors, 12/12 CML including both lymphoid and myeloid blast crisis, 15/15 ALL, 9/9 CLL, and normal and stimulated peripheral blood cells.

Recently, Ball et al. [137] described five monoclonal antibodies (AML-1-21, AML-2-23, AML-2-30, AML-1-99, CML-75) that were cytotoxic to acute myelocytic leukemia cells and/or chronic myelocytic leukemia in blast crisis. These antibodies were all capable of binding to normal cell populations, but were unable to mediate complement-dependent lysis of these normal cells.

12.6 *Megakaryoblastic leukemias*

Of the few monoclonal antibodies that react with malignancies of the megakaryocyte-platelet lineage (as well as with their normal counterparts) several seem to detect the normal glycoproteins of the I, II, and III series [105, 138, 139]. One such antibody is AN51 [139] which detects glycoprotein Ib in platelets and can precipitate this 150 kD component there from [139]. AN51 is also reactive with some CML-BC with megakaryoblastic features and with some mature megakaryocytes from normal bone marrow or from cultured human megakaryocyte colonies. AN51 was not reactive with the small precursor-like cells of normal bone marrow nor with the following malignancies: null cell lymphoblastic leukemia, promyelocytic leukemia, acute myelomonocytic leukemia, monoblastic leukemia, and myeloblastic leukemia.

12.7 *Viral antigens related to leukemias and lymphomas*

The discovery of EBV particles associated with Burkitt's lymphoma and the more recent finding of C-type retrovirus particles in association with human cutaneous T-cell lymphoma (mycosis fungoides) present another category of leukemia/lymphoma antigens. Investigations on only the human T-lymphoma virus (HTLV)-associated antigens will be considered here; for a review of EBV-associated antigens, see reference [140].

The apparently novel HTL virus is characterized by (i) mature, immature, and budding virus particles of typical C morphology as determined by elec-

tron microscopy; (ii) its exclusively extracellular location on 2 cutaneous T cell lymphoma (CTCL) cell lines and PBL, all isolated from the same mycosis fungoides patient; (iii) a reverse transcriptase (RT) activity whih has a cation optimum slightly different from that of other such enzymes and which is not immunologically related to the RT of FeLV, SSV (SSAV), GALV, RC-114, MuLV, AMV and other retroviruses; (iv) low nucleic acid homology with known type B, C and D viruses and (v) six major proteins of 10K, 13K, 19K, 24K, 42K and 52K daltons, which do not constitute the typical molecular weight distribution of type C RNA viruses [141, 142].

An IgG1k monoclonal antibody to HTLV p19 has been made [143] which was reactive with 3/12 HTLV-producing cutaneous T-cell lymphoma cell lines and with 1/2 producing cutaneous T-cell leukemia lines and non-reactive with the non-producer line HUT-78. Anti-p19 was also non-reactive on the following, as assayed by immunofluorescence: 9/9 animal retrovirus-infected cell lines; 7/7 B cell lines; 9/9 normal peripheral blood lymphocytes cultured with TCGF and PHA; 5/5 ALL lines; 8/8 fresh ALL, 3/3 cultured and fresh hairy cell leukemia; 6/6 cultured and fresh CLL, 2/2 cultured AML, one cultured Hodgkins, 3/3 cultured promyelocytic leukemias, 6/6 fresh and cultured T-cell lymphomas. Eight out of 8 fresh cutaneous T cell leukemia and 6/6 fresh cutaneous T cell lymphomas were negative. Two of the B-cell lines that were negative for anti-p19 were EBV positive.

13. MISCELLANEOUS TUMOR MARKERS

13.1 *Carcinoembryonic antigen (CEA)*

Conventional polyclonal anti-CEA sera have sufficiently high affinity constants to make them useful in radioimmunoassays and they effectively distinguish CEA from cross-reacting normal glycoproteins. Possible reasons for producing monoclonal antibodies to CEA would seem to be (i) the production of standardized reagents in virtually unlimited amounts and (ii) for investigating the possibility that subpopulations of CEA exist that have epitopes that are restricted to tumor CEA. None of the moAbs yet produced to CEA [144–146] seem to meet the latter criterion although one moAb bound more strongly to CEA in the serum than to CEA extracted from tumors and may therefore be useful in immunodiagnosis [144].

13.2 *Ca antigen*

A novel antigen (optimistically named Ca) that is present on many different tumor types has recently been described [147]. The IgM antibody (Ca 1) detecting this antigen was developed from mice immunized with a wheat germ agglutinin-binding fraction from extracts of the laryngeal carcinoma

cell line H.Ep.2. Its specificity was analyzed by measuring binding to a variety of tumor cell lines and normal fibroblasts. The antibody was also tested against matched pairs of hybrid cell lines derived from crosses between cells of a cervical carcinoma and diploid fibroblasts; one member of each pair was malignant as measured by its ability to grow progressively in nude mice whereas in the other member the ability to grow in nude mice was suppressed. The Ca antigen was found to be expressed only on the malignant hybrids and on all tumor cell lines tested except two lymphomas. The analysis was extended to an immunohistological study on a range of normal human tissues and tumors in paraffin-embedded sections [148]. The great majority of tumors tested expressed Ca antigen (these were mainly epithelial cancers), although prostatic and testicular cancers, some sarcomas, some lymphomas, brain tumors and melanomas were negative. Of the normal tissues examined only fallopian tube epithelium and transitional epithelium of the urinary tract gave positive staining.

Ca antigen is an integral membrane glycoprotein with a high carbohydrate content. Two components, with molecular weights of 380,000 and 350,000, were immunoprecipitated from extracts of ^{125}I-labeled tumor cells and malignant hybrids. The determinant detected by moAb Ca 1 seems to be carbohydrate in nature as it is heat stable, resistant to extraction with organic solvents and susceptible to neuraminidase and endo-β-galactosidase treatments.

This antigen is of considerable interest as its expression seemed to be correlated with malignancy (or, at least, growth in nude mice) and not, as is the case with most other tumor antigens, with the differentiation state of a particular tissue or group of embryologically-related tissues.

14. CLASSIFICATION OF TUMOR ANTIGENS

14.1 *Tumor-specific antigens*

Tumor specific antigens can be defined as those antigens that are expressed solely on tumor cells and not on any other cell or tissue in the body at any stage of differentiation or development. A less restrictive, but possibly more realistic usage, would be to define tumor-specific antigens as those that are expressed on tumors and are recognized as foreign by the immune system of the tumor-bearing host. A basic tenet of tumor immunology is that such antigens exist and can be detected using suitable assays. As is well known, the firmest evidence for tumor-specific antigens comes from the demonstration of strong tumor antigens on chemically or UV-induced tumors in mice as detected by transplantation techniques [149–151]. Some investigators have argued, however, that these experimentally-induced tu-

mors are special cases and that most spontaneous tumors do not carry strong tumor-specific transplantation antigens [152–154]. The best evidence for tumor-specific antigens on human tumors has come from the demonstration that some cancer patients produce antibodies that detect cell surface antigens that are restricted to their own tumors (reviewed in reference [3]). However, since the biochemical or genetic basis for these antigenic specificities is unknown, the full significance of these findings cannot yet be determined. Neither have mouse or human monoclonal antibodies been produced that identify these unique tumor antigens.

The hope that antigens restricted to human tumor cells could be detected by producing hybridoma antibodies from mice immunized with such cell has not yet been clearly fulfilled. None of the new antigens so far detected using mouse monoclonal antibodies have, upon rigorous examination, been shown to be completely restricted to tumor cells. Nevertheless, a number of promising systems are still under investigation and as new antigens are continuously being discovered, it is premature to conclude that this approach will not eventually demonstrate the existence of tumor-restricted antigens. In considering the limited success to date, three reasons can be suggested for the failure to detect restricted antigens: (i) human tumor specific-antigens do not exist (see above), (ii) human tumor-specific antigens exist but human-mouse immunizations are not capable of producing antibodies to them, and (iii) human tumor-specific antigens exist but they are extremely minor components of the cell surface, or are poor immunogens, so that whole tumor cells or crude extracts are not sufficiently immunogenic for the stimulation of antibody-producing clones of this specificity. It should also be emphasized at this point that many moAbs give an appearance of restricted specificity during early stages of an analysis because of the difficulty in completely elucidating the tissue distribution of an antigen. To complete such an analysis, a quantitative assay must be developed to the antigen in order to set a minimum level for its expression in apparently antigen-negative cells. In addition, a large variety of tissues must be screened by immunofluorescence or immunoperoxidase techniques in order to confirm expression of the antigen in tumor samples and to rule out its expression on normal tissues not available for study as cell lines.

Even with these precautions, it is difficult, or even impossible, to prove that an antigen is 'tumor-specific' in the sense that it is not possible to prove a negative, i.e. the complete absence of antigen from normal tissue. It would seem best to conclude that tumor specificity is not a useful concept except perhaps in the context of an immune response in the cancer patient. Monoclonal antibodies, and the antigens they detect, should be considered individually in terms of their particular properties and the uses to which they can be applied.

14.2 *Differentiation antigens*

Most of the tumor antigens thus far detected by mouse monoclonal antibodies can be characterized as differentiation antigens. Differentiation antigens were originally defined as those categorizing '... different cell types in a single individual' [155]. However, as recognized by the original authors, this is rather a broad definition and at least three sub-categories can be recognized:

(1) *Antigens characteristic of cells of a common embryologic origin.* Examples of this class are the antigens discussed in Sections 2 and 3 that are common to melanomas, melanocytes, astrocytomas, and neural cells. These antigens may represent the vestigial expression of markers that were involved in the formation and migration of neuroectodermal cells in embryonic development.

(2) *Antigens defining cells of a particular tissue.* Leucocyte common antigen (Leu 200), an antigen widely expressed on leucocytes (being found on T- and B-lymphocytes, thymocytes, macrophages and granulocytes, but not on other tissues), is a typical tissue-specific antigen [156, 157]. Several of the antigens of the kidney system, e.g. gp160 and S_{25}, also fall into this group (see Section 8). Similarly, moAb R_{24} (recognizing G_{D3} ganglioside) reacts with all cells of the melanocyte lineage. Presumably these antigens have a function related to the specialized functions of the tissue on which they are expressed.

(3) *Antigens distinguishing cells at a particular differentiation or maturation stage within cells of a given lineage.* Several examples of maturation antigens have already been given; these include antigens in the proposed melanocyte differentiation pathway (Section 2.13) and antigens of astrocyte differentiation (Section 3). An interesting aspect of these studies is that they use tumor cells to gain information about normal cell differentiation. Some antigens serve as differentiation markers in two different tissues, e.g. HLA-DR in both melanocyte and lymphocyte differentiation.

As most of the monoclonal antibodies that have apparent specificity for tumor cells detect quantitative differences in antigen expression, tumors must express much larger amounts of some differentiation antigens than do the corresponding normal cells. The mechanism for this enhanced expression is unclear but recent advances in related areas of molecular biology provide some basis for speculation. The concept is developing that oncogenes are derived from their counterparts (proto-oncogenes) in the normal genome and that transformation is caused by the activation of such genes; this results in the enhanced production of a normal cell protein usually held

under tight control [158–160]. According to this view, malignancy is a dosage effect and results from the elevated expression of normal gene products. These findings have clear implications for the nature of cell surface antigen in tumor cells even though the primary gene products of oncogenes are thought to be intracellular. Although the function of these products is not yet understood, their presumed effect on the synthesis of other cell components could result in changes in cell surface composition. Moreover, the recent demonstration that the transformation of B- and T-lymphocytes results from the activation of specific oncogenes corresponding to specific stages of differentiation within the cell lineage [161], provides an explanation for the amplified production of the differentiation-related products in human tumors that are detected by mouse monoclonal antibodies.

14.3 *Jumping and random antigens*

Milstein and Lennox [162], and later Springer [163], defined a category of differentiation antigens that they termed 'jumping' antigens. This description was used to describe antigens that appear, disappear and reappear in cells as they proceed along their differentiation pathway. Often the last stage is correlated with the migration of the cells to a different tissue, e.g. mouse erythroblasts loose Forssman antigen before they leave the bone marrow and enter the circulation. Several other examples of jumping antigens were cited; these included Thy-1 antigen (in T-lymphocyte and brain development) and asialo-G_{M1} (as a marker for peripheral T-lymphocytes that is absent from thymocytes). To this list can be added SSEA-1 antigen [70, 164] and blood group I and i antigens [165] which show characteristic cyclic expression during early embryogenesis in the mouse.

The concept of jumping antigens is valuable when applied to cells of a single lineage, however since many of the antigens are also expressed on some unrelated cell types the term is not very precise. For example, Thy-1 is found on brain and fibroblasts as well as thymocytes in the mouse. Likewise, Forssman antigen is expressed on cells that are not on a direct differentiation pathway. Some antigens have an even more random distribution and are expressed on cell types having no apparent relationship. For example, gp95 in addition to being strongly expressed on melanoma cells (but not on melanocytes) is also found on sweat gland epithelia [26]. Likewise, S_6, which was considered to be a kidney tissue-specific antigen [96], has recently been found to be expressed on cultured melanocytes [49]. Undoubtedly, these apparently random patterns have their basis in shared common functions that will become clear once the antigens have been fully characterized. In the case of the expression of gp95 on melanoma and sweat gland epithelia, the basis for the shared antigen expression may even be the common embryologic origin of these cell types from primitive ectoderm. Such exam-

ples indicate that classification schemes must be used with some caution as many antigens have functions that overlap any proposed categories.

14.4 *Fetal antigens*

Although the re-expression of fetal or embryonic antigens is often considered to be a characteristic of cancer cells, few examples of the detection of this type of antigen by monoclonal antibodies can be cited. The gastrointestinal cancer antigen, GICA, may, however, be one example, as it is found in meconium as well as in tumors [64].

14.5 *Common antigens*

Widely-distributed antigens may be of interest as cancer markers when their normal structure is altered or if they show anomolous expression in tumors. The unexpected detection of A blood group [166, 167], P blood group [168] and Forssman [169] antigens in A, P, or Forssman-negative individual, respectively, are examples.

15. CONCLUDING REMARKS

A remarkable array of tumor antigens has already been identified using the hybridoma antibody approach. Very few of these antigens had previously been recognized using polyclonal antisera, thus illustrating the power of the technique to select monoclonal antibodies directed to antigens having some sought-after characteristic. As discussed in the previous section, the antigens detected fall into several categories and it is likely the general range of tumor antigens that can be detected using mouse monoclonal antibodies has already been identified.

Monoclonal antibodies have already provided powerful new insights into the characteristics of tumor cell components and into tumor cell biology in general. A major conclusion of this review is that very few mouse monoclonal antibodies to human tumor cells detect antigens that fit into precise, defined categories, e.g. tumor-specific; rather they show individual patterns of reactivity and each antibody has to be considered on its own merit and used according to its particular characteristics. Future advances in the application of monoclonal antibodies to the diagnosis and therapy of human cancers will, therefore, depend on the imaginative use of reagents which may not have the ideal property of complete tumor specificity. The production of human-human monoclonal antibodies has barely been mentioned in this review, but this is obviously another area in which rapid advances will be made in the future.

ACKNOWLEDGEMENTS

I am grateful to Ms. Linda Roux for help in preparing sections of this review and to Dr. Alan Houghton for critically reading the text. I also thank Ms. Yvette Johnson for expert assistance in typing the manuscript. Work in the author's laboratory is supported by the National Cancer Institute grants CA-08748, CA-21445 and CA-26184 and by the Borden and Gorrett funds.

REFERENCES

1. Köhler G, Milstein C: Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256:495–496, 1975.
2. Yelton DE, Schraff M: Monoclonal antibodies: A powerful tool in biology and medicine. Ann Rev Biochem 50:657–680, 1981.
3. Old LJ: Cancer immunology: The search for specificity. Cancer Res 41:361–375, 1981.
4. Eisinger M, Marko O: Selective proliferation of normal melanocytes *in vitro* in the presence of phorbol ester and cholera toxin. Proc Natl Acad Sci USA 79:2018–2022, 1982.
5. Herlyn M, Herlyn D, Guerry D: *In vitro* growth and characterization of cells from non-malignant melanocytic lesions. Abstr American Assoc Cancer Res 23:274, 1982.
6. Albino AP, Lloyd KO, Houghton AN, Oettgen HF, Old LJ: Heterogeneity in surface antigen and glycoprotein expression of cell lines derived from different melanoma metastases of the same patient. J Exp Med 154:1764–1778, 1981.
7. Kanzaki T, Hashimoto K, Bath DW: Heterotransplantation of human malignant melanoma cell lines in athymic nude mice. J Nat Cancer Inst 62:1151–1158, 1979.
8. Lloyd KO, Travassos LR, Takahashi T, Old LJ: Cell surface glycoproteins of human tumor cell lines. J Nat Cancer Inst 63:623–634, 1979.
9. Mishima Y: Melanocytic and nevocytic malignant melanoma: Cellular and subcellular differentiation. Cancer 20:632–649, 1967.
10. Koprowski H, Steplewski Z, Herlyn D, Herlyn M: Study of antibodies against human melanoma produced by somatic cell hybrids. Proc Natl Acad Sci USA 75:3405–3409, 1978.
11. Proceedings of National Cancer Institute Workshop on 'Malignant Antibodies to Human Melanoma Antigens' Hybridoma 1:379–482, 1982.
12. Carrel S, Accolla RS, Carmagnola RL, Mach J-P: Common human melanoma-associated antigen(s) detected by monoclonal antibodies. Cancer Res 40:2523–2528, 1980.
13. Imai K, Ng AK, Ferrone S: Characterization of monoclonal antibodies to human melanoma-associated antigens. J Nat Cancer Inst 66:489–496, 1981.
14. Morgan AC, Galloway DR, Reisfeld RA: Production and characterization of monoclonal antibody to a melanoma specific glycoprotein. Hybridoma 1:27–36, 1981.
15. Wilson BS, Imai K, Natali PG, Ferrone S: Distribution and molecular characterization of a cell-surface and a cytoplasmic antigen detectable in human melanoma cells with monoclonal antibodies. Int J Cancer 28:293–300, 1981.
16. Natali PG, Imai K, Wilson BS, Bigotti A, Cavaliere R, Pellegrino MA, Ferrone S: Structural properties and tissue distribution of the antigen recognized by the monoclonal antibody 653.40S to human melanoma cells. J Nat Cancer Inst 67:591–601, 1981.
17. Bumol TF, Reisfeld RA: Unique glycoprotein - proteoglycan complex defined by monoclonal antibody on human melanoma cells. Proc Natl Acad Sci USA 79:1245–1249, 1982.

18. Loop SM, Nishiyama K, Hellström I, Woodbury RG, Brown JP, Hellström KE: Two human tumor-associated antigens, P155 and P210, detected by monoclonal antibodies. Int J Cancer 27:775–781, 1981.
19. Dippold WG, Lloyd KO, Li LTC, Ikeda H, Oettgen HF, Old LJ: Cell surface antigens of human malignant melanoma: Definition of six antigenic systems with mouse monoclonal antibodies. Proc Natl Acad Sci USA 77:6114–6118, 1980.
20. Saxton RE, Mann BD, Irie RF, Morton DL, Burk MW: Monoclonal antibodies to human melanoma: Identification of two unique proteins on the tumor cell membrane. Abstr American Assoc Cancer Res 23:263, 1982.
21. Albino AP, Lloyd KO, Old LJ: In preparation.
22. Thompson JJ, Herlyn MF, Elder DE, Clark WC, Steplewski Z, Koprowski H: Use of monoclonal antibodies in detection of melanoma-associated antigens in intact human tumors. Amer J Pathol 107:357–361, 1982.
23. Mitchell KF, Fuhrer JP, Steplewski Z, Koprowski H: Biochemical characterization of human melanoma cell surfaces: Dissection with monoclonal antibodies. Proc Natl Acad Sci USA 77:7287–7291, 1980.
24. Mitchell KF, Fuhrer JP, Steplewski Z, Koprowski H: Structural characterization of the 'melanoma-specific' antigen detected by monoclonal antibody 69115NU-4-B. Mol Immunol 18:207–218, 1981.
25. Woodbury RG, Brown JP, Yey M, Hellström I, Hellström KE: Identification of a cell surface protein, p97 in human melanomas and certain other neoplasms. Proc Natl Acad Sci USA 77:2183–2187, 1980.
26. Garrigues HJ, Tilgen W, Franke W, Hellström KE: Detection of a human melanoma-associated antigen, p97, in histological sections of primary human melanomas. Int J Cancer 29:511–515, 1982.
27. Brown JP, Woodbury RG, Hart CE, Hellström I, Hellström KE: Quantitative analysis of melanoma-associated antigen p97 in normal and neoplastic tissues. Proc Natl Acad Sci USA 78:539–543, 1981.
28. Brown JP, Nishiyama K, Hellström KE: Structural characterization of human melanoma-associated antigen p97 with monoclonal antibodies. J Immunol 127:539–546, 1981.
29. Brown JP, Hewick RM, Hellström I, Hellström KE, Doolittle RF, Dreyer WJ: Human melanoma-associated antigen p97 is structurally and functionally related to transferrin. Nature 296:171–173, 1982.
30. Imai K, Wilson BS, Bitotti A, Natali PG, Ferrone S: A 94,000 dalton glycoprotein expressed by human melanoma and carcinoma cells. J Natl Cancer Inst 68:761–767, 1982.
31. Natali PG, Wilson BS, Imai K, Bigotti A, Ferrone S. Tissue distribution, molecular profile, and shedding of a cytoplasmic antigen identified by the monoclonal antibody 465.12S to human melanoma cells. Cancer Res 42:583–589, 1982.
32. Chee DO, Yomemoto RM, Leong SPI, Richards RG, Smith VR, Klotz JL, Goto RM: Mouse monoclonal antibodies to a melanoma-carcinoma associated antigen synthesized by a human melanoma cell line propagated in serum free medium. Cancer Res. 42:3142–3147, 1982.
33. Bumol TF, Chee DO, Reisfeld RA: Immunochemical and biosynthetic analysis of a monoclonal antibody defined melanoma-associated antigen. Hybridoma 1:283–292, 1982.
34. Johnson JP, Demmer-Dieckman M, Meo T, Hadam MR, Reithmuller G: Surface antigens of human melanoma cells defined by monoclonal antibodies: I. Biochemical characterization of two antigens found on cell lines and fresh tumors of diverse origin. Europ J Biochem 11:825–831, 1981.
35. Yeh M-Y, Hellström I, Brown JP, Warner GA, Hansen JA, Hellström KE: Cell surface antigens of human melanoma identified by monoclonal antibody. Proc Nat Acad Sci USA 76:2927–2931, 1979.

36. YehM-Y, Hellström I, Hellström KE: Clonal variation in expression of a human melanoma antigen defined by a monoclonal antibody. J Immunol 126:1312–1317, 1981.
37. Hellström I, Hellström KE, Yeh M-Y: Lymphocyte-dependent antibodies to antigen 3.1, a cell-surface antigen expressed by a subgroup of human melanomas. Int J Cancer 27:281–285, 1981.
38. Pukel CS, Lloyd KO, Travassos LR, Dippold WG, Oettgen HF, Old LJ: G_{D3}, a prominent ganglioside of human melanoma: Detection and characterization by mouse monoclonal antibody. J Exp Med 155:1133–1147, 1982.
39. Yeh M-Y, Hellström I, Abe K, Hakomori S, Hellström KE: A cell-surface antigen which is present in the ganglioside fraction and shared by human melanomas. Int J Cancer 29:269–275, 1982.
40. Real F, Houghton AN, Urmacher C, Melamed M, Oettgen H, Old LJ: Monoclonal antibodies detecting melanoma antigen in tissue sections. Abstrs American Assoc Cancer Res 23:225, 1982.
41. Nudelman E, Hakomori S, Kannagi R, Levery S, Yeh M-Y, Hellström KE, Hellström I: Characterization of a human melanoma-associated ganglioside antigen defined by a monoclonal antibody, 4.2. J Biol Chem 257:12752–12756, 1982.
42. Portoukalian J, Zwingelstein G, Dore J: Lipid composition of human malignant melanoma tumors at various levels of malignant growth. Eur J Biochem 94:19–23, 1979.
43. Brown JP, Wright PW, Hart CE, Woodbury RG, Hellström KE, Hellström I: Protein antigens of normal and malignant human cells identified by immunoprecipitation with monoclonal antibodies. J Biol Chem 255:4980–4983, 1980.
44. Winchester RJ, Wang C-Y, Gibofsky A, Kunkel HG, Lloyd KO, Old LJ: Expression of Ia-like antigens on cultured human malignant melanoma cell lines. Proc Nat Acad Sci USA 75:6235–6239, 1978.
45. Wilson BS, Indiveri F, Pellegrino MA, Ferrone S: DR (Ia-like) antigens on human melanoma cells: Serological detection and immunochemical characterization. J Exp Med 149:658–668, 1979.
46. Lloyd KO, Ng J, Dippold WG: Analysis of the biosynthesis of HLA-DR glycoproteins in human malignant melanoma cell lines. J Immunol 126:2408–2413, 1981.
47. Burchiel SW, Martin JC, Imai K, Ferrone S, Warner NL: Heterogeneity of HLA-A, B, Ia-like and melanoma associated antigen expression by human melanoma cell lines analyzed with monoclonal antibodies and flow cytometry. Cancer Res 42:4110–4115, 1982.
48. Johnson JP, Meo J, Riethmuller G, Schendel DJ, Wank R: Direct demonstration of an HLA-DR allotypic determinant on the low molecular weight (beta) subunit using a mouse monclonal antibody specific for DR3. J Exp Med 156:104–111, 1982.
49. Houghton AN, Eisinger M, Albino AP, Cairncross JG, Old LJ: Surface antigens of melanocytes and melanomas: Markers for melanocyte differentiation and melanoma subsets. J Exp Med 156:1735-1766, 1982.
50. Kennett RH, Gilbert E: Hybrid myelomas producing antibodies against a human neuroblastoma antigen present on fetal brain. Science 203:1120–1121, 1979.
51. Schnegg JF, Diserens AC, Carrel S, Accolla RS, De Tribolet N: Human glioma-associated antigens detected by monoclonal antibodies. Cancer Res 41:1209–1213, 1981.
52. Cairncross JG, Mattes MJ, Beresford HR, Albino AP, Houghton AN, Lloyd KO, Old LJ: Cell surface antigens of human astrocytoma defined by mouse monoclonal antibodies: Identification of astrocytoma subsets. Proc Natl Acad Sci USA 79:5641–5643, 1982.
53. Seeger RC, Danon YL, Rayner SA, Hoover F: Definition of a Thy-1 determinant of human neuroblastoma, glioma, sarcoma and teratoma cells with a monoclonal antibody. J Immunol 128:983–989, 1982.
54. Momoi M, Kennett RH, Glick MC: A membrane glycoprotein from human neuroblasto-

ma cells isolated with the use of a monoclonal antibody. J Biol Chem 255:11914–11921, 1980.

55. Seeger RC, Rosenblatt HM, Imai K, Ferrone S: Common antigenic determinants on human melanoma, glioma, neuroblastoma and sarcoma cells defined with monoclonal antibodies. Cancer Res 41:2714–2717, 1981.
56. Liao SK, Clarke BJ, Kwong PC, Brickenden A, Gallie BL, Dent PB: Common neuroectodermal antigens on human melanoma, neuroblastoma, retinoblastoma, glioblastoma and fetal brain revealed by hybridoma antibodies raised against melanoma cells. Eur J Immunol 11:450–454, 1981.
57. Carrel S, de Tribolet N, Mach J-P: Expression of neuroectodermal antigens common to melanoma, gliomas and neuroblastomas: I. Identification by monoclonal anti-melanoma antibodies. Acta Neuropathol 57:158–164, 1982.
58. Sikora K, Phillips J: Human monoclonal antibodies to glioma cells. Brit J Cancer 43:105–107, 1981.
59. Sikora K, Alderson T, Phillips J, Watson JV: Human hybridomas from malignant gliomas. Lancet i:11–14, 1982.
60. Herlyn M, Steplewski Z, Herlyn D, Koprowski H: Colorectal carcinoma-specific antigen: Detection by means of monoclonal antibodies. Proc Natl Acad Sci USA 76:1438–1442, 1979.
61. Koprowski H, Steplewski Z, Mitchell K, Herlyn M, Herlyn D, Fuhrer P: Colorectal carcinoma antigens detected by hybridoma antibodies. Somatic Cell Genet 5:957–972, 1979.
62. Brockhaus M, Magnani LJ, Blaszczyk M, Steplewski Z, Koprowski H, Karlsson K, Larson G, Ginsburg V: Monoclonal antibodies directed against the human Le^b blood antigen. J Biol Chem 256:13223–13225, 1981.
63. Hakomori S, Andrews HD: Sphingoglycolipids with Le^b activity, and the co-presence of Le^a and Le^b glycolipids in human tumor tissue. Biochim Biophys Acta 202:225–228, 1970.
64. Magnani JL, Brockhaus M, Smith DF, Ginsburg V, Blaszczyk M, Mitchell KF, Steplewski Z, Koprowski H: A monosialoganglioside is a monoclonal antibody-defined antigen of colon carcinoma. Science 212:55–56, 1981.
65. Magnani J, Nilsson B, Brockhaus M, Zopf D, Steplewski Z, Koprowski H, Ginsburg V: The antigen of a tumor-specific monoclonal antibody is a ganglioside containing sialylated lacto-N-fucopentaose II. Fed Proc 41:898, 1982.
66. Chang TH, Steplewski Z, Sears HF, Koprowski H: Detection of monoclonal antibody-defined colorectal carcinoma antigen by solid-phase binding inhibition radioimmunoassay. Hybridoma 1:37–45, 1981.
67. Herlyn M, Sears HF, Steplewski Z, Koprowski H: Monoclonal antibody detection of a circulating tumor-associated antigen: I. Presence of antigen in sera of patients with colorectal, gastric and pancreatic carcinoma. J Clin Immunol 2:135–140, 1982.
68. Koprowski H, Blaszczyk M, Steplewski Z, Brockhaus M, Magnani J, Ginsburg V: Lewis blood-type may affect the incidence of gastrointestinal cancer. Lancet i:1332–1333, 1982.
69. Brockhaus M, Magnani J, Herlyn M, Blaszczyk M, Ginsburg V: Monoclonal antibodies directed against the sugar sequence of lacto-N-fucopentaose III obtained from mice immunized with human tumors. Fed Proc 41:897, 1982.
70. Gooi HC, Feizi T, Kapadia A, Knowles BB, Solter D, Evans MJ: Stage-specific embryonic involves antigen involves α (1$\rightarrow$3) fucosylated type 2 blood group chains. Nature 292:156–158, 1981.
71. Hakomori S, Nudelman E, Levery S, Solter D, Knowles BB: The hapten structure of a

developmentally regulated glycolipid antigen (SSEA-1) isolated from human erythrocytes and adenocarcinoma: A preliminary note. Biochem Biophys Acta 100:1579–1586, 1981.

72. Hounsell EF, Gooi HC, Feizi T: The monoclonal antibody anti-SSEA-1 discriminates between fucosylated type 1 and type 2 blood group chains. FEBS Letts 131:279–282, 1981.
73. Cuttitta F, Rosen S, Gazdar AF, Minna JD: Monoclonal antibodies that demonstrate specificity for several types of human lung cancer. Proc Natl Acad Sci USA 78:4591–4595, 1981.
74. Kasai M, Saxton RE, Holmes EC, Burk MW, Morton DL: Membrane antigens detected on human lung carcinoma cells by hybridoma monoclonal antibody. J Surg Res 30:403–408, 1981.
75. Iwaki Y, Kasai M, Terasaki PI, Bemoco D, Park MS, Ciccicrelli J, Henitz B, Saxton RE, Burk MW, Morton DL: Monoclonal antibody against A_1 Lewis^d antigen produced by the hybridoma immunized with a pulmonary carcinoma. Cancer Res 42:409–411, 1982.
76. Anger BR, Lloyd KO, Oettgen HF, Old LJ: Mouse monoclonal IgM antibody against human lung cancer line SK-LC-3 with specificityfor H (O) blood group antigen. Hybridoma 1:139–147, 1982.
77. Young WW, Portoukalian J, Hakomori S: Two monoclonal anticarbohydrate antibodies directed to glycophingolipids with a lacto-N-glycosyl Type II chain. J Biol Chem 256:10967–10972, 1981.
78. Knowles RW, Bai Y, Daniels GL, Watkins W: Monoclonal anti-type 2H: An antibody detecting a precursor of the A and B blood group antigens. J Immunogen 9:69–76, 1982.
79. Mazauric T, Mitchell KF, Letchworth GJ, Koprowski H, Steplewski Z: Monoclonal antibody defined human lung cell surface protein antigens. Cancer Res 42:150–154, 1982.
80. Lloyd KO: Human ovarian tumor antigens. In: Serological analysis of human cancer antigens, Rosenberg SA (ed). New York: Academic Press, 1980, pp 515–524.
81. Bhattacharya M, Barlow JJ: Tumor markers for ovarian cancer. Int Adv Surg Oncol 2:155–176, 1979.
82. Bast RC, Feeney M, Lazarus H, Nadler LM, Colvin RC, Knapp RC: Reactivity of a monoclonal antibody with human ovarian carcinoma. J Clin Invest 68:1331–1337, 1981.
83. Kabawat SE, Bast RC, Welch WR, Knapp RC, Colvin RB: Immunopathologic characterization of a monoclonal antibody that recognizes common surface antigens of human ovarian tumors of serous, endometroid and clear cell types. Amer J Pathol 79:98–104, 1983.
84. Bhattacharya M, Chatterjee SK, Barlow JJ, Fuji H: Monoclonal antibodies recognizing tumor-associated antigen of human ovarian mucinous cystadenocarcinomas. Cancer Res 42:1650–1654, 1982.
85. Colcher D, Hand PH, Nuti M, Schlom J: A spectrum of monoclonal antibodies reactive with human mammary tumor cells. Proc Natl Acad Sci USA 78:3199–3203, 1981.
86. Yuan D, Handler FJ, Vitetta ES: Characterization of a monoclonal antibody reactive with a subset of human breast tumors. J Nat Cancer Inst 68:719–729, 1982.
87. Taylor-Papadimitriou J, Peterson JA, Arklie J, Burchell J, Ceriani RL: Monoclonal antibodies to epithelium-specific components of the human milk fat globule membrane: Production and reactions with cells in culture. Int J Cancer 28:17–21, 1981.
88. Arklie J, Taylor-Papadimitriou J, Bodmer WF, Egan M, Millis R: Differentiation antigens expressed by epithelial cells in the lactating breast are also detectable in breast cancers. Int J Cancer 28:23–29, 1981.
89. Foster CS, Edwards PA, Dinsdale EA, Neville AM: Monoclonal antibodies to the human

mammary gland: I. Distribution of determinants in non-neoplastic mammary and extra mammary tissues. Virchows Arch Pathol Anat 394:279–293, 1982.

90. Foster CS, Dinsdale EA, Edwards PA, Neville AM: Monoclonal antibodies to the human mammary gland: II. Distribution of determinants in breast carcinomas. Virchows Anat Pathol Anat 394:295–305, 1982.
91. Nuti M, Teramoto YA, Mariani-Constantini R, Hand PH, Colcher D, Schlom J. A monoclonal antibody (B72.3) defines patterns of distribution of a novel tumor associated antigen in human mammary carcinoma cell populations. Int J Cancer 29:539–545, 1982.
92. Schlom J, Wunderlich D, Teramoto YA: Generation of human monoclonal antibodies reactive with human mammary carcinoma cells. Proc Natl Acad Sci USA 77:6841–6845, 1980.
93. Gold P, Freedman SO: Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. J Exp Med 121:439–462, 1964.
94. Neville AM, Laurence DJR: Report of the workshop on the carcino-embryonic antigen (CEA): The present positions and proposals for future investigation. Int J Cancer 14:1–18, 1974.
95. Colcher D, Hand PH, Nuti M, Schlom J: Differential binding to human mammary and non-mammary tumors of monoclonal antibodies reactive with carcinoembryonic antigen. Cancer Invest 1:127–138, 1983.
96. Ueda R, Ogata S, Morrissey DM, Finstad CL, Szkudlarek J, Whitmore WF, Oettgen HF, Lloyd KO, Old LJ: Cell surface antigens of human renal cancer defined by mouse monoclonal antibodies: Identification of tissue-specific glycoproteins. Proc Natl Acad Sci USA 76:5122–5126, 1981.
97. Bander N, Finstad C, Old LJ. In preparation.
98. Metzgar RD, Gaillard MT, Levine SJ, Tuck FL, Bossen EH, Borowitz MJ: Antigens of human pancreatic adenocarcinoma cells defined by murine monoclonal antibodies. Cancer Res 42:601–608, 1982.
99. Frankel AE, Rouse RV, Herzenberg LA: Human prostate specific and shared differentiation antigens defined by monoclonal antibodies. Proc Natl Acad Sci USA 79:903–907, 1982.
100. Ware JL, Webb KS, Paulson DF: Monoclonal antibodies recognizing human prostatic carcinoma. Fed Proc 40:982, 1981.
101. Wright GL, Starling JL, Sieg SM: Monoclonal antibodies to tumor-associated antigens in human prostate and bladder cancer. Fed Proc 40:995, 1981.
102. Embleton MJ, Gunn B, Byers VS, Baldwin RW: Antitumor reactions of monoclonal antibody against a human osteogenic-sarcoma cell line. Br J Cancer 43:582–587, 1981.
103. Hosoi S, Nakamura T, Higashi S, Yamamuro T, Toyama S, Shinomiya K, Mikawa H: Detection of human osteosarcoma-associated antigens detected by monoclonal antibodies. Cancer Res 42:654–659, 1982.
104. Schroff RW, Foon KA, Billing RJ, Fahey JL: Immunologic classification of lymphocytic leukemias based on monoclonal antibody-defined cell surface antigens. Blood 59:207–215, 1982.
105. Foon KA, Schroof RW, Gale RP: Surface markers on leukemia and lymphoma cells: Recent advances. Blood 60:1–19, 1982.
106. Haynes BF, Hemler M, Cotner T, Mann DL, Eisenbarth GS, Strominger JL, Fauci AS: Characterization of a monoclonal antibody (5E9) that defines a human cell surface antigen of cell activation. J Immunol 127:347–351, 1981.
107. Omary MB, Trowbridge IS, Minowada J: Human cell-surface glycoprotein with unusual properties. Nature 286:888–891, 1980.

108. Billing R, Lucero K, Shi BJ, Terasaki PI: A new acute leukemia-associated blast cell antigen detected by a monoclonal antibody. Blood 59:1203–1206, 1982.
109. Terhorst C, van Agthoven A, LeClair K, Snow P, Reinherz E, Schlossman S: Biochemical studies of the hunam thymocyte cell-surface antigens T6, T9 and T10. Cell 23:771–780, 1981.
110. Billing R, Terasaki PI, Clark B: Heteroantisera against a blast cell antigen. Clin Immol Immolpath 13:435–443, 1979.
111. Reinherz EL, Schlossman SF: The differentiation and function of human T lymphocytes. Cell 19:821-827, 1980.
112. Reinherz EL, Schlossman SF: Derivation of human T-cell leukemias. Cancer Res 41:4767–4770, 1981.
113. Reinherz EL, Kung PD, Goldstein G, Levey RH, Schlossman SF: Discrete stages of human intrathymic differentiation: Analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage. Proc Natl Acad Sci USA 77:1588-1592, 1980.
114. Trowbridge IS, Omary MB: Human cell surface glycoprotein related to cell proliferation is the receptor for transferrin. Proc Natl Acad Sci USA 78:3039–3043, 1981.
115. Schneider C, Sutherland R, Newman R, Greaves M: Structural features of the cell surface receptor for transferrin that is recognized by the monoclonal antibody OKT9. J Biol Chem 257:8516–8255, 1982.
116. Bramwell ME, Harris H: An abnormal membrane glycoprotein associated with malignancy in a wide range of different tumors. Proc Roy Soc (London) B201:87–106, 1981.
117. Bramwell ME, Harris H: Some further information about the abnormal membrane glycoprotein associated with malignancy. Proc Roy Soc (London) B203:93–98, 1978.
118. Knowles RW, Bodmer WF: A monoclonal antibody recognizing a human thymus leukemia-like antigen associated with β_2-microglobulin. Eur J Immunol 12:676–681, 1982.
119. Ziegler A, Milstein C: A small polypeptide different from β_2-microglobulin associated with a human cell surface antigen. Nature 279:243–244, 1979.
120. McMichael AJ, Pilch JR, Galfre G, Mason DY, Fabre JW, Milstein C: A human thymocyte antigen defined by a hybrid myeloma monoclonal antibody. Eur J Immunol 9:205–210, 1979.
121. Tanigaki N, Tokuyama H, Fukunishi T, Minowada J, Pressman D: Human cell membrane components dominant in T-cell lineage: Identification and characterization of human TL-like antigens. J Immunol 123:2906–2914, 1979.
122. Deng C-T, Chia J, Terasaki P, Billing R. Monoclonal antibody specific for human T acute lymphoblastic leukaemia. Lancet i:11, 1982.
123. Catovsky D, Cherchi M, Brooks D, Bradley J, Zola H: Heterogeneity of B-cell leukemias demonstrated by the monoclonal antibody FMC7. Blood 58:406–408, 1981.
124. Brooks DA, Beckman IGR, Bradley J, McNamara PJ, Thomas ME, Zola H: Human lymphocyte markers defined by antibodies derived from somatic cell hybrids: IV. A monoclonal antibody reacting specifically with a subpopulation of human B-lymphocytes. J Immunol 126:1372–1377, 1981.
125. Ritz J, Nadler LM, Bhan AK, Notis-McConarty J, Pesando JM, Schlossman SF: Expression of common acute lymphoblastic leukemia antigen (CALLA) by lymphomas of B-cell and T-cell lineage. Blood 58:648–652, 1981.
126. Carrel S, de Tribolet N, Gross N: Expression of HLA-DR and common acute lymphoblastic leukemia antigens on glioma cells. Eur J Immunol 12:354–357, 1982.
127. Metzgar RS, Borowitz MJ, Jones NH, Dowell BL: Distribution of common acute lymphoblastic leukemia antigen in non-hematopoietic tissues. J Exp Med 154:1249–1254, 1981.
128. Janossy G, Bollum FJ, Bradstock KF, McMichael A, Rapson N, Greaves MF: Terminal transferase-positive human bone marrow cells exhibit the antigenic phenotype of common acute lymphoblastic leukemia. J Immunol 123:1525-1529, 1979.

129. Greaves M, Delia D, Janossy G, Rapson N, Chessells G, Woods M, Prentice G: Acute lymphoblastic leukemia associated antigen: IV. Expression on non-leukemic 'lymphoid' cells. Leuk Res 4:15–32, 1980.
130. Ritz J, Pesando J, Notis-McConarty J, Lazarus H, Schlossman SF: A monoclonal antibody to human acute lymphoblastic leukemia antigen. Nature 283:583–585, 1980.
131. Ritz J, Pesando JM, Notis-McConarty J, Clavell LA, Sallan SE, Schlossman SF: Use of monoclonal antibodies as diagnostic and therapeutic reagents in acute lymphoblastic leukemia. Cancer Res 41:4771–4775, 1981.
132. Knapp W, Majdic O, Bettelheim P, Liszka K: VIL-A1, a monoclonal antibody reactive with common acute lymphatic leukemia cells. Leuk Res 6:137–147, 1982.
133. Pesando JM, Ritz J, Levine H, Terhorst C, Lazarus H, Schlossman SF: Human leukemia-associated antigen: relation to a family of surface glycoproteins. J Immunol 124:2794–2799, 1980.
134. Kersey JH, Le Bien TW, Abramson CS, Newman R, Sutherland R, Greaves M: p24: A human leukemia-associated and lymphohemopoietic progenitor cell surface structure identified with monoclonal antibody. J Exp Med 153:726–731, 1981.
135. Ueda R, Tanimoto M, Takahashi T, Ogata S-i, Nishida K, Namikawa R, Nishizuka Y, Ota K: Serological analysis of cell surface antigens of null cell acute lymphocytic leukemia by mouse monoclonal antibodies. Proc Natl Acad Sci USA 79:4386–4390, 1982.
136. Linker-Israeli M, Billing RJ, Foon KA, Terasaki PI: Monoclonal antibodies reactive with acute myelogenous leukemia cells. J Immunol 127:2473–2477, 1981.
137. Ball ED, Kadushin JM, Schacter B, Fanger MW: Studies on the ability of monoclonal antibodies to selectively mediate complement-dependent cytotoxicity of human myelogeneous leukemia blast cells. J Immunol 128:1476–1481, 1982.
138. Vainchenker W, Deschamps JF, Bastin J, Guichard J, Titeux M, Breton-Gorius J, McMichael AJ: Two monoclonal anti-platelet antibodies as markers of human megakaryocyte maturation: Immunofluorescent staining and platelet peroxidase detection in megakaryocyte colonies and in *in vivo* cells from normal and leukemic patients. Blood 52:514–521, 1982.
139. McMichael AJ, Rust NA, Pilch JR, Sochynsky R, Morton J, Mason DY, Ruan C, Tobelem G, Caen J: Monoclonal antibody to human platelet glycoprotein II. Br J Haem 49:501–509, 1982.
140. Thorley-Lawson DA, Edson CM, Geilinger K: Epstein-Barr virus antigens - a challenge to modern biochemistry. Adv Cancer Res 36:295–348, 1982.
141. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC: Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci USA 77:7415–7419, 1980.
142. Kalyanaraman VS, Sarngadharan MG, Poiesz B, Ruscetti FW, Gallo RC: Immunological properties of a type C retrovirus isolated from cultured human T-lymphoma cells and comparison to other mammalian retroviruses. J Virol 38:906–915, 1981.
143. Robert-Guroff M, Ruscetti FW, Posner LE, Poiesz BJ, Gallo RC: Detection of the human T-cell lymphoma virus p19 in cells of some patients with cutaneous T-cell lymphoma and leukemia using a monoclonal antibody. J Exp Med 154:1957–1964, 1981.
144. Accolla RS, Carrel S, Mach J-P: Monoclonal antibodies specific for carcino-embryonic antigen and produced by two hybrid cell lines. Proc Natl Acad Sci USA 77:563–566, 1980.
145. Rogers GT, Rawlins GA, Bagshawe KD: Somatic-cell hybrids producing antibodies against CEA. Br J Cancer 43:1–4, 1981.
146. Kupchik HZ, Zurawski VR, Hurrel JGR, Zamcheck N, Black PH: Monoclonal antibodies to carcino-embryonic antigen produced by somatic cell fusion. Cancer Res 41:3306–3310, 1981.

147. Ashall F, Bramwell ME, Harris H: A new marker for human cancer cells: 1. The Ca antigen and the Ca 1 antibody. Lancet ii:1-6, 1982.
148. McGee J O'D, Woods JC, Ashall F, Bramwell ME, Harris H: A new marker for human cancer cells: 2. Immunohistochemical detection of the Ca antigen in human tissues with the Ca 1 antibody. Lancet ii:7-10, 1982.
149. Gross L: Intradermal immunization of C3H mice against a sarcoma originating in an animal of the same line. Cancer Res 3:326-333, 1943.
150. Foley EJ: Antigenic properties of methylcholanthrene-induced tumors in mice of the strain of origin. Cancer Res 13:835-837, 1953.
151. Prehn RT, Main JM: Immunity to methylcholanthrene-induced sarcomas. J Nat Cancer Inst 18:769-778, 1957.
152. Hewitt HB, Blake ER, Walder AS: A critique of the evidence for active host defense against cancer, based on personal studies of 27 murine tumors of spontaneous origin. Br J Cancer 33:241-259, 1976.
153. Weiss DM: The questionable immunogenicity of certain neoplasms. Cancer Immunol Immunother 2:11-19, 1977.
154. Klein G, Klein E: Rejectability of virus-induced tumors and non-rejectability of spontaneous tumors: A lesson in contrasts. Transpln Proc IX:1095-1104, 1977.
155. Boyse EA, Old LJ: Some aspects of normal and abnormal cell surface genetics. Ann Rev Genet 3:269-290, 1969.
156. Omary MG, Trowbridge IS, Battitura HA: Human homologue of murine T200 glycoprotein. J Exp Med 152:842-852, 1980.
157. Morishima Y, Ogata S, Collins NH, Dupont B, Lloyd KO: Carbohydrate differences in human high molecular weight antigens of B- and T-cell lines. Immunogen 15:529-535, 1982.
158. Bishop JM: Enemies within: The genesis of retrovirus oncogenes. Cell 23:5-6, 1981.
159. Neel BG, Hayward WS, Robinson HL, Fang J, Astrin SM: Avian leukosis virus-induced tumors have proviral integration sites and synthesize discrete new RNAs: Oncogenesis by promoter insertion. Cell 23:323-334, 1981.
160. Blair DG, Oskarrsson M, Wood TG, McClements WL, Fischinger PJ, van de Woude GG: Activation of the transforming potential of a normal cell sequence: A molecular model for oncogenesis. Science 212:941-944, 1981.
161. Lane MA, Sainten A, Cooper GM: Stage-specific transforming genes of human and mouse B- and T-lymphocyte neoplasms. Cell 28:873-880, 1982.
162. Milstein C, Lennox E: The use of monoclonal antibody techniques in the study of developing cell surface. Curr Top Devel Biol 14:1-32, 1980.
163. Springer TA: Cell-surface differentiation in the mouse. In: Monoclonal Antibodies, Kennett RH, McKearn TJ, Bechtol KB (eds). New York: Plenum Press, 1980, pp 185-217.
164. Solter D, Knowles BB: Monoclonal antibody defining a stage-specific mouse embryonic antigen (SSEA-1). Proc Natl Acad Sci USA 75:5565-5569, 1978.
165. Kapadia A, Feizi T, Evans MJ: Changes in the expression and polarization of blood group I and i antigens in post-implantation embryos and teratocarcinomas of mouse associated with cell differentiation. Exp Cell Res 131:185-195, 1981.
166. Hakkinen I: A-like blood group antigen in gastric cancer cells of patients of blood groups O or B. J Nat Cancer Inst 44:183-193, 1970.
167. Yokota M, Warner GA, Hakomori S: Blood group A-like glycolipid and a novel Forssman antigen in the hepatoma of a blood group O individual. Cancer Res 41:4185-4190, 1981.
168. Levine P: Illegitimate blood group antigens P_1, A, and MN (T) in malignancy-A possible therapeutic approach with anti-Tj^a, anti-A, and anti-T. Ann NY Acad Sci 277:428-435, 1976.

169. Hakomori S, Wang SM, Young WW: Isoantigenic expression of Forssman glycolipids in human gastric and colonic mucosa: Its possible identity with 'A-like antigen' in human cancer. Proc Natl Acad Sci USA 74:3023-3027, 1977.
170. Yu RK, Ando S: Structures of some new gangliosides of fish brain. Adv Exp Med Biol 125:33-45, 1980.

6. Immunocompetence in Cancer Patients

LINDA M. KARAVODIN and SIDNEY H. GOLUB

INTRODUCTION

One of the most popular areas of clinical investigation has been the determination of immune status of cancer patients. Almost every issue of cancer oriented journals and of clinical immunology oriented journals contains at least one article in which some immunologic parameter is defined for a population of cancer patients. Of course, such studies are not confined to malignant disease as an extraordinary volume of literature has accumulated on the measurement of the immune parameters in a whole spectrum of pathological conditions. It is not the intent of this review to catalogue all such studies. In contrast, it is our intention to critically review the usefulness and methodology for the assessment of immunocompetence in cancer patients. Therefore, we have tended to concentrate in this review on studies with reasonable numbers of patients, where appropriate statistical analysis was included, and where reproducible methodology was employed. Even so, we have undoubtedly failed to include a large number of completely respectable scientific efforts.

In undertaking such a critical review, we have asked why this area of investigation has been so popular. There are three probable explanations for the extraordinary output of information on the immune status of cancer patients. The first is the indelible influence that the concept of 'immune surveillance' has had on the field of tumor immunology. Although the concept that the immune system protects the host against the initiation of neoplasia has been in existence since the time of Ehrlich, the notion was formalized and popularized by Burnet [1]. 'Immune surveillance' gave a theoretical basis for the belief that there was an intimate relationship between immunologic function and the neoplastic process. The findings of Gatti and Good [2] that patients with some immunologic deficiencies appear to have an increased susceptibility to malignant disease gave further impetus to the

R.B. Herberman (ed), Basic and Clinical Tumor Immunology. ISBN-13: 978-1-4613-3875-8
© 1983. Martinus Nijhoff Publishers, Boston.

belief that the study of immunologic function was relevant to the study of human malignancy. It is not the intent of this review to deal with the question of surveillance and whether failure of the immune system results in cancer. In fact, it is the intention of this review to deal with the opposite question, i.e. what is the effect of cancer on the immune system? For those interested in the surveillance problem, the excellent recent review by Stutman [3] and the issue of Transplantation Reviews [4] devoted to this question are excellent sources of information. However, it should be kept in mind that the surveillance issue was one of the driving forces promoting this area of research.

The second reason for the remarkable level of activity in this area of research is the possibility that assays of immune function will provide information of prognostic significance. Perhaps the most influential paper in this regard was the important work of Eilber and Morton [5] indicating that patients with an inability to make a delayed type hypersensitivity response to a new skin test antigen had a poor prognosis. This work, and similar studies, suggested that relevant and important information could be obtained about the prognosis of the patient by a simple immunologic procedure. The problem with this approach is that it appears most likely that compromises in immune function are a symptom of cancer rather than a reason for its progression. Thus, even the best immunologic assays are likely to give only an approximation of the tumor burden in the patient. With refinements in diagnostic procedures, it seems likely that much more precise measurements of residual tumor burden can be developed with conventional diagnostic procedures than can be accomplished by nonspecific measures of immunocompetence. Nevertheless, this remains one of the compelling reasons for pursuing research in this area. It certainly is possible that immunologic assays may reveal the nature of the biology of the host-tumor relationship in individual patients and in those patients for whom their immune response is readily and quickly suppressed by the presence of a tumor the prognosis may be affected. Identification of such patients would, at a minimum, save them from unnecessary and futile procedures. However, this still points to a very limited role for immunocompetence testing in cancer patients.

The third reason which has prompted so much research in this area is the simple fact that such studies are remarkably easy to perform. All one needs to publish a paper is a set of patients and an immunologic assay. This has led to considerable sarcasm [6], much of which is justified. It probably also explains why so many of the studies have used the technically easiest assays (delayed type hypersensitivity skin tests or mitogen response) rather than

more complex or technically demanding systems. Unfortunately, most of these studies have really missed the essential question. What needs to be examined is the biology of the interaction between the host immune system and the tumor. This has been addressed to some degree in studies of immune responses to tumor associated antigens, but has been largely ignored in studies of the general functioning of the immune system in cancer patients.

This review will focus on an evaluation of the current state of the art of the measurement of immune competence of cancer patients. We have restricted the scope of this review to studies in humans, although it must certainly be admitted that animal models can and should be effectively used to study the biology of the effects of the neoplastic disease upon the immune system. We have simply elected not to include such studies in our review because of space considerations. The review is organized by the various methodologies that have been used to evaluate immunocompetence. This inevitably leads to some compartmentalization of the immune response without sufficient attention to the interactions of these various components. However, this format does facilitate comparisons of similar studies and an analysis of which areas are most likely to be productive in the future. In selecting areas to cover, we have had to make some quite arbitrary decisions. For example, we have not extensively reviewed the critically important area of non-specific immunosuppressive factors. This is not because we deemed such factors irrelevant, but because there is such an extensive literature on immunoregulatory products of the host or immunosuppressive products of the tumor that we feel that this area could not be properly treated in a review of this dimension. Furthermore, the authors' interests are focused primarily on the functional activities of T cells, B cells, and other lymphoid cells. Hopefully, an analysis of these components will provide sufficient perspective to allow us to make some general conclusions about the usefulness of measuring immunocompetence in cancer patients.

1. DELAYED TYPE HYPERSENSITIVITY (DTH)

The delayed hypersensitivity reaction is an immunologically specific cell-mediated response which develops after a test antigen is injected intradermally or applied topically to the skin. If the patient has been previously sensitized to this antigen, an inflammatory reaction, characterized by erythema and induration, will occur at the site 24 to 48 hours later. There are two general categories of skin test antigens: neo-antigens and recall antigens.

Testing with a new antigen requires that the patient be sensitized and challenged with a substance to which he has not been previously exposed, such as 2.4 dinitrochlorobenzene (DNCB). This type of test allows for the clinical evaluation of all aspects of DTH response from initial antigen recognition to cellular infiltration. The use of recall antigens such as tuberculin, candida, and mumps, assumes that the patient has already had contact with these common antigens and became sensitized. The patient's response to recall antigens measures only the efferent response and not the ability to process and become sensitized to the antigen.

Testing patients with new antigens has been the method of choice for most *in vivo* DTH studies. These primary antigens are synthetic contact-sensitizing agents such as DNCB, DNFB, although some work had been done with biological agents such as keyhole limpet haemocyanin and α-haemocyanin of Helix pomatia. These antigens will induce an immune response in almost all normal individuals. Brown et al. [7] found that 95% of the normal population tested were able to develop a positive skin test to DNCB.

Studies on DTH responses of cancer patients to new antigens have been carried out since the mid 1960's. The vast literature which has accumulated on this subject has yielded several basic conclusions. The first of these findings has been that as the severity of the disease increased, the incidence of positive DTH reactions has decreased. In one detailed study [8], 234 patients with various types of cancer and 17 patients with non-malignant neoplasms were tested for DNCB reactivity. Patients without metastases gave a positive reaction more often (83%) than those with regional metastases (67%) or with distant metastases (41%). In a study of 173 bladder cancer patients [9] response to DNCB was found in 48% of the patients with superficial tumors while only 17% of the patients with metastatic disease responded. Patients with transitional cell carcinoma of the bladder were found to have a stepwise decrease in response to DNCB with advancing stage of disease, with 75% positive among those with superficial tumors and 35% positive among those with metastatic disease [10]. Delayed type hypersensitivity was found to correlate well with extent of malignancy in a study of 116 malignant melanoma patients and 40 skeletal and soft tissue sarcomas [11]. Eighty per cent of Stage I patients had a positive response while 37% of Stage III patients responded. A decreased ability to be sensitized to DNCB was shown in a study [12] of patients with carcinoma of the lung, all in good condition and not receiving chemotherapy or radiation. In addition, there was a strong correlation between nonresectable disease and unresponsiveness to DNCB. Patients with Hodgkins disease have a marked inability to be sensitized to DNCB [13], yet even among these patients the degree of

impairment may correlate with the stage of disease. Hodgkins disease patients with early Stage I disease have been found to have an intact response [7], but in another study, at a lower sensitizing dose, a decreased reactivity was shown in patients with early disease [14].

A second observation has been that the incidence of positive reactions varies according to the type of cancer. Patients with certain solid tumors have a marked impairment in their ability to be sensitized to DNCB [5, 8, 12]. Pinsky et al. [9] found that patients with localized head and neck cancer had a much lower reactivity (42%) than patients with sarcoma (73%) and lung cancer (80%). Eilber and Morton [5] studied 100 patients with a variety of carcinomas and sarcomas and found a correlation of DNCB reactivity to clinical status which was more pronounced in patients with squamous cell carcinoma of the head and neck than in those with sarcoma or melanoma. Bolton [15] found that reactivity was impaired at all stages of colon and gastric cancer, while breast cancer patients suffered no major impairment unless the disease was disseminated. Therefore, although DNCB reactivity correlates with the extent of disease in general, the likelihood of unresponsiveness at a given stage seems to depend on the type of cancer. Among the hematologic malignancies, patients with Hodgkins disease are hyporeactive to DTH testing, and advancing disease is accompanied by more frequent anergy [16]. In a study of patients with Burkitts lymphoma [17] no impairment to DNCB reactivity was found, although a later study noted a decreased reactivity when large tumors were present [18]. It can be concluded that defects in the DTH response to DNCB are frequent in patients with all types of cancer, but somewhat more frequent in patients with Hodgkins disease, non-Hodgkins lymphoma, and squamous cell carcinoma of the head and neck area.

In addition to correlations to tumor burden and histology, an association has been seen between patient reactivity to DNCB and the recurrence of disease. Seventy-three patients tested with DNCB were followed for at least 6 months after definitive surgery [8]. Of those patients with a positive DNCB, only 3 of 57 developed recurrence; however, 7 of 16 patients with negative DNCB test developed recurrent disease. In another study, 83 patients with a variety of malignant tumors were tested prior to surgery [5]. Cancer patients were grouped into those who were disease free at 6 months and those who were inoperable or had recurrence at that time. In the disease free group 92% were DNCB positive, compared with only 7% in the recurrent or inoperable group. Other workers have confirmed the impairment of the DNCB response in advanced cancer patients [19], but their results were not as clearcut as those of Eilber and Morton [5] since 47% of patients with known distant metastases were DNCB positive. This association with prog-

nosis has been observed in patients at all stages of disease. Brosnan et al. [9] found that the survival of patients with metastases was significantly better if they were initially DNCB skin test positive. Pinsky et al. [20] found that DNCB testing at the time of surgery appeared to be useful as a means of identifying a group of patients without metastases whose prognosis was poorer than expected. It appears that a negative response to DNCB is usually associated with a poor prognosis in cancer, but the reverse is not necessarily true [15].

In contrast to DNCB, patients' reactions to skin tests with the common or recall antigens have been less closely correlated with the subsequent course of the malignancy. Investigators have combined the use of several tests in order to ensure that a patient will have been previously exposed to at least one of them. Healthy individuals will usually react to at least one of: Dermatophytin 'O', mumps skin test antigen, tuberculin (PPD), and streptokinase/streptodornase. Among 80 patients with a variety of malignancies, an increased anergy to recall antigens was seen in those with lymphorecticular malignancies, but not in patients with solid tumors [21]. In a study of bladder cancer patients, no recall antigens were found to correlate with prognosis, and only streptokinase/streptodornase was related to tumor stage [9]. In an age-adjusted study of 179 malignant melanoma and acute leukemia patients tested with a variety of recall antigens [22], Stage IV patients showed decreased reactivity only with PPD when compared to normals. However, other investigators [23] found that patients with Stage I melanoma and a positive DTH to recall antigens had a lower recurrence rate and a longer disease free interval than those with a negative response.

It should be remembered that interpretation of skin test results can vary widely between studies, since dilution of antigen, reader variability, patient's prior exposure to antigen, booster effects of repeated antigen administration, time course of the reaction, and definition of a positive reaction are all variations which can affect the results [24]. The observation that skin test reactivity can be normal in early cancer and decreases as the disease advances suggests that tumor dissemination precedes the impairment of general immunocompetence, and that immunosuppression is the result rather than the cause of formation of metastases. Of the available agents, only DNCB has been a consistently useful skin test agent. However, even DNCB is an awkward reagent to employ as repeated tests will clearly yield anamnestic responses. Thus, only the original test on an individual can provide clear information on the responsiveness to a neoantigen. Even with this limitation, DNCB is superior to common microbial antigens, which have so little usefulness as measures of general immune competence that most laboratories have abandoned them.

2. ANTIBODY FORMATION

Quantitation of the level of immunoglobulins in the sera is one of the most easily assessed immune parameters, and for this reason was included in many of the earliest studies of cancer patient immunity. The single radial diffusion method was often used. Changes in the level of immunoglobulins were assumed to indicate either a disproportionate number of B lymphocytes, dysfunction of regulatory mechanisms, or a defect in Ig biosynthetic pathways. Studies of non-specific (or non-tumor specific) humoral immunity have been done in two ways: (1) by measurement of total Ig and Ig classes, and (2) by measurement of primary or secondary antibody response to various antigens.

The first method has produced many reports of variations in the serum Ig levels of cancer patients. Patients with a variety of hematologic malignancies were found to have decreased mean total Ig compared with normal controls in one study [25]. Other studies showed low levels of total Ig to be characteristic of chronic lymphocytic leukemia [26] while patients with acute lymphocytic leukemia or chronic myelocytic leukemia demonstrated only decreased IgA levels. Increased IgG levels were reported in patients with bronchogenic cancer [27]. A large study of 984 patients with cancer of nonhematopoetic tissues found no general trends [28] but did find increases in IgG and IgA in males with skin and lung tumors, increases in IgM in males with sarcoma and females with melanoma, decreases in IgM in patients with primary ovarian cancer, and increased IgA in patients with cancer of the mouth, gut, or uterus. Increased IgA has also been demonstrated in patients with oropharyngeal cancer [29], prostate cancer [30], and cancer of the nasopharynx [31]. Increased levels of IgA have not been found to correlate with disease stage. In a study of breast cancer and malignant melanoma, Cochran [32] found raised IgG and IgA levels but no difference between patients with primary disease and those with metastases. Plesnicar [33] found IgA elevated in all three stages of cancer of the uterine cervix, with an elevation of IgG in Stage I and a decrease in IgM in Stage III.

The second method of measurement of primary or secondary antigen response to a known antigen has also given conflicting results. Decreased antibody response has been observed in some patients with chronic lymphatic leukemia [34], lymphosarcoma [35], and multiple myeloma [36]. Libansky [37] tested 113 patients with a variety of malignant blood disorders with Brucella endotoxin and found a decreased antibody response only in chronic lymphadenosis. Patients with non-lymphomatous malignancies tested with Salmonella extract were found to have a decreased antibody response which correlated with their survival [38]. Jansen [39] immunized patients with squamous-cell bronchial carcinoma with *Helix pomatia* haemocyanin, and

found that patients with disseminated disease showed a significant deficit in IgG and IgA. However, no decreased response has been reported for Hodgkins disease [40] and for other lymphomas and non-lymphoid tumors [41].

Many of these discrepancies may be due in part to the choice of different antigens for different studies, some antigens being more immunogenic in these patients than others. Fluctuations in the patients' serum Ig levels could be due to infections or malnutrition to which they are predisposed in advanced disease. A decreased antibody response may be more related to these underlying complications than to the cancer itself.

A more fundamental reason for the variations among different studies may simply be that the B cell compartment is not the appropriate place to look for immunologic clues to a patient's disease outcome. While antibody responses to tumor-associated antigens may be quite important in the host-tumor relationship, it is unlikely that tumor directed antibodies will constitute more than a small fraction of the total serum Ig pool. Thus, perterbations of total Ig levels are unlikely to be caused by anti-tumor humoral responses and ongoing antibody responses to non-tumor (environmental) antigens appear to be only marginally affected by neoplastic disease. Therefore, the simplest approach of quantitation of immunoglobulin production in cancer patients has essentially no clinical value.

In contrast, quantitation of circulating immune complexes may be of considerable use. Although this area does not fall into the general category of 'immunocompetence' testing, it should be noted that several laboratories [25–27] have shown correlations between the levels of circulating immune complexes and the degree of tumor burden or prognosis of cancer patients. This illustrates that gross measurements, such as Ig levels, can be non-informative while other non-tumor specific parameters can be useful if they reflect the host-tumor relationship.

3. LYMPHOCYTE PROLIFERATIVE FUNCTIONS

Lymphocyte transformation is an *in vitro* technique commonly used to measure cellular immunocompetence. This term was first used to describe morphologic changes that occurred when small, resting lymphocytes were transformed into large lymphoblastic cells by exposure to a mitogen, such as phytohemagglutinin (PHA). Blastogenesis also refers to the formation of blast cells in lymphocyte cultures stimulated by either nonspecific mitogens or specific antigens. This activation *in vitro* parallels the *in vivo* response of sensitized lymphocytes to an antigen in the host. Lymphocyte transformation to mitogens measures the functional capacity of lymphocytes to proli-

ferate in response to antigen stimulation. It thus provides a measurement which is restricted to the efferent aspect of the immune response, unlike the *in vivo* skin test response, which relies on a more complex series of cellular interactions. A variety of plant lectins have been used to assess lymphocyte function. The most popular have been PHA and concanavalin A (Con A), both primarily T cell mitogen, and pokeweed mitogen (PWM), which activates both B and T cells.

An extensive literature has accumulated on the use of the lymphocyte transformation assay as a measure of cellular immunocompetence. Because this assay is relatively simple, it has become one of the most popular methods for immunological survey studies of cancer patients. Unfortunately, it is this very simplicity that has led to the failure of many investigators to control for a number of variables inherent in the assay. These variables will be discussed later in this section and may account for the substantial inconsistencies in the published data.

Many investigators have reported a correlation of PHA response to stage of disease. Among those patients with tumors arising in lymphoreticular tissues, there have been reports of a diminished transformation response in patients with chronic lymphatic leukemia and lymphosarcoma [42], acute lymphatic leukemia [43], plasma cell myeloma [14], and other lymphoproliferative disorders [45]. In a study of 35 patients with previously untreated Hodgkins disease, lymphocyte response to PHA was deficient at all stages [46] indicating the early presence of a fundamental lymphocyte defect.

Patients with a large variety of nonlymphoid malignant diseases also have been reported to have a depressed lymphocyte response to mitogens. PHA induced blastogenesis, as measured by the proportion of cells with blastoid morphology, was associated with disease stage in 179 patients with carcinoma of the stomach [47]. Eighty-six percent of patients at Stage I and II had greater than 40% blasts, while 49% of those in Stage III and only 24% of those in Stage IV showed this response. A study of 154 patients with carcinoma of the lung showed that the lymphocyte response to PHA, PWM, and Con A was significantly decreased in Stage III disease but not in Stages I and II, as compared with normal controls [48]. Among 76 patients with colon adenocarcinoma and age matched controls, an age related decline in blastogenic response was observed in both patient and control groups, but the rate of decline of this response was greater in magnitude in the patient group, suggesting that their tumor burden contributed to this loss of transformation capability [49]. A study of breast cancer patients by stage [50] showed a decreased response to PHA among those with Stage III disease.

Other investigators have reported a correlation between response to mitogens and recurrence of disease. Bjokholm et al. [51] studied that blastog-

enic response of 81 patients with previously untreated Hodgkins disease to Con A, PHA, PWM, and purified protein derivative of tuberculin. By deriving a total score from all of these assays they were able to closely correlate disease outcome with test results and found that these *in vitro* assays were better predictors of outcome than age, clinical stage, histopathology and symptoms. In a study of 94 patients with clinically operable nonlymphoid malignant tumors [52], PHA reactivity was quantitated prior to surgery and patients were followed for three years to determine clinical outcome. All patient groups had lower lymphocyte reactivity than the age matched controls, but in the clinically 'cured' group this reduction was significantly less than those with inoperable or recurrent conditions. Han and Takita [53] found that 45% of patients with operable carcinoma of the lung and 15% of inoperable patients had lymphocyte responses to PHA that were in the normal range. Among 44 patients surgically resected for Stage B and C carcinoma of the colon, Payne et al. [54] found a diminished PHA transformation in 90% of those patients who had recurrence of tumor. In a study of patients with all stages of squamous cell carcinoma of the head and neck [55], PHA and Con A blastogenesis was found to be significantly depressed in patients in whom clinically apparent recurrences developed, regardless of extent of disease or regional lymph node involvement. Lymphocytes of 154 operable breast cancer patients were tested for *in vitro* stimulation by PPD and PHA and *in vivo* reactivity to PPD and DNCB, then followed for 3 to 6 years to determine the relationship of these assay results to recurrence of disease [56]. PPD and PHA *in vitro* proliferative responses were of higher predictive value for disease recurrence than the *in vivo* tests. An integrated score of immunocompetence based on all tests showed that disease recurrence was significantly more likely for suboptimal responders (61%) than for optimal responders (28%).

Although these results have been quite impressive, there are many other investigators who have reported no correlation between blastogenic response and stage or outcome of disease. In a study of 44 breast cancer patients, Roberts [57] found no difference in PHA reactivity between patient and control populations. Among 120 patients with a variety of nonlymphoid tumors, Sample et al. [58] observed an age related decline which did not differ from that found in the controls. Butterworth et al. [59] studied 71 patients with melanoma and found no significant differences in their lymphocyte transformation ability from that of the normal group. Similarly, Golub et al. [60], in a study of 94 melanoma patients, could find only slight and statistically insignificant suppression of PHA, PWM, Con A, and PPD responses when compared to 96 healthy controls. Although mixed lymphocyte culture (MLC) responses were significantly lower among the melanoma patients than among the controls, neither MLC nor the mitogen responses

were able to distinguish between melanoma patients who recurred within 12 months and those who remained disease free.

Golub et al. [61] found a decreased response to the mitogen Con A in a group of 52 cancer patients, but their responses to PHA and pokeweed mitogen were in the normal range. Twomey and co-workers [62] reported impairment of response to PHA and DNCB among a high percentage of 100 patients 'cured' of squamous cell carcinoma of the lung. These results were similar to those of 200 pre-operative squamous carcinoma patients, indicating that PHA transformation assays in patients with bronchial carcinoma do not correlate with treatment results.

The MLC is another type of lymphocyte activation assay in which lymphocytes respond to foreign histocompatibility antigens on unrelated lymphocytes. The proliferative response in this case is activated by the antigens of the human major histocompatibility complex (the HLA antigens). MLC closely parallels the *in vivo* DNCB test in that the MLC measures both the afferent and efferent lines of the immune response *in vitro*, unlike testing with mitogens, which measures only the efferent response. In fact, in a study of 52 melanoma, sarcoma, and adenocarcinoma patients, Golub et al. [61] found MLC results strongly correlated with response to DNCB in that all patients who were DNCB nonreactors also showed a markedly reduced response in MLC. However, this correlation did not extend to melanoma patients [60].

A number of studies have reported a correlation between lymphocyte response in MLC and stage or prognosis of disease. Among lung cancer patients, Holmes and Golub [63] found a significant decrease in the MLC reactivity of patients with Stage II and Stage III disease. Butterworth et al. [59] studied 71 patients with melanoma and found a diminished MLC reactivity in 44%. Of these, MLC reactivity was absent in only 10% of Stage I patients, but was absent in 53% of Stage II patients, and 45% of Stage III melanoma patients. In a study of 94 melanoma patients [60] MLC responsiveness was shown to be substantially depressed, and stage of disease and age had additional effects on MLC. Thirty patients with head and neck cancer were studied by Berlinger and Good [64] who found that of the 47% who had deficient MLC response, all had advanced tumors, recurrent tumors, poor response to therapy, or multiple primary tumors of different histological types. Of the 53% with normal MLC response, all patients had new, small, or established non-aggressive tumors. In a study of 60 patients with a variety of non-lymphoid malignant tumors, Suciu-Foca et al. [65] tested lymphocytes against a battery of allogeneic cells and found that MLC reactivity was lower than controls in all disease stages. Thirty-five Stage I lung carcinoma patients were tested postoperatively against allogeneic cells in MLC [66] and a depressed response was found to be associated with a

significantly shorter disease free interval. The MLC response also appeared to be a better predictor of disease recurrence than PHA blastogenesis in this study. Another correlation with disease outcome was seen by Han and Takita [67], again with lung cancer patients, in which only 43% of the patients had a normal MLC response. Although histologic type did not correlate with these results, MLC and PHA response both correlated fairly well with subsequent survival.

Several investigators have examined the correlation of various mitogenic stimuli to each other and to other common tests of immunocompetence in cancer patients. For example, Catalona et al. [68] found that PHA response paralleled results of skin testing with DNCB since both tests were normal in patients with early bladder cancer, while both were significantly impaired in patients with advanced disease. PHA response was found not to correlate with quantitation of T cells in patients with thyroid cancer. These patients showed depressed PHA-induced blastogenesis despite adequate T cell numbers [69]. In a study of 48 patients with anaplastic bronchogenic carcinoma [70], the data suggest that response to Con A, PHA, and MLC are more often suppressed than are skin test responses. Graze et al. [71] found with 27 Hodgkins disease patients, that those with negative delayed type hypersensitivity reactions showed a diminished PHA and MLC blastogenic response while those with intact DTH showed a decreased PHA response but a normal MLC response. Thus, the proliferation assays do not clearly correlate to each other or to other readily assessed parameters. Presumably, each assay involves sufficiently different cellular mechanisms that none are clearly redundant.

The great variability seen within the results from different sources is probably due to two factors: differences in patient populations and precise standardization for this deceptively simple technique. More recent studies have attempted to pinpoint and control for these experimental variables. Teasdale et al. [72] in an analysis of the PHA assay pointed out the importance of setting up a dose response curve, establishing quantitation criteria for maximum response of each patient, and determining the optimal time course for the assay, which appears to vary among individuals. Dean [73] examined variation in all lymphoproliferative assays and noted problems related to the assay itself - inherent variation, nonstandardization of methods and measurement parameters, and those related to study design - lack of normal controls, differences in patients disease groupings, and influences of chemotherapy and immunotherapy on lymphoproliferative responses.

In addition to study design and test population differences, there has been no consensus among investigators on how to express proliferation data. This is an important point, as the means of calculating this data will greatly affect its interpretation. The simplest method to express proliferation data is as

counts per minute (or disintegrations per minute) of radiolabelled thymidine incorporated into DNA for a standard number of cells. Although this approach has the attractiveness of simplicity, many investigators have felt uncomfortable with it since it does not include the 'background' or unstimulated response in the formulation. This has led many investigators to use net counts/minute (stimulated cpm – unstimulated cpm) or more frequently to use a 'stimulation index' (stimulated cpm – unstimulated cpm). There are several major problems with the use of a 'stimulation index'. Foremost is a statistical problem, related to the fact that the unstimulated cpm will be a small number and therefore will be statistically less reliable than the larger stimulated cpm. When one divides by the unstimulated cpm (or subtracts the unstimulated cpm), one is taking a statistically highly reliable number and transforming it by a statistically less reliable number. Therefore, one is taking good data and transforming it into less reliable data. The second problem is a biological one as a stimulation index makes the assumption that there is a direct relationship between the unstimulated activity in thymidine incorporation and the mitogen stimulated activity. For example, the stimulation index assumes that an individual who has an unstimulated response of 100 cpm and a PHA stimulated response of 10,000 cpm is equivalent to another individual who has an unstimulated response of 1,000 cpm and a PHA stimulated result of 100,000 cpm. Both individuals would have a stimulation index of 100, yet their PHA stimulated responses are obviously quite different. The data to support the assumption that the stimulated response is a function of the background cpm is lacking and we have felt that it is safest to deal with the stimulated responses without modification by the unstimulated results. Instead, we simply calculate the unstimulated response as a separate parameter of responsiveness.

The final way that data has been analyzed is by some mathematical transformation of the cpm results. We have examined a number of such transformations [74] and found that $\log_{10}$ provided the most reliable transformation. Repeated samplings from the same individual showed the greatest reproducibility when the data was expressed as $\log_{10}$ units. This is a particularly appealing transformation as proliferation is a logarithmic function and probably should be expressed in logarithmic units.

Considering the extraordinary output of information relating to lymphocyte proliferative activity in cancer patients, one would hope that this area has some real significance. Indeed, the results have pointed out some interesting clues to the biology of neoplastic disease. There is no question that the lymphocyte transformation studies have shown that certain disease types, such as head and neck cancers and lymphoproliferative malignancies, are frequently associated with defective lymphocyte proliferation functions while other malignancies such as malignant melanoma and breast carcino-

ma are seldom associated with profound defects in proliferative capacity. The use of different stimuli to analyze the nature of such defects can provide important clues as to the biology of these diseases. However, it has seldom been shown that lymphocyte proliferation activity has the type of clinical significance that can be used to assess an individual patient's prognosis. The hope that one could send a blood sample to a clinical immunology laboratory and receive a PHA response result that would tell the physician the prognosis of the patient is clearly unrealistic. The assay shows too great a variability in the normal population and most importantly has shown too little relationship to clinical features to be statistically reliable for individual patient assessment. It seems quite unlikely that mitogen responses need to be added to the normal battery of blood tests as non-specific diagnostic aids. Instead, they should remain as basic research tools to examine the influence of neoplastic disease on immune function.

4. T CELL QUANTITATION

The quantitation of lymphocytes in the peripheral blood of cancer patients has been studied as a measure of immunocompetence since the early 1970's, when lymphocytopenia was found to be common in patients with malignancies [75] and was correlated to a poor prognosis in patients with nonlymphoid tumors [76]. Other studies found an inverse correlation between total lymphocyte count and stage of disease in breast [77], lung [48], and head and neck cancer [78].

The observation that human T lymphocytes could be identified by a binding reaction to sheep red blood cells (SRBC), coupled with the emerging recognition of the importance of the T cell in tumor rejection in animal models, quickly led to numerous studies of E rosette forming cells (E-RFC) in patients with malignant disease. Lymphocytes which bind at least 3 SRBC are by convention considered to be T lymphocytes. The most common technique to measure rosette forming cells (RFC) involves formation of E-rosettes by mixing SRBC and PBL at a ratio of 100:1, briefly incubating at 37 °C, pelleting the cells, and further incubation at 4 °C for 1 to 24 hours. Cells are counted as percent of lymphocytes forming E-rosettes or by multiplication with the total lymphocyte count as absolute numbers of E-rosettes. A modification of the E-rosette test, the 'active' E-rosette test, has been developed and measures rosettes formed after five minutes of incubation between low ratios of SRBC and lymphocytes. Other investigators have distinguished between two rosette forming populations on the basis of relative rosette forming affinity. 'High affinity' rosettes were identified by their ability to form rosettes at elevated temperatures (29 °C) and at low

SRBC/lymphocyte ratios. 'Low affinity' E-RFC required optimal conditions (4 °C and SRBC excess) to form rosettes. The level of low affinity rosettes in normal individuals has been found to correlate with the proportion of Fc receptor-bearing cells and reactivity in ADCC assay [79].

Several studies have found an association between levels of E-rosettes and other tests of immune competence, but the results have been far from unanimous. In 102 patients with untreated solid carcinomas, Kerman et al. [80] found a relationship between positive microbial skin test results and numbers of active E-rosette forming cells. In a series of 52 patients with urologic malignancies, Catalona et al. [81] found a significant positive correlation between DNCB skin testing and E-rosettes, and PHA response and E-rosettes. Bobrove et al. [82] also showed a relationship between a decreased percent of E-rosetting cells in the peripheral blood and a diminished proliferative response to PHA among a group of Hodgkin's disease patients. However, when T cells were enumerated by complement dependent anti-T cell cytotoxicity, they concluded that the patients had normal levels of T lymphocytes and that the defect was in the ability of these cells to form rosettes or respond to PHA [82]. In another study of lung and breast cancer patients, this relationship was studied by regression analysis [83] and it was concluded that although some cancer patients may have depressed lymphoproliferative responses in association with low levels of T lymphocytes, others have depressed functional activity with completely normal E-rosette levels.

Percent E rosette forming cells have been determined for patients with a variety of malignancies, and several investigators have found an association with the clinical stage of the disease. In 31 patients with bronchial carcinoma, Anthony et al. [84] observed a reduction in the proportion of E-rosetting cells in the majority of patients with oat cell tumors and frequently among patients with squamous cell tumors. Silverman et al. [85] reported below normal % E-RFC in patients with disseminated melanoma, but not in those with melanoma confined to the primary site. Whitehead et al. [86] studied 100 women with breast cancer and found a decreased percentage of E-rosettes at all stages except locally advanced disease. Among patients with cervical carcinoma, Rand et al. [87] found a significant depression in % T cells in association with invasive, but not pre-invasive, conditions.

Other studies have examined absolute numbers of T cells as well as percentages in relation to disease stage. Among 62 patients with Hodgkins disease, 86% had a significantly lower % E-rosettes and 65% had a lower absolute T cell count [88]. T cell levels of 50 patients with colorectal adenocarcinoma were quantitated prior to surgery and 60% of these showed a significant decrease in both % and absolute numbers of E-rosetting cells [89]. Catalona et al. [68] found an inverse correlation between absolute

count of T cells and tumor stage in patients with bladder carcinoma. Among 154 patients with primary carcinoma of the lung, Wanebo et al. [48] reported that absolute T cell numbers were significantly decreased in 44% of patients with Stage III disease. The report of 112 patients with bronchial carcinoma by Dellon et al. [90] describes a positive correlation between E-rosette forming cell numbers, but not percentages, with disease stage in those patients with squamous cell carcinoma. Among patients with adenocarcinoma, there was no significant difference among those with localized disease and those with regional and metastatic disease.

Modifications of the E-rosette technique also showed an association with disease stage in some investigations. Using the high affinity rosette technique, West et al. [91] studied 50 patients with solid tumors and found that 82% had decreased high-affinity E-RFC numbers compared to a normal population. Weese et al. [92] also used the high affinity technique to study 105 breast cancer patients and found that in groups of Stage II patients and patients with metastatic disease 37% and 60%, respectively, had abnormally low rosette levels, compared to a level of 10% in the control group. A decrease in % 'active' rosette forming cells was reported by Wybran and Fudenberg [93] in 68% of patients with newly diagnosed solid tumors, and in 72% of untreated patients undergoing relapse. In both categories, patients with metastases had fewer RFC than patients with localized disease. In contrast, Cochran et al. [32] found an increase in 'active' T cells among patients with malignant melanoma.

Other reports have demonstrated a correlation between E-RFC and clinical outcome. Dellon et al. [90] found that absolute T cell numbers declined progressively among patients with advanced stages of squamous cell carcinoma, oat cell carcinoma, and undifferentiated carcinoma, but not among patients with adenocarcinoma. A fall in T cell numbers preceded clinically evident metastases, while postoperative patients with rising T cell levels remained clinically free of disease [90]. This has been confirmed in a study by Shirakusa et al. [94] who also studied patients with bronchogenic cancer and found that the absolute number of T cells, and not the T cell percentages, correlated well with the postoperative course. Using the high affinity rosette technique, Oldham et al. [95] serially tested 29 lung cancer patients who subsequently developed metastases, and 15 of these showed a decline in E-rosette percentages prior to disease recurrence. Among patients with malignant melanoma, Bernego et al. [96] reported that although active rosettes were decreased only in metastatic patients, a sequential study of these values as closely linked to the clinical course. Patients whose values were constant tended to remain free of disease while patients whose values declined tended to develop metastases.

In contrast to these positive trends, many other investigators have found

no correlation of T cell numbers to disease stage or prognosis. Nemoto et al. [97] did not observe any significant decrease in the total RFC number according to disease stage or course in 55 patients with breast cancer. Stein et al. [99] studied breast cancer among 255 women and found that % E-RFC did not correlate with prognosis. There was no association with stage among 360 patients with gastric carcinoma in a study by Orita et al. [47]. Bjorkholm et al. [51] was unable to find any relationship between total lymphocyte counts or T lymphocyte counts and disease outcome in previously untreated patients with Hodgkins disease. Among 102 patients with solid tumors, Kerman et al. [80] studied 'active' E-RFC and found normal percentages at all stages, although absolute counts were somewhat depressed.

The current focus of these quantitation studies has centered on the recognition of different subsets of human T lymphocytes. A portion of T cells from normal individuals bear surface Fc receptors for IgG ($T\gamma$), and others have T cell receptors with high affinity for IgM ($T\mu$) [99, 100]. $T\mu$ receptor cells have helper activity for B cell differentiation and T cells express suppressor activity in a similar system [101]. Measurement of these T cell subsets has been recently studied in patients with malignant disease. Among 24 patients with Hodgkins disease, peripheral blood $T\gamma$ were significantly increased, resulting in low $T\mu/T\gamma$ ratios, while in the spleen there was an increase in the proportion of $T\mu$, resulting in an abnormally high $T\mu/T\gamma$ ratio [102]. In this study, $T\mu$ cell proportions in the peripheral blood were significantly lower in patients with combined Stage III and IV than for I and II. However, proportions of $T\mu$ and $T\gamma$ cells must be viewed with considerable caution, as the expression of these markers can vary extensively depending on *in vitro* conditions [103].

In two separate studies of patients with chronic lymphocytic leukemia [104, 105], $T\gamma$ cells were reported to be significantly higher in patients compared to healthy controls. Among 30 patients with urogenital cancer, the proportion of $T\mu$ cells was found to decrease to 50% of normal values in early disease and decreased to 25% of normal in advanced disease [106]. Forty-five patients with untreated solid tumors and 24 patients with lymphomas were found to have an increase in the percentage of peripheral blood $T\gamma$ cells and a decrease in $T\mu$ cells when compared with 30 normal controls [107].

The recent development of monoclonal antibodies defining helper and suppressor cell types in T cell populations promises to contribute even more data to the analysis of T cells and T cell subsets. However, these reagents have not yet been extensively applied to malignant disorders. Posner et al. [108] examined the lymphocyte subpopulations of patients with untreated Hodgkins disease and found no alteration in cells bearing markers associated with helper T cells (OKT4) or suppressor T cells (OKT8) in the

peripheral blood. Relative and absolute numbers of T cells were decreased only in those patients with a poor prognosis, as defined by the presence of B symptoms. Our study of T cell subsets in the peripheral blood of malignant melanoma patients [109] revealed no correlation of OKT4 and OKT8 markers with stage of disease. In addition, no correlation was seen with clinical outcome, either by analysis of melanoma patients' cryopreserved lymphocytes obtained several years earlier before the determination of clinical outcome, or by sequential studies from patients with subsequent recurrent disease. This study indicates that, at least among patients with malignant melanoma, analysis of T cell subsets in peripheral blood cannot predict tumor progression.

The problem of interpreting this enormous amount of conflicting data seems to be three-fold. Technical variables are the first to be considered. Slight changes in temperature of incubation, ratio of SRBC to lymphocytes, freshness of the SRBC, and the use and choice of serum as a stabilizing source can considerably alter rosette results. There is such a lack of standardization for this method that virtually every study of rosette formation in cancer patients employs a somewhat different technique. The second problem concerns the assumption that the measurement of a cell type by the presence of a receptor is a direct indication of function. Because a receptor is expressed does not mean that the cell expressing it is capable of carrying out specific functional activities. On the other hand, a completely functional lymphocyte might develop a modification of the lymphocyte membrane which prevents rosette formation. And finally, the peripheral blood of the cancer patient may not be the most appropriate place to study T cell subset imbalances, especially among patients with more localized disease. These studies did demonstrate that there can be a considerable imbalance of T cell numbers in patients with metastases. However, it might be better to study the earlier disease stages by examining a lymphatic compartment closer to the tumor itself, such as the adjacent lymph nodes. The microenvironment of the tumor might be a more suitable place to determine the importance of T cell and T cell subset quantitation to the stage of disease and clinical outcome. The fact that most of the positive correlations to stage or prognosis have been consistantly observed only in patients with certain cancers or with advanced cancers indicates that the abnormalities of T cell levels seen in these patients are secondary to the malignant process rather than a preliminary event.

5. LYMPHOCYTE CYTOTOXIC FUNCTIONS

The measurement of non-disease related lymphocyte mediated cytotoxicity can be used as a determination of functional immunocompetence. The

functions to be considered include K cells active in antibody dependent cellular cytotoxicity and natural killer (NK) cells. Both processes involve non-immune cells which do not require previous exposure to the antigen. It is this lack of specificity for any single target antigen which qualifies these as measures of general immune function. In contrast, measures of specific tumor cytotoxicity have been excluded from this section. 'Specific' cytotoxic T cells have been extensively studied in patients with malignant disease. The data on cytotoxic T lymphocytes (CTL) against human tumor associated antigens is difficult to evaluate because of the variety of targets and techniques employed. Furthermore, CTL are not directly relevant to the central question of this chapter, i.e. of what value is the determination of immune competence in the cancer patient? While CTL specifically reactive to the tumor may be among the most important factors in determining the host-tumor relationship, their measurement is not a function of 'immunocompetence' nor are methods currently available that would allow standardized testing of CTL against a single known 'tumor antigen'. Thus, general T cell functional properties are considered elsewhere in this chapter, but cytotoxic T cell functions are excluded. Similarly, we have examined the non-specific component of ADCC (i.e. the function of the effector K cells) but not the specificity of the antibodies involved in ADCC against human tumor associated antigens.

Antibody dependent cell mediated cytotoxicity can be mediated by various populations of cells in the peripheral blood, and the type of target cell affects the type of cell which mediates the cytotoxic reaction. Antibody-coated avian and mammalian erythrocytes can be lysed by polymorphonuclear cells, monocytes, and lymphocytes [110], while antibody-coated lymphocyte targets or tissue culture cell lines are generally killed only by lymphoid effector cells (designated as killer or K cells) [111]. K cells are non-adherent, non-phagocytic lymphoid cells which do not possess T or B cell markers although they may sometimes have low affinity sheep erythrocyte receptors or other T markers. They do have Fc receptors which interact with the specific IgG bound to the surface of the target cell [112].

Interpretation of clinical studies using ADCC has been hampered by several complications. The target cell used in the assay determines the effector cell type, so results cannot be compared between nucleated targets and erythroid targets since they measure the activity of different effector populations. Secondly, there have been fewer clinical investigations with less patients per study because of the technical complexity of the ADCC assay compared to other measures of general immune function.

Reports on ADCC effector functions mediated by K cells in cancer patients are conflicting. ADCC directed against Chang cell targets was found to be impaired in one study of 100 cancer patients with a variety of

tumors [113], with 69% of the patients having lower mean values than the normal controls. Another study tested lymphocytes of patients with various tumors against allogeneic PBL as targets using antibody with activity against HLA specificities [114]. It was found that the lymphocytes from cancer patients demonstrated less killing than lymphocytes from controls but that this decrease did not correlate with stage of the disease. In assays against a mouse mastocytoma target, K cell activity was found to be the same in patients with early stage cancers as in healthy individuals and those with benign disease [115]. In this same study, a decrease of 42% in cytotoxicity was noted in patients with advanced cancer, although this decrease was also seen in patients with sepsis and did not correlate with prognosis. No significant difference in ADCC activity against Chang targets was found in 37 patients with colorectal cancer compared to 23 healthy controls [116], although some patients did show an increase in cytotoxicity after tumor resection. Using two other tumor targets, K cell activity from lymphocytes of breast cancer patients at Stages I and II was similar to the activity seen in healthy controls [117]. Deficiencies in Chang target cell killing among chronic lymphocytic leukemia patients returned to normal values after removal of adherent cells and was attributed to a difference in actual numbers of killer cells and not to a defect in their function [118].

Investigations of adherent cell mediated ADCC by cancer patients also show conflicting results. Studies of Hodgkins patients leukocytes against the CRBC target have shown responses ranging from an impaired cytotoxicity [119], to normal cytotoxicity [120], to an increased monocyte mediated cytotoxicity [121]. Other investigations of patients with various solid tumors have shown no difference in their adherent cell ADCC as compared to normal controls [113]. Clearly, the problem with many of these assays has been that the operating effector cell has not been well characterized and may have included a mixed population of cell types. The discrepancies seen in the literature on all ADCC clinical studies are undoubtedly due to the variation imposed on the system by the use of several different target cells and an undefined effector population.

Natural killer cells are a group of cells of lymphoid appearance whose cytotoxic capabilities are not dependent on prior sensitization. NK effectors are non-phagocytic, non-adherent cells that are quite heterogeneous with regard to other markers. For example, natural cytotoxicity has been found in populations which are both E-rosette positive and negative [122, 123]. Most investigators agree that NK activity in peripheral blood is mediated by cells with Fc receptors for IgG [124, 125] although some activity has been associated with cells bearing Fc receptors for IgM [126, 127]. This heterogeneity may indicate that NK activity resides in different subsets of lymphocytes. Alternately, it has been proposed that these markers may not be

stable indicators of different lineages and that a single cell type with functional NK activity might express different markers at various stages of maturation or activation [128]. The 'specificity' of NK cytotoxicity is a complex problem beyond the scope of this chapter. Suffice it to say that some NK 'sensitive' target cell lines such as the myeloid leukemia cell line K562 probably represent the most common NK target structures. Unfortunately, standardization of NK methodology or choice of target cells has not occurred, although K562 has become the most popular target cell.

Several laboratories have compared the NK cytotoxicity of cancer patients and normal donors, and most have found a decreased reactivity among patients with malignant disease. Takasugi et al. [129] studied 289 patients with a wide variety of solid tumors and found that in comparison to normal individuals, cancer patients showed a small decline in average NK reactivity which correlated with increasing tumor involvement. Pross and Baines [130] found no significant difference in NK activity between healthy controls and patients with clinically localized malignancies of many different histologic types. However, these investigators did find that patients with metastatic disease, and patients with untreated chronic lymphocytic leukemia, had very significant decreases in NK cytotoxicity. This association with stage of disease was again demonstrated in a study by Kadish et al. [131]. She found that 31% of 51 patients with solid tumors had decreased killing against the cell line K562 compared to 7% in the normal controls. When patients with metastases were considered separately this number increased to 50%. Of particular interest in the study of Kadish et al. is the finding that low NK activity is not due to abnormally low interferon production. Since interferon is a potent activator of NK cytotoxicity, this finding suggests that low NK activity in cancer patients is under regulatory controls other than interferon. Vose et al. [132] reported that 47% of patients with confirmed malignant disease failed to give significant cytotoxicity to the cell line K562, but these investigators did not find a correlation to stage of disease. They also did not observe significant differences in the proportion of Fc receptor cells or E-rosetting cells among these patients [132]. Patients with chronic lymphocytic leukemia exhibited a similar decrease in NK activity [133], and these investigators were not able to increase NK cytotoxicity by increasing the effector to target cell ratio. They concluded that low NK cytotoxicity was not due to a decrease in the number of immunocompetent cells.

Other investigators have not observed a decline in NK activity among cancer patients. Women with clinically localized mammary carcinoma had a wide range of NK cytotoxicity which was comparable to that seen in healthy individuals [134]. This is in contrast to a study of 83 women with primary untreated breast cancer [135] in which one-third of these patients

gave poor NK reactivity to K562 cells. Among 74 malignant melanoma patients, no differences in natural cytotoxicity levels were found against non-melanoma target cells compared to control values [136]. No decrease in the NK activity against K562 and CCRF-CEM cell lines was evident among 72 patients with Stage III primary lung cancer and metastatic tumors prior to chemotherapy [137]. However, there was a decrease in all patients following chemotherapy, and this recovery of NK activity seemed to correlate with the effect of therapy [137]. Other data obtained from patients with small cell carcinoma of the lung and Stage I and II melanoma indicated the NK activity is associated more with the amount of clinically detected tumor than with the results of chemotherapy [138].

The relationship of NK activity to other measures of immunocompetence has been examined by a number of investigators. Pross and Baines [130] reported a lack of association between delayed type hypersensitivity to PPD and NK cytotoxicity. Vose et al. [132] found that although tumor infiltrating lymphocytes (TIL) and lymph node lymphocytes (LNL) gave diminished NK reactivity, LNL expressed good reactivity to PHA and in MLC. Niitsua et al. [139] also observed very poor reactivity in NK and MLC-CML assays with TIL from pulmonary tumors while LNL and peripheral blood lymphocytes from the same patients had normal or near normal levels of activity. Menon and Stephani [140] have reported that the observed decrease in NK response in cancer patients is paralleled by a decrease in PHA responsiveness. Good correlations between NK and ADCC reactivity have been seen in studies of normal donors and cancer patients [124, 141] as would be expected for tests that appear to measure two overlapping but non-identical cell populations.

The results obtained from the many laboratories studying ADCC and/or NK cytotoxicity in cancer patients are similar to other immunological assays in terms of providing an overall picture of immune function. NK and ADCC appear in general to be depressed in the cancer patient, especially when there is extensive metastatic disease, but the tests certainly cannot be used as accurate indicators of either diagnosis or prognosis. The lack of standardized technology aggravates this problem but even if all laboratories were using identical techniques, this would not eliminate the fact that cancer patients differ from healthy subjects in such assays by a relatively small factor. Given the enormous degree of variability within the human population, such tests can only be of limited value in assessing any individual patient. Once again, they point to decreased reactivity with increased tumor burden and support the hypothesis that impairment of immune functions is a consequence of the malignant process.

Analysis of the distribution of reactivity in any single immunologic assay, such as NK cytotoxicity, can be extremely useful as a probe of the biology of the disease. For example, Pattengale et al. [142] showed a correlation

between susceptibility of leukemia cells to NK effectors and the ultimate clinical progress of the leukemia. In an extensive study of 194 melanoma patients, Hersey et al. [143] showed a correlation between NK activity and certain histopathological features of the primary melanoma such as the presence of high NK activity in the peripheral blood being associated with less lymphocyte infiltration at the base of the tumor. These types of observations, while they fail to provide a complete explanation of the biological significance of NK cytotoxicity, do point to possible fruitful areas of investigation into the host-tumor immunobiology. The use of immunologic immunocompetence assessment to address questions about the natural history of a malignant disease are more likely to be of long-range usefulness than attempts to determine prognosis of individual patients.

6. SUPPRESSOR CELLS

It is now clear that immunology has entered an age of 'regulation' and no discussion of immune function would be complete without an evaluation of the role of suppressor cells. Since this is a topic of extraordinary interest in contemporary immunology, it would be quite easy to assemble a vast literature survey on suppressor cells and malignancy. Instead, we have chosen to address only two relatively narrow issues – are 'non-specific' suppressor cells the cause of impaired immunologic function in the cancer patient and is the measurement of suppressor cells of use and significance in assessing the immunocompetence of the cancer patient? We will not be addressing the biology of suppressor T cells which are antigen-specific and affect the function of rejection mechanisms specific for tumor associated antigens. This area has been reviewed by others [144] and implicit in such studies is the requirement for the demonstration of a 'tumor-specific' immune response. Since demonstration of such specific responses are beyond the capacity of most clinical immunology laboratories, and certainly do not lend themselves to standardization, this area of research is unlikely to be part of the routine analysis of the cancer patient in the foreseeable future. A second area of considerable importance that will not be further discussed are neoplastic diseases of regulatory cells. There is little doubt that neoplasms of helper or suppressor lymphocytes will prove as illuminating to the cellular immunologists as myelomas proved to be for the immunochemists. This area has been reviewed by Broder et al. [145] and will not be further discussed here as they represent very infrequent clinical entities.

Three cell types have generally been evaluated as suppressors of immune functions. These include spontaneous T cell suppressors of immune responses, inducible T cell suppressors of responses with the induction of the suppressive activity usually generated by a polyclonal T cell mitogen such as

Con A, and spontaneous suppressors of monocyte/macrophage phenotype. Each of these cell types will be discussed separately, although frequently techniques have been employed that will not distinguish among these cell types.

Spontaneous T cell suppressors (i.e. suppressor cells which do not require an activational event for them to express suppressive activity) have been most thoroughly studied in chronic lymphocytic leukemia (CLL). The simple question addressed by most laboratories has been is the hypogammaglobulinemia associated with CLL due to excessive suppressor T cell activity? In this case, the target of the suppressor T cells would be the normal B cells. Hersey et al. [146] suggested that both regulatory T cell functions and B cell functions were abnormal in CLL patients. Six of eight patients tested showed diminished Ig production by B cells, 5 of 8 showed impaired T helper cell function when tested with normal B cells, and 6 of 8 showed excessive T suppressor function on normal B cell Ig synthesis. Kay [147] also showed diminished T helper and excessive T suppressor function in CLL patients when B cell DNA synthesis was evaluated. He identified the T suppressor function with Tγ cells. Catovsky et al. [148] in a study of 40 CLL patients also concluded that excessive T suppressor activity resulted in hypogammaglobulinemia and also ascribed the suppressor activity to Tγ cells. However, Inoshita and Whiteside [149] suggested that the primary defect in CLL was in B cell function rather than in dysfunction of immunoregulatory T cells. Their study, based on 12 patients, did find abnormal proportions of Tμ and Tγ cells but failed to detect abnormal T functional capacities for the regulation of Ig synthesis. This is an important point as functional activities are clearly more relevant than surface markers in determining suppressor cell function.

Other than CLL, suprisingly little has been done to evaluate the role spontaneous T cell suppressors in human malignant disease. Hersh et al. [150] examined suppressor activity for mitogen induced proliferative responses using peripheral blood lymphocytes from cancer patients as the regulatory cell and peripheral blood lymphocytes from healthy donors as the target. They identified suppressor cells in 71% of the 35 patients they studied and concluded this activity had a T cell component on the basis of sensitivity of some of the suppressor activity to thymic hormone preparations and/or ionizing radiation. However, interpretation of this type of study is difficult as a variety of patients were used and the abrogation of activity by thymic hormone containing preparations is not the clearest criteria for identification of a suppressor cell as a T suppressor cell. The commonly used experimental design of testing for suppression on normal allogeneic cells, as was done by Hersh et al. [150], presents several problems. First of all, some degree of mixed lymphocyte reaction between suppressor and indicator cells is likely and such responses will be difficult to identify

and quantitate. Secondly, suppressor reactions that are restricted by the major histocompatibility complex will not be detected. Unfortunately, it is difficult to find alternative designs. Testing on the patients own cells is probably better, but these cells may have been already subjected to suppressive influences and may not be good indicators. Lymphocytes from HLA matched siblings would be an excellent, albeit limiting and inconvenient, choice.

A much more direct indication of T suppressor cells was provided by Moroz and Kupfer [151] in breast cancer patients. They demonstrated that a subpopulation of T cells (the lymphocytes bearing ferritin on their surface) would suppress the mixed lymphocyte reaction of the patient's own lymphocytes and that the presence of such cells was associated with lymphocytes from early stage breast cancer donors but not peripheral blood lymphocytes from normal women or women with benign breast diseases. Further exploration of this system may be quite productive in determining the role of suppressor cells in the development of human breast cancer.

T cell suppressors for Ig synthesis have also been shown in patients with melanoma [152]. Radiosensitive R-rosette forming cells from the peripheral blood of normal donors were able to suppress the pokeweed mitogen driven Ig synthesis of purified B cells of the same donors. Increased activity in this assay was detected in patients with localized melanoma. Surgical removal of the tumor appeared to result in a significant decrease in suppressor activity against IgA and IgM but not against IgG production. Although the authors were optimistic that this type of approach could explain the failure of many patients to produce anti-melanoma antibodies, the facts remain that cancer patients in general have normal Ig levels and the melanoma is not generally associated with profound immunologic anergy. Thus, the significance of this finding remains uncertain.

A much more complex system is the Con A induced suppressor system. In such systems, 'pre-suppressor cells' are cultured with a T cell mitogen such as Con A and an active suppressor cell is generated in culture. These induced suppressor cells are then tested for their ability to suppress a response of autologous or allogeneic lymphocytes. The target responses are usually proliferative responses to mitogens but can also include other T or B cell functions.

The pattern which appears to be emerging from studies with Con A induced suppressor cells is of deficient suppressor activity in lymphoproliferative malignancies and excessive suppressor activity in 'solid' tumors. Whether this generalization will withstand critical analysis remains to be determined, and may represent an optimistic viewing of the literature rather than an established fact. The suggestive evidence in this direction is provided by studies of 20 Hodgkins disease patients [153] in which 7 of the 20 were deficient in Con A induced suppressor cell activity. Five of nine

advanced disease patients showed deficient suppressor activity compared to only 2 of 11 early stage patients. No correlation could be obtained between Con A induced suppressor activity for PHA responses and circulating lymphocyte or T cell numbers or PHA response of separated lymphocytes. Suppressor activity also appears to be somewhat diminished in children with acute lymphoblastic leukemia [154]. In contrast, Con A induced suppressor activity appears to be higher than normal in the peripheral blood lymphocytes of urologic cancer patients [155] and gastric cancer patients [156]. However, no difference was found between 22 melanoma patients and age-matched controls for Con A induced suppressor activity as measured by autologous lymphocyte responses to Con A [157]. The lack of suppressor activity in melanoma was interesting in that the proportion of Tγ cells was increased. Thus, the proportion of cells with 'suppressor phenotype' will not necessarily indicate presence of suppressor cells in a population of cancer patients. In a study of 13 Hodgkins disease patients, Vanhaelen and Fisher [158] found no difference between the patients and controls in ability to generate Con A induced suppressors although the patients lymphocytes were more sensitive to such suppression. The mechanism of suppression in such systems remains obscure although the recent suggestion that the mediator of the human Con A induced suppressor cell is interferon certainly deserves further attention [159], as the biological and clinical significance of Con A induced suppressor cells can only be fully understood when the mechanism of suppression is better understood. However, several serious reservations have been expressed about the Con A system. Some of these reservations relate to interpretation of results and the necessity for appropriate controls. For example, Schulof et al. [160] pointed out the necessity of using age-matched controls as the ability to suppress allogeneic response, although not autologous responses, declined in the elderly population. A similar concern has been raised about the suppressor macrophage system [161]. The problem of appropriate selection of controls is a difficult one that is beyond the scope of this discussion but should be kept in mind when evaluating all human studies. A much more fundamental question has been raised by Fernandez and MacSween [162]. These authors questioned wheter a Con A inducible suppressor cell really exists since lymphocytes cultured in the absence of Con A actually show an augmentation of proliferative responses when compared to lymphocytes not subjected to culture. They proposed that culture of the cells in the presence of Con A results in the loss of augmentation rather than the induction of suppression. This further emphasizes the need for much more comprehensive understanding of the nature of the suppressor cells and the mechanism of suppression before it is even useful to assess whether they are an important factor in the development of a unique host-tumor relationship.

The most extensively studied suppression system in human malignant

disease has been the adherent cell capable of suppressing normal immunologic functions. These cells are usually assessed for the inhibition of proliferative responses although Broder et al. [163] identified circulating monocytes capable of suppressing Ig synthesis in patients with multiple myeloma. This finding may help explain the impairment of normal polyclonal Ig synthesis in patients with myeloma and may reflect a normal homeostatic mechanism attempting to deal with the excessive Ig production inherent in the disease.

Hodgkins disease has probably been the most extensively studied malignancy for the presence of suppressor macrophages. Goodwin et al. [164] identified prostaglandin secreting macrophages as the suppressive cell in Hodgkins patients and Han [165] found this activity to be much more pronounced in patients with active and generalized disease as opposed to patients with localized disease or disease in remission. Other laboratories have indicated that the monocyte mediated suppression in Hodgkins disease correlates with the poor proliferative response characteristic of lymphocytes from patients with this disease [166] and lymphocytes from Hodgkins disease patients may exhibit increased sensitivity to monocyte mediated suppression by lymphocytes [167]. Again, such data must be viewed with caution as the excess suppression mediated by monocytes in Hodgkins disease may be due in part to the greater tendency of monocytes from the peripheral blood of patients with this disease to accumulate in the standard purification procedure for mononuclear cells when compared to mononuclear cells from normal individuals [166]. Thus, a small change in the density of the monocytes would result in accumulation of these cells in the mononuclear preparations and yield a false result suggestive of excessive suppression.

Non-specific suppression mediated by adherent cells has also been studied in patients with non-lymphoid malignancies. Quan and Burtin [168] tested peripheral blood lymphocytes from 169 cancer patients and found an overall significant diminution of response to PHA. They found the presence of suppressor cells in 6 of the most suppressed samples and concluded that the suppressor cells were monocytes based on sensitivity to carrageenan. More convincing evidence of monocyte mediated suppression has been obtained using indomethacin treatment of mononuclear cells to prevent prostaglandin synthesis. This approach has been used to show the presence of suppressor monocytes in melanoma [169] and lung cancer patients [170]. In the study of 33 melanoma patients [169], indomethacin increased the proliferative response by 35% to 85% whereas this drug increased the Con A response from normal subjects by only 5% to 15%. No correlation could be obtained between suppressor activity and stage of disease, age, or proportion of blood monocytes. The proportion of monocytes is an important variable which is all too often ignored in such studies. Braun and Harris [171 showed that cancer patients with poor PHA responses tend to have

higher monocyte counts than do normal controls or patients with strong PHA responses. This does not mean that only monocyte numbers affect this response, since differences in regulatory function clearly exist [171], but that quantitative differences in monocytes can have important effects. In another study of 25 melanoma patients [172], adherent indomethacin-sensitive suppressor cells were found more often in patients with metastatic disease than in patients with localized disease and the presence of adherent suppressor cells 'tended to correlate' with more rapidly progressing disease. Similarly, 26 of 30 lung cancer patients studied had suppressor activity of monocyte-enriched cells and this activity was most noticeable among patients with more extensive disease [173]. In addition to proliferative responses, indomethacin reversal of suppression of the ability to manifest a local graft-vs.-host reaction has been reported [174]. This latter study is of interest in that the indomethacin effect was not related to inhibition of synthesis of prostaglandin. This raises the important point, also emphasized by Schechter et al. [175], that excessive production of prostaglandins is probably not the sole mechanism of inhibition by monocytes. Other monocyte products are likely to have an involvement in the regulation of lymphocyte responses and even if a response is indomethacin-sensitive one cannot assume that this is an unequivocal indication of prostaglandin involvement in the regulatory process.

A number of other malignancies have been reported to have adherent suppressor cells demonstrable in the peripheral blood. For example, adherent suppressor cells have been reported in bladder carcinoma [176, 177], prostate cancer [178], and head and neck cancer [179]. Particularly interesting among these diseases is the finding of adherent suppressor cell activity in healthy relatives of patients with hereditary colon cancer [180]. Whether this finding indicates the involvement of suppressor cells in the etiology of the disease or is simply a potentially useful marker for those at risk has yet to be determined.

With the variety of types of suppressor cells identified and a variety of techniques available to measure each type of suppressor cell, there is inevitable confusion as to what a 'suppressor cell' really is and what suppressor cell activity means biologically. Several laboratories have addressed this problem by comparing several assays for suppressor cells. The major problem in doing such experiments is that most of the systems are technically demanding and require a substantial number of cells. Thus, it is difficult to do multiple assays on any one sample and the end result is that most published studies have included relatively few patients. Therefore, these studies must be taken as indications of promising areas of further research rather than definitive comparisons of the activity of various types of suppressor cells. For example, Jerrells et al. [181] studied 38 lung and breast cancer

patients and found 15 that had suppressor cell activity. Fourteen of these were further characterized and included 7 patients with predominately adherent cells mediating the suppression (5 lung and 2 breast cancers) and 7 with predominately non-adherent suppressor cells (3 lung and 4 breast). In a similar study, Uchida and Hoshino [182] studied 15 patients with either stomach or lung cancer. They identified two types of suppressor cells including a Sephadex G10 adherent monocyte and a nylon wool non-adherent cell (persumably a T lymphocyte). Hubbard et al. [183] compared spontaneous and Con A induced suppressor activity with lymphocytes obtained from 13 head and neck or colorectal cancer patients. When compared to 18 healthy control donors, the cancer patients exhibited higher spontaneous suppressor activity and diminished Con A inducible suppressor activity for mixed lymphocyte culture responses. Schulof et al. [184] compared a wide variety of parameters of immunocompetence including Con A induced suppressor activity in 31 untreated Hodgkins disease patients. Con A induced suppressor activity did correlate to PHA response and T cell colony forming capacity. However, there were no characteristic abnormalities in any single assay or combination of assays.

It must be kept in mind that studies designed to search for suppressor cells are more likely to find suppressor cell activity than studies designed to question their existence. The operational paradigm for many tumor immunologists is that suppressor cells exist and are responsible for tumor-induced immunosuppression. Therefore, experiments are structured to test for suppressor cells rather than to disprove their existance. The danger in this type of thinking is that we will assume that suppressor cells can explain all instances of impaired immune function. Yet, there are several studies which indicate that suppressor cells do not exist in all malignant conditions. Bankhurst et al. [185] were unable to identify suppressor cells in the peripheral blood of cancer patients using three different assay systems including the measurement of prostaglandin secreting adherent cells, spontaneous suppressor cells, or Con A induced suppressor cells. Although these investigators tested only 5 to 10 patients in each assay system, all the patients tested were anergic. This study once again indicates that mechanisms other than suppressor cells can result in defective T cell functions. Fernandez et al. [186] were unable to find lymphocytes of chronic lymphocytic leukemia (CLL) patients able to suppress T cell proliferative responses to mitogens. They also used several different methods to detect suppressor cells and were unable to find indomethacin-sensitive or spontaneous suppressors of T cell functions. This result is in strong contrast to the previously cited results in CLL of suppressor cells for B cell functions. It is possible that the suppressor functions specific for B cell responses in CLL represent a normal immunologic mechanism attempting to deal with excessive B cell proliferation and

that induction of suppressor activity is not a generalized phenomenon in either CLL or in all malignant diseases.

The suppressor cell functions described thus far have been detected in cells of the peripheral blood. However, other lymphoid compartments such as the lymph nodes or the tumor infiltrating lymphocytes (TIL) may be of even greater significance since they represent lymphoid compartments in closer proximity to the actual tumor site. Several laboratories have examined regulatory functions among lymph node lymphocytes (LNL) draining the tumor site. Catalona et al. [187] found Con A inducible suppressor cells in 11 of 14 regional lymph nodes draining urological tumors and only in 1 of 4 control nodes studied. The function being measured was suppression of autologous peripheral blood responses to Con A. It is interesting to note that they were unable to find suppressor cells without Con A activation while Herr [188] found spontaneous suppressors of peripheral blood lymphocyte MLC reactivity in the pelvic nodes of three patients with advanced bladder cancer. However, Callery et al. [189] used a very similar protocol to examine LNL from melanoma patients and were unable to detect suppressor cells for MLC in any of 12 samples tested. Vose and Moore [190] found suppressor cell activity among TIL of human lung and breast cancers. Responses being measured were peripheral blood lymphocyte proliferative responses to PHA and the suppressor cells required no induction by Con A. In contrast, Uchida and Micksche [191] found Con A inducible suppressors but not spontaneous suppressors in the pleural effusions of 18 of 20 patients with lung cancer. Again, the response being measured was autologous or allogeneic proliferative responses to PHA or Con A. Why should spontaneous suppressors be detected among TIL of lung or breast tumors while only Con A inducible 'pre-suppressors' are found in pleural effusions? The answer is obscure at present but may involve the responses being measured, the source and method of preparation of the lymphocytes, or the selection of patients. The indicator response being suppressed is probably a very important variable. For example, TIL may show suppressor activity for proliferative responses but neither TIL nor LNL have been shown to have suppressor activity for NK cytotoxicity [139, 192]. The lack of suppressor activity among TIL and LNL is interesting considering that both TIL and LNL have rather low NK activity. This emphasizes that the absence of a response or the presence of a low level response cannot be assumed to be evidence for the presence of suppressor cells.

CONCLUSIONS

The stated purpose of this chapter was to examine studies of immunocompetence in cancer patients to determine if such studies have been of any

practical use for the development of better patient care or of any scientific usefulness in better defining the host-tumor relationship. Despite the extraordinary effort devoted to studies of immunocompetence in cancer patients, and the thorough analysis of many systems for correlations between immunologic function and tumor stage or progression, the practical usefulness of 'non-specific' immunologic testing in cancer patients remains highly doubtful. Simple immunologic tests such as skin tests may help to identify the anergic patient. Identification of the profoundly immunosuppressed individual can be of obvious usefulness as this group of patients may require special considerations such as measures to prevent opportunistic infections. The profoundly immunosuppressed patients probably represent subsets of patients with large tumor burdens or with immune systems particularly sensitive to immunosuppressive factors produced by or induced by the tumor. As such, they represent a special challenge to the therapist and are particularly poor candidates for immunotherapy. Beyond these considerations, it is hard to pinpoint any specific advantage to routine immunologic testing of cancer patients. No single assay can accurately predict prognosis for an individual patient and any attempt to individualize therapy based on PHA response or some comparable immunologic parameter is unlikely to succeed. If no single assay can provide more than a general indication of the effects of the tumor on the immune system, can a collection of assays be used to accurately predict which patients are doing well and which will do poorly? Many laboratories have used multiple assays and sophisticated statistical techniques to construct 'immunograms' for a set of patients. While the last word is not in on such attempts, they strike us as being unlikely to be able to predict the outcome for individual patients. The assumption underlying such an attempt is that all of these assays have some relevance and validity. However, for any individual patient some assays will be entirely irrelevant and may even be totally deceptive. For example, consider an individual who has always had a PHA response which was 50% higher than age-matched controls. If this person now has Hodgkins disease and presents with a PHA response which is at 'normal' levels, he would be identified as an individual likely to do well, but, he may really be expressing a profound deficit in immunologic function. Thus, the extraordinary spread of responses among the normal population greatly limits the usefulness of almost all these assays. Furthermore, attempts to use combinations of assays presume that we can assess the relative importance of each assay system. This has proven to be impossible thus far and probably will continue to be impossible since the relative importance may vary with the individual patient. There is an obvious experiment to determine if any collection of assays of immunocompetence can predict clinical outcome. One needs to test a set of patients with the panel of assays and construct a for-

mula so that the immunologic test results divide the patients into those who recurred and those who did not recur. There is no doubt that any set of assays can do this since any collection of assays must find some which will describe any subset of the patients. The rigorous test is to then discard the assays which appear to have no value in the first set of patients for prediction of prognosis and utilize the same formula generated from this first set on an entirely new set of patients. So far, no such formula has been generated which can be used reproducibly from one patient population to the next. It is possible that the introduction of specific anti-tumor responses into the formulation would allow a much more precise determination of prognosis or diagnosis. In this case, assays of immunocompetence may prove to be a useful secondary part of an immunologic evaluation of a patient. However, there remains little evidence that assays of immunocompetence alone are of sufficient accuracy or relevance to provide useful information.

The other central question is whether assays of immunocompetence can provide useful information concerning the biology of the host-tumor relationship. Here, one can be much more optimistic. Research in this area has already proven itself to be of considerable significance. For example, the overwhelming body of evidence indicates that cancer is an immunosuppressive disease. With this as an established fact, investigators were encouraged to explore the various immunoregulatory properties of host factors. Thus, the role of such immunoregulatory molecules as α-feto-protein, prostaglandins, histamine, interferon, and many others have come to light and been carefully analyzed. Many other examples abound where fundamental studies and basic information of considerable value have been generated from efforts to determine why and how immunologic function is impaired in cancer patients. The exact benefits to be gained from such studies cannot be predicted in advance, but a prediction that such studies will be useful is a very safe one.

ACKNOWLEDGMENT

We thank Ms. Lisa DeTournay for her excellent assistance in the preparation of the manuscript.

REFERENCES

1. Burnet FM: The concept of immunological surveillance. Prog Exp Tumor Res 13:1–27, 1970.
2. Gatti RA, Good RA: Occurrence of malignancy in immunodeficiency diseases: A literature review. Cancer 28:89–98, 1971.

3. Stutman O: Immunological surveillance and cancer. In: The handbook of cancer immunology, Waters H (ed). New York: Garland STPM Press, 1981, Vol 7, pp 1-25.
4. Transplantation Review, Moller G (ed). Stockholm, 1976. Vol 28, Immunological Surveillance.
5. Eilber FR, Morton DL: Impaired immunologic reactivity and recurrence following cancer surgery. Cancer 25:362-367, 1970.
6. Goodwin JS: OKT3, OKT4, and all that. JAMA 246:947-948, 1981.
7. Brown RS, Haynes HA, Foley TH, Goodwin HA, Berard CW, Carbone PP: Hodgkins disease: Immunologic, clinical and histologic features of 50 untreated patients. Ann Intern Med 67:291-302, 1967.
8. Pinsky CM, El-Domeiri A, Caron AA, Knapper WH, Oettgen HF: Delayed hypersensitivity reactions in patients with cancer. In: Recent results in cancer research, Vol 47, Mathe G, Weiner R (eds). New York: Springer-Verlag, 1974, pp 37-41.
9. Brosman S, Elhilali M, Vescura C, Fahey J: Immune response in bladder cancer patients. J Urology 121:162-169, 1979.
10. Bean MA, Schellhammer PS, Herr HW, Pinsky CM, Whitmore WF: Immunocompetence of patients with transitional cell carcinoma as measured by DNCB skin tests and *in vitro* lymphocyte function. Natl Cancer Inst Monogr 49:111-114, 1978.
11. Eilber FR, Nizze JA, Morton DL: Sequential evaluation of general immune competence in cancer patients: Correlation with clinical course. Cancer 35:660-665, 1975.
12. Wells SA Jr, Burdick JF, Christiansen C, Ketcham AS, Adkins PC: Demonstration of tumor associated delayed cutaneous hypersensitivity reactions in lung cancer patients and in patients with carcinoma of the cervix. Natl Cancer Inst Monogr 37:197-203, 1973.
13. Aisenberg A: Manifestations of immunological unresponsiveness in Hodgkins disease. Cancer Res 26:1152-1160, 1966.
14. Kaplan HS: Role of immunological disturbance in human oncogenesis: Some facts and fancies. Br J Cancer 25:620-634, 1971.
15. Bolton PM: DNCB sensitivity in cancer patients: A review based on sequential testing in 430 patients. Clin Oncol 1:59-69, 1975.
16. Eltringham JR, Kaplan HS: Impaired delayed hypersensitivity responses in 154 patients with untreated Hodgkins disease. Natl Cancer Inst Monogr 36:107-115, 1973.
17. Ziegler JL, Cohen MH, Morrow RH, Kyalwazi SK, Carbone PP: Immunologic studies in Burkitt's lymphoma. Cancer 25:734-739, 1970.
18. Stjernsward J, Clifford P, Svedmyr E: In: General and tumor-distinctive cellular immunological reactivity in Burkitt's lymphoma, Burkitt DP, Wright DH (eds). Edinburgh: E and S Livingstone, Ltd, 1970, p 164.
19. Pinsky CM, Oettgen HF, El-Domeiri A, Old LJ, Beattie EJ, Burchenal JH: Delayed hypersensitivity reactions in patients with cancer. Proceedings of the American Association for Cancer Research 12:100, 1971.
20. Pinsky CM, Wanebo H, Mike V, Oettgen H: Delayed cutaneous hypersensitivity reactions and prognosis in patients with cancer. Ann NY Acad Sci 276:407-410, 1976.
21. Zuckerman KS, Lo Buglio AF: Phytohemagglutanin skin tests in cancer patients. Cancer 39:2355-2361, 1977.
22. Morris DL, Hersh EM, Hsi BP, Gutterman JU, Marshall MM, Mavligit GM: Recall antigen delayed type hypersensitivity skin testing in melanoma and acute leukemia patients and their associates. Cancer Res 39:219-226, 1979.
23. Aranha GV, McKhann CF, Simmons RL, Grage TB: Recall skin test antigens and the prognosis of stage I melanoma. J Surg Oncol 11:13-16, 1979.
24. Bates SE, Suen JY, Tranum BL: Immunological skin testing and interpretation: A plea for uniformity. Cancer 43:2306-2314, 1979.
25. Bernier GM: Adult hypogammaglobulinemia. Am J Med 36:618-623, 1964.

26. Fahey JL: Antibodies and immunoglobulins. J Am Med Assn 194:255-258, 1965.
27. Barkas T, Al-Khateeb SF, Irvine WJ, Davidson NM, Roscoe P: Inhibition of antibody dependent cell mediated cytotoxicity (ADCC) as a means of detection of immune complexes in the sera of patients with thyroid disorders and bronchogenic carcinoma. Clin Exp Immunol 25:270–279, 1976.
28. Hughes NR: Serum concentrations of γG, γA and γM immunoglobulins in patients with carcinoma, melanoma, and sarcoma. J Natl Cancer Inst 46:1015–1028, 1971.
29. Mandel MA, Dvorak K, Decasse JJ: Salivary immunoglobulins in patients with oropharyngeal and bronchopulmonary cancer. Cancer 31:1408–1413, 1973.
30. Albin RJ, Gordon MJ, Soane WA: Levels of immunoglobulins in the serum of patients with carcinoma of the prostate. Neoplasma 19:57–60, 1972.
31. Wara WM, Wara DW, Phillips TL, Ammann AJ: Elevated IgA in cancer of the nasopharynx. Cancer 35:1313–1315, 1975.
32. Cochran AJ, Mackie RM, Grant RM, Ross CE, Connell MD, Sandilands G, Whaley K, Hoyle DE, Jackson AM: An examination of the immunology of cancer patients. Int J Cancer 18:298–309, 1976.
33. Plesnicar S: Immunoglobulins in carcinoma of the uterine cervix. Acta Radiol 11:37–47, 1972.
34. Shaw RK, Swzed C, Boggs DR, Fahey JL, Frei E, Morrison E, Utz JP: Infection and immunity in chronic lymphocytic leukemia. Arch Inter Med 106:467–478, 1960.
35. Barr M, Fairley GH: Circulating antibodies in reticuloses. Lancet 1:1305–1310, 1961.
36. Cone L, Uhr JW: Immunological deficiency disorders associated with chronic lymphocytic leukemia and multiple myeloma. J Clin Invest 43:2241–2248, 1964.
37. Libansky J: Study of immunologic reactivity in hemoblastosis: Circulating antibody formation as a response to antigenic stimulus in leukemia, malignant lymphoma, myeloma, and myelofibrosis. Blood 25:169–178, 1965.
38. Lee AKY, Rowley M, MacKay IR: Antibody-producing capacity in human cancer. Br J Cancer 24:454–463, 1970.
39. Jansen HM, The TH, deGast GC, Esselink MT, Pastoor G, Orie NG: The primary immune response of patients with different stages of squamous-cell bronchial carcinoma. Thorax 33:755–760, 1978.
40. Aisenberg AC, Leskowitz S: Antibody formation in Hodgkins disease. N Eng J Med 268:1269–1272, 1963.
41. Levin AG, Cunningham MP, Steers AK, Miller DG, Southham CM: Production of 19S and 7S antibodies by cancer patients. Clin Exp Immunol 7:839–849, 1970.
42. Quaglino D, Cowling DC: Cytochemical studies on cells from chronic lymphatic leukaemia and lymphosarcoma cultured with phytohaemagglutinin. Br J Haematol 10:358–364, 1964.
43. Melief CJM, Schweitzer M, Zeylemaker WP, Verhagen EH, Eijsvoogel VP: Some immunological properties of lymphoid cells from patients with acute lymphatic leukemia (ALL). Clin Exp Immunol 15:131–143, 1973.
44. Mellstedt H, Holm G: *In vitro* studies of lymphocytes from patients with plasma cell myeloma: I. Stimulation by mitogens and cytotoxic activities. Clin Exp Immunol 15:309–320, 1973.
45. Hershorn K, Schreibman RR, Bach FH, Silzbach LE: *In vitro* studies of lymphocytes from patients with sarcoidosis and lymphoproliferative diseases. Lancet 2:842–843, 1964.
46. Fauget GB: Quantitation of immunocompetence of Hodgkins disease. J Clin Invest 56:951–957, 1975.
47. Orita K, Miwa H, Fukuda H, Yumura M, Uchida Y, Mamnami T, Konaga E, Tanaka S: Pre-operative cell mediated immune status of gastric cancer patients. Cancer 38:2343–2348, 1976.

48. Wanebo HJ, Rao B, Miyazawa N, Martini N, Middleman MP, Oettgen HF, Beattie EJ: Immune reactivity in primary carcinoma of the lung and its relation to prognosis. J Thorac Cardiovasc Surg 72:339–347, 1976.
49. Goldrosen MH, Han T, Jung D, Smolev J, Holyoke ED: Impaired lymphocyte blastogenic response in patients with colon adenocarcinoma: Effects of disease and age. J Surg Oncol 9:229–234, 1977.
50. Humphrey LJ, Humphrey MA, Singla O, Volenec FJ: Immunologic responsiveness of patients with cancer: Relationship to tumor type, stage and prognosis. Ann Surg 193:574–578, 1981.
51. Bjorkholm M, Holm G, Mellstedt H, Johansson B, Killander D, Sunblad R, Soderberg G: Prognostic factors in Hodgkins disease. Scand J Hematol 20:306–318, 1978.
52. Chretien PB, Crowder WL, Gertner HR, Sample WF, Catalona WJ: Correlation of preoperative lymphocyte reactivity with the clinical course of cancer patients. Surg Gynecol Obstet 136:380–384, 1973.
53. Han T, Takita H: Immunologic impairment in bronchogenic carcinoma: A study of lymphocyte responses to phytohemagglutinin. Cancer 30:616–620, 1972.
54. Payne JE, Meyer JH, Macpherson JG, Nelson DS, Walls RS, Pheils MT: The value of lymphocyte transformation in carcinoma of the colon and rectum. Surg Gynecol Obstet 150:687–693, 1980.
55. Ryan RE Jr, Neel HB, Ritts RE: Correlation of pre-operative immunologic test results with recurrence in patients with head and neck cancer. Otolaryngol Head Neck Surg 88:58–63, 1980.
56. Adler A, Stein JA, Ben-Efraim S: Immunocompetence, immunosuppression, and human breast cancer: III. Prognostic significance of initial level of immunocompetence in early and advanced disease. Cancer 45:2074–2083, 1980.
57. Roberts MM: Lymphocyte transformation in breast cancer. Br J Surg 57:381, 1970.
58. Sample WF, Gertner HR, Chretien PB: Inhibition of phytohemaglutinin induced *in vitro* lymphocyte transformation by serum from patients with carcinoma. J Natl Cancer Inst 46:1291–1297, 1971.
59. Butterworth C, Oon CJ, Westbury G, Hobbs JR: T lymphocyte response in patients with malignant melanoma. Eur J Cancer 10:639–646, 1977.
60. Golub SH, Rangel DM, Morton DL: *In vitro* assessment of immunocompetence in patients with malignant melanoma. Int J Cancer 20:873–880, 1977.
61. Golub SH, O'Connell TX, Morton DL: Correlation of *in vivo* and *in vitro* assays of immunocompetence in cancer patients. Cancer Res 34:1833–1837, 1974.
62. Twomey PL, Catalona WJ, Chretien PB: Cellular-immunity in cured cancer patients. Cancer 33:435–440, 1974.
63. Holmes EC, Golub SH: Immunologic defects in lung cancer patients. J Thorac Cardiovasc Surg 71:161–167, 1976.
64. Berlinger NT, Good RA: Concommitant immunopathology with squamous cell carcinoma of the head and neck regions. Trans Am Acad Opthalmol Otolaryngol 82:588–594, 1976.
65. Suciu-Foca N, Buda J, McManus J, Theim T, Reemtsma K: Impaired responsiveness of lymphocytes and serum-inhibitory factors in patients with cancer. Cancer Res 33:2373–2377, 1973.
66. Cannon GB, Dean JH, Herberman RB, Perlin E, Reid J, Milter C, Lang NP: Association of depressed postoperative lymphoproliferative responses to alloantigens with poor prognosis in patients with stage I lung cancer. Int J Cancer 25:9–17, 1980.
67. Han T, Takita H: Impaired lymphocyte response to allogeneic cultured lymphoid cells in patients with lung cancer. N Eng J Med 286:605–606, 1972.

68. Catalona WJ, Tarpley JL, Potvin C, Chretien PB: Host immunocompetence in genitourinary cancer: Relation to tumor stage and prognosis. Natl Cancer Inst Monogr 49:105–110, 1978.
69. Liaw KY, Chang-Chien Y, Chen YC, Deny JS: Impaired cell mediated immunity function in thyroid cancer. Cancer 46:285–288, 1980.
70. Alsabti EA: *In vivo* and *in vitro* assays of immunocompetence in bronchogenic carcinoma. Oncology 36:171–175, 1979.
71. Graze PR, Perlin E, Royston IL: *In vitro* lymphocyte dysfunction in Hodgkins disease. J Natl Cancer Inst 56:239-243, 1976.
72. Teasdale C, Roux L, Whitehead RH, Bolton RM, Hughes LE: Analysis of PHA responsiveness of lymphocytes in cancer patients. Clin Oncol 2:306, 1976.
73. Dean JH: Application of the microculture lymphocyte proliferation assay to clinical studies. In: Immunodiagnosis of cancer, part 2, Herberman RB, McIntire KR (eds). New York: Marcel Dekker Inc, 1979, p 738.
74. Golub SH, Sulit HL, Morton DL: The use of viable frozen lymphocytes for studies in human tumor immunology. Transplantation 19:195–202, 1975.
75. Zacharski LR, Linman JW: Lymphocytopenia: Its causes and significance. Mayo Clin Proc 46:168–173, 1971.
76. Riesco A: Five year cancer cure: Relation to total amount of peripheral lymphocytes and neutrophils. Cancer 25:135–140, 1970.
77. Papatestas AE, Kark AE: Peripheral lymphocyte counts in breast carcinoma: An index of immune competence. Cancer 34:2014-2017, 1974.
78. Wanebo HJ, Jun M, Strong E, Oettgen HF: T cell deficiency in patients with squamous cell cancer of head and neck. Am J Surg 130:445–449, 1975.
79. West WH: E-rosette formation in immunodiagnosis. In: Immunodiagnosis of cancer, Part 2, Herberman RB, McIntire KR (eds). New York: Marcel Dekker Inc, 1979, p 725.
80. Kerman RH, Smith R, Stefani SS: Active T-rosette forming cells in the peripheral blood of cancer patients. Cancer Res 36:3274–3280, 1976.
81. Catalona WJ, Tarpley JL, Potvin C, Chretien PB: Correlations among cutaneous reactivity to DNCB, PHA-induced lymphocyte blastogenesis and peripheral blood E-rosettes. Clin Exp Immunol 19:327–332, 1975.
82. Bobrove AM, Fuks S, Strober S, Kaplan HS: Quantitation of T and B Lymphocytes and cellular immune function in Hodgkins disease. Cancer 36:169–179, 1975.
83. Jerrells TR, Dean JH, Herberman RB: Relationship between T lymphocyte levels and lymphoproliferative responses to mitogens and alloantigens in lung and breast cancer patients. Int J Cancer 21:282–290, 1978.
84. Anthony HM, Kirk JA, Madsen KE, Mason MK, Templeman GH: E and EAC rosetting lymphocytes in patients with carcinoma of the bronchus: I. Some parameters of the test and its prognostic significance. Clin Exp Immunol 20:29–40, 1975.
85. Silverman NA, Alexander JC, Potvin C, Chretien PB: *In vitro* lymphocyte reactivity and T cell levels in patients with melanoma: Correlations with clinical and pathological stage. Surgery 79:332–339, 1976.
86. Whitehead RH, Thatcher J, Teasdale C, Rogerts GP, Hughes LE: T and B lymphocytes in breast cancer: Stage relationship and abrogation of T lymphocyte depression by enzyme treatment *in vitro*. Lancet 1:330–333, 1976.
87. Rand RJ, Jenkins DM, Bulmer R: T and B lymphocyte subpopulations in pre-invasive and invasive carcinoma of the cervix. Clin Exp Immunol 30:421–428, 1977.
88. Bensa JC, Micouin C, Schaerer R, Sotto JJ, Hollard D: Quantitative study of T and B lymphocytes in Hodgkins disease. Biomedicine 26:137–144, 1977.
89. Hernandez JL, Sanz ML, Crisi CD, Voltas J, Subira ML, Zornoza G, Oehling A: Lym-

phocyte subpopulations in colorectal cancer – postoperative evaluation. Allergy Immunopathol 6:339–344, 1978.

90. Dellon AL, Potvin C, Chretien PB: Thymus dependent lymphocyte levels in bronchogenic carcinoma: Correlations in histology, clinical stage, and clinical course after surgical treatment. Cancer 35:687–694, 1975.
91. West WH, Sienknecht CW, Townes AS, Herberman RB: Performance of a rosette assay between lymphocytes and sheep erythrocytes at elevated temperatures to study patients with cancer and other diseases. Clin Immunol Immunopathol 5:60–66, 1976.
92. Weese JL, Oldham RK, Tormey DC, Barbock AL, Morales A, Cohen MH, Alford JC, Sharb PE, Tsangaris NT, West WH, Cannon GB, Dean JH, Djeu J, McCoy JL, Herberman RB: Immunologic monitoring in carcinoma of the breast. Surg Gynecol Obstet 145:209–218, 1977.
93. Wybran J, Fudenberg HH: Thymus-derived rosette-forming cells in various human disease states: Cancer, lymphoma, bacterial and viral infections, and other diseases. J Clin Invest 52:1026–1032, 1973.
94. Shirakusa T, Shigematsu N, Yosida T, Saido R, Katayama N, Inokuchi K: Changes in T cell population in patients with bronchogenic carcinoma. J Thorac Cardiovasc Surg 76:262–265, 1978.
95. Oldham RK, Weese JL, Herberman RB, Perlin E, Mills M, Heim W, Blom J, Green D, Reid J, Bellinger S, Law I, McCoy JL, Dean JH, Cannon GB, Djeu J: Immunologic monitoring and immunotherapy in carcinoma of the lung. Int J Cancer 18:739–749, 1976.
96. Bernengo MG, Capella G, Peruccio M, Zina G: Relationship between T and B lymphocyte values and prognosis in malignant melanoma. Br J Dermatol 98:655–662, 1978.
97. Nemoto T, Han T, Minowada J, Angkur V, Chamberlain A, Dao T: Cell mediated immune status of breast cancer patients: Evaluation by skin test, lymphocyte stimulation and counts of rosette-forming cells. J Natl Cancer Inst 53:641–645, 1974.
98. Stein JA, Alder A, Shlomo BE, Moshe M: Immunocompetence, immunosuppression, and human breast cancer. Cancer 38:1171–1187, 1976.
99. Moretta L, Ferrarini M, Durante ML, Mingari MC: Expression of a receptor for IgM by human T cells *in vitro*. Eur J Immunol 5:565–570, 1975.
100. Ferrarini M, Moretta L, Abrlle R, Durante ML: Receptors for IgG molecules on human lymphocytes forming spontaneous rosettes with sheep red cells. Eur J Immunol 5:70–77, 1975.
101. Moretta L, Mingari MC, Morètta A: Human T cell subpopulations in normal and pathologic conditions. Immunological Review 45:163–193, 1979.
102. Gupta S: Subpopulations of human T lymphocytes: XVI. Maldistribution of T cell subsets associated with abnormal locomotion of T cells in untreated adult patients with Hodgkins disease. Clin Exp Immunol 42:186–195, 1980.
103. Golightly MG, Golub SH: The *in vitro* culture characteristics and requirements for expression of receptors for IgM on human peripheral blood lymphocytes. Clin Exp Immunol 39:222–232, 1980.
104. Lauria F, Foa R, Catovsky D: Increase in T gamma lymphocytes in B cell chronic lymphocytic leukemia. Scand J Haematol 24:187–190, 1980.
105. Platsoucas CD, Kamoin S. Karanas A, Clarkson BD, Good RA, Gupta S: Receptors for immunoglobulin isotype on T and B lymphocytes from patients with untreated chronic lymphocytic leukemia. Clin Exp Immunol 40:256–263, 1980.
106. Umeda T, Yokoyama H, Kobayashi K, Akaza H, Niijima T: Subsets of T lymphocytes of patients with malignancies of the urogenital tract. Cell Mol Biol 25:95–99, 1979.
107. Kaszubowski PA, Husky G, Tung KS, Williams RC Jr: T lymphocyte subpopulations in peripheral blood and tissues of cancer patients. Cancer Res 40:4648–4657, 1980.

108. Posner MR, Reinherz EL, Breard J, Nadle LM, Rosenthal DS, Schlossman SF: Lymphocyte subpopulations of peripheral blood and spleen in untreated Hodgkins disease. Cancer 48:1170–1176, 1981.
109. Karavodin LM, Guiliano AE, Golub SH: T lymphocyte subsets in patients with malignant melanoma. Cancer Immunol Immunother 11:251–254, 1981.
110. Nelson DL, Bundy BM, Pitchon HE, Blease RM, Stober W: The effector in human peripheral blood mediating mitogen induced cellular cytotoxicity and antibody dependent cellular cytotoxicity. J Immunol 117:1472–1481, 1976.
111. MacDonald HR, Bonnard GD, Sordat B, Zawodruk SA: Antibody dependent cell mediated cytotoxicity: Heterogeneity of effector cells in human peripheral blood. Scand J Immunol 4:487–497, 1975.
112. Moller G, Suehag S: Specificity of lymphocyte-mediated cytotoxicity induced by in vitro antibody-coated target cells. Cell Immunol 4:1–19, 1972.
113. Catalona WJ, Ratliff TL, McCool RE: Discordance among cell-mediated cytolytic mechanisms in cancerous patients: Importance of the assay system. J Immunol 122:1009–1014, 1979.
114. Ting A, Terasaki PI: Depressed lymphocyte mediated killing of sensitized targets in cancer patients. Cancer Res 34:2694–2698, 1974.
115. McCredie JA, MacDonald HR: Antibody dependent cellular cytotoxicity in cancer patients: Lack of prognostic value. Br J Cancer 41:880–885, 1980.
116. Waller CA, Gill PG, MacLennon IC: Enhancement of lymphocyte mediated cytotoxicity after tumor resection in patients with colorectal cancer. JNCI 65:223–230, 1980.
117. Eremin O, Ashby J, Franks D: Killer (K) cell activity in human normal lymph node, regional tumor lymph node, and inflammatory lymph node. Int Arch Allergy Appl Immunol 54:210–220, 1977.
118. Smith JB, Knowlton RP, Koons LS: Normal antibody dependent killer cell function in patients with chronic lymphocytic leukemia. JNCI 58:1497–1499, 1977.
119. Kohl S, Pickering LK, Sullivan MP, Walter DL: Impaired monocyte-macrophage cytotoxicity in patients with Hodgkins disease. Clin Immunol Immunopathol 15:577–585, 1980.
120. Gupta S, Fernandes G: Spontaneous and antibody dependent cellular cytotoxicity by lymphocyte subpopulations in peripheral blood and spleen from adult untreated patients with Hodgkins disease. Clin Exp Immunol 45:205–214, 1981.
121. Pehamberger H, Ludwig H, Potzi P, Knapp W: Increased monocyte mediated ADCC in Hodgkin disease. Br J Cancer 41:778–781, 1980.
122. deVries JE, Cornain S, Rumke P: Cytotoxicity of non-T versus T lymphocytes from melanoma patients and healthy donors on short and long term cultured melanoma cells. Int J Cancer 14:427–434, 1974.
123. Levin AC, Massey RJ, Deinhardt F, Schauf V, Wolter Jb: Use of a ^{51}Cr release microcytotoxicity assay for the study of lymphocytotoxicity and inhibitors of lymphocytotoxicity in human breast cancer. In: Neoplasm immunity: Theory and application, Crispen RG (ed). Chicago: ITR, 1975, pp 107–126.
124. Peter HH, Kalden JR, Seeland P, Diehl V, Eckert G: Humoral and cellular immune reaction 'in vitro' against allogeneic and autologous human melanoma cells. Clin Exp Immunol 20:193–207, 1975.
125. Hersey P, Edwards A, Edwards J, Adams AE, Milton GW, Nelson DS: Specificity of cell mediated cytotoxicity against human melanoma lines: Evidence for non-specific killing by activated T cells. Int J Cancer 16:173–183, 1975.
126. Merrill JE, Ullberg M, Jondal Mb: Influence of IgG and IgM receptor triggering on human natural killer cell cytotoxicity measured on the level of the single effector cell. Eur J Immunol 11:536–541, 1981.

127. Golightly MG, D'Amore P, Golub SH: Studies on cytotoxicity generated in mixed lymphocyte reaction: III. Natural killer-like cytotoxicity mediated by human lymphocytes with receptors for IgM. Cell Immunol 70:219–230, 1982.
128. Golub SH: In situ, *in vitro,* and systemic regulation of NK cytotoxicity. Inb: Fundamental mechanisms in human cancer immunology, Saunders JP, Daniels JC, Serrou B, Rosenfeld C, Denney CB (eds). Amsterdam: Elsevier/North-Holland, 1981, pp 477-497.
129. Takasugi M, Ramseyer A, Takasugi J: Decline of natural non-selective cell-mediated cytotoxicity in patients with tumor progression. Cancer Res 37: 413–418, 1977.
130. Pross HF, Baines MG: Spontaneous human lymphocyte mediated cytotoxicity against tumor target cells: I. The effect of malignant disease. Int. J Cancer 18: 593–604, 1976.
131. Kadish AS, Doyle AT, Steinhaur EH, Ghossein NA: Natural cytotoxicity and interferon production in human cancer: Deficient natural killer activity and normal interferon production in patients with advanced disease. J Immunol 127:1817–1822, 1981.
132. Vose BM, Vanky F, Argrov S, Klein E: Natural cytotoxicity in manb: Activity of lymph node and tumor-infiltrating lymphocytes. Eur J Immunol 7:753–757, 1977.
133. Behelak Y, Banerjee D, Richter M: Immunocompetent cells in patients with malignant disease: I. The lack of naturally occurring killer activity in the unfractionated circulating lymphocytes from patients with chronic lymphocytic leukemia (CLL). Cancer 38:2274–2227, 1976.
134. Eremin O, Ashby J, Stephens JP: Human natural cytotoxicity in the blood and lymphoid organs of healthy donors and patients with malignant disease. Int J Cancer 21:35–41, 1978.
135. Cunningham-Rundles S, Filippa A, Braun DW, Antonelli P, Ashikari H: Natural cytotoxicity of peripheral blood lymphocytes and regional lymph node cells in breast cancer in women. JNCI 67:585–590, 1981.
136. Hersey P, Edwards A, Milton GW, McCarthy WH: Relationship of cell mediated cytotoxicity against melanoma cells to prognosis in melanoma patients. Br J Cancer 37:505–513, 1978.
137. Saijo N, Shimizu E, Irimajiri N, Ozaki A, Kimura K, Takizawa T, Niitani H: Analysis of NK activity and ADCC in healthy volunteers and in patients with primary lung carcinoma and metastatic pulmonary tumors. J Cancer Reg Clin Oncol 102:195–214, 1982.
138. Forbes JT, Greco FA, Oldham RK: Human natural cell mediated cytotoxicity: II. Levels in neoplastic disease. Cancer Immunol Immunother 11:147–153, 1981.
139. Niitsuma M, Golub, SH, Edelstein R, Holmes EC: Lymphoid cells infiltrating human pulmonary tumors: Effect of intralesional BCG injection. JNCI 67:997–1003, 1981.
140. Menon M, Stephani SS: Lymphocyte-mediated natural cytotoxicity in neoplasia. Oncology 35:63–67, 1978.
141. Santoli D, Trichieri G, Zmjiewski CM, Koprowski H: HLA-related control of spontaneous and antibody dependent cell mediated cytotoxic activity in humans. J Immunol 117:765–770, 1976.
142. Pattengale PK, Gidlund M, Nilsson K, Sundstrom C, Sallstrom J, Simonsson B, Wigzell H: Lysis of fresh human B-lymphocyte-derived leukemia cells by interferon-activated natural killer (NK) cells. Int J Cancer 29:1–7, 1982.
143. Hersey P, Hobbs A, Edwards A, McCarthy WH, McGovern VJ: Relationship between natural killer cell activity and histological features of lymphocyte infiltration and partial regression of the primary tumor in melanoma patients. Cancer Res 42:363–368, 1982.
144. Naor D: Suppressor cells: Permitters and promoters of malignancy? Adv Cancer Res 29:45–125, 1979.
145. Broder S, Uchiyama T, Waldmann TA: Neoplasms of immunoregulatory cells. Am J Clin Pathol 72: 724–731, 1979.
146. Hersey P, Wotherspoon J, Reid G, Gunz FW: Hypogammaglobulinaemia associated with

abnormalities of both B and T lymphocytes in patients with chronic lymphatic leukaemia. Clin Exp Immunol 39:698–707, 1980.

147. Kay NE: Abnormal T-cell subpopulation function in CLL: Excessive suppressor (T gamma) and deficient helper (T mu) activity with respect to B-cell proliferation. Blood 57:418–420, 1981.
148. Catovsky D, Lauria F, Matutes E, Foa R, Mantovani V, Tura S, Galton DA: Increase in T gamma lymphocytes in B-cell chronic lymphocytic leukaemia: II. Correlation with clinical stage and findings in B-prolymphocytic leukaemia. Br J Haematol 47:539–544, 1981.
149. Inoshita T, Whiteside TL: Imbalance of T-cell subpopulations does not result in defective helper function in chronic lymphocytic leukemia. JNCI 67: 1277-1282, 1981.
150. Hersh EM, Patt YZ, Murphy SG, Dicke K, Zander A, Adegbite M, Goldman R: Radiosensitive, thymic hormone-sensitive peripheral blood suppressor cell activity in cancer patients. Cancer Res 40:3134-3140, 1980.
151. Moroz C, Kupfer B: Suppressor cell activity of ferratin-bearing lymphocytes in patients with breast cancer. Isr J Med Sci 17: 879–881, 1981.
152. Werkmeister J, McCarthy W, Hersey P: Suppressor cell activity in melanoma patients: I. Relation to tumor growth and immunoglobulin levels in vivo. Int J Cancer 15:1–9, 1981.
153. Schulof RS, Lee BJ, Lacher MJ, Straus DJ, Clarkson BD, Good RA, Gupta S: Concanavalin A-induced suppressor cell activity in Hodgkin disease. Clin Immunol Immunopathol 16:454–462, 1980.
154. Eibl M, Krepler P, Schmidmeier W, Zielinski C, Winterleitner H: Con A-induced suppressor cells in children with acute lymphoblastic leukaemia. Clin Exp Immunol 40:586-592, 1980.
155. Catalona WJ, Ratcliff TL, McCool RE: Concanavalin A-activated suppressor cell activity in peripheral blood lymphocytes of urologic cancer patients. JNCI 65:553–557, 1980.
156. Toge T, Yanagawa E, Nakanishi K, Yamada Y, Niimoto M, Hattori T: Concanavalin A-activated suppressor cell activity in gastric cancer patients. Gan 71:784–789, 1980.
157. Werkmeister J, Phillips G, McCarthy W, Hersey P: Suppressor cell activity in melanoma patients: II. Concanavalin A-induced suppressor cells in relation to tumor growth and suppressor T-cell subsets. Int J Cancer 28:11–15, 1981.
158. Vanhaelen CP, Fisher RI: Increased sensitivity of lymphocytes from patients with Hodgkin disease to concanavalin A-induced suppressor cells. J Immunol 127:1216–1220, 1981.
159. Kadish AS, Tansey FA, Yu GS, Doyle AT, Bloom BR: Interferon as a mediator of human lymphocyte suppression. J Exp Med 151:637–650, 1980.
160. Schulof RS, Garofalo JA, Good RA, Gupta S: Concanavalin A-induced suppressor cell activity for T-cell proliferative responses: Autologous and allogeneic suppression in aging humans. Cell Immunol 56:80–88, 1980.
161. Treves AJ, Horowitz A, Biran S, Brufman G, Fuks Z: Comparison of the response to PHA and suppressor macrophage function in different age groups of cancer patients and healthy controls. Cancer 48:2606–2611, 1981.
162. Fernandez LA, MacSween JM: Generation of suppressor cells by concanavalin A: A new perspective. J Immunol 125:267–269, 1980.
163. Broder S, Humphrey R, Durm M, Blackman M, Meade D, Goldman C, Strober W, Waldmann T: Impaired synthesis of polyclonal (non-paraprotein) immunoglobulins by circulating lymphocytes from patients with multiple myeloma. N Engl J Med 293:887–892, 1975.
164. Goodwin JS, Messner RP, Bankhurst AD, Peake GT, Saiki JH, Williams RC: Prostaglandin-producing suppressor cells in Hodgkins disease. N Engl J Med 297:963–968, 1977.
165. Han T:Role of suppressor cells in depression of T lymphocyte proliferative response in untreated and treated Hodgkins disease. Cancer 45:2102–2108, 1980.

166. Twomey JJ, Laughter AH, Rice L, Ford R: Spectrum of immunodeficiencies with Hodgkins disease. J Clin Invest 66:629–637, 1980.
167. Fisher RI, Vanhaelen C, Bostick F: Increased sensitivity to normal adherent suppressor cells in untreated advanced Hodgkins disease. Blood 57:830–835, 1981.
168. Quan PC, Burtin P: Demonstration of nonspecific suppressor cells in the peripheral lymphocytes of cancer patients. Cancer Res 38:288–296, 1978.
169. Tilden AB, Balch CM: Indomethacin enhancement of immunocompetence in melanoma patients. Surgery 90:77–84, 1981.
170. Han T, Takita H: Indomethacin-mediated enhancement of lymphocyte response to mitogens in healthy subjects and lung cancer patients. Cancer 46:2416–2420, 1980.
171. Braun DP, Harris JE: Relationship of leukocyte numbers, immunoregulatory cell function, and phytohemagglutinin responsiveness in cancer patients. JNCI 67:809–814, 1981.
172. Murray JL, Springle C, Ishmael DR, Lee ET, Longley R, Kollmorgen GM, Nordquist RL: Adherent idomethacin-sensitive suppressor cells in malignant melanoma: Correlation with clinical status. Cancer Immunol Immunother 11:165–172, 1981.
173. Han T, Takita H: Depression of T lymphocyte response by non-T suppressor cells in lung cancer patients: A possible prognostic value of suppressor-cell activity. Cancer 44:2090–2098, 1979.
174. Mavligit GM, Raphael LS, Calvo DB 3d, Wong WL: Indomethacin-induced, monocyte-dependent restoration of local graft-versus-host reaction among cells from cancer patients. JNCI 65:317–320, 1980.
175. Schechter GP, Wahl LM, Oppenheim JJ: Suppressor monocytes in human disease: A review. Adv Exp Med Biol 121B:283–298, 1979.
176. Herr HW: Adherent suppressor cells in the blood of patients with bladder cancer. J Urol 126:457–460, 1981.
177. Spina CA, Dorey F, Vescera C, Brosman S, Fahey JL: Depression of the generation of cell-mediated cytotoxicity by macrophage-like suppressor cells in bladder carcinoma patients. Cancer Res 41:4324–4330, 1981.
178. Herr HW: Suppressor cells in immunodepressed bladder and prostate cancer patients. J Urol 123:635–639, 1980.
179. Zigelboim J, Corey F, Parker NH, Calcatera T, Ward P, Fahey JL: Immunologic evaluation of patients with advanced head and neck cancer receiving weekly chemoimmunotherapy. Cancer 44:117, 1979.
180. Berlinger NT, Good RA: Suppressor cells in healthy relatives of patients with hereditary colon cancer. Cancer 45:1112–1116, 1980.
181. Jerrells TR, Dean JH, Richardson GL, McCoy JL, Herberman RB: Role of suppressor cells in depression of in vitro lymphoproliferative responses of lung cancer and breast cancer patients. JNCI 61:1001–1009, 1978.
182. Uchida A, Hoshino T: Clinical studies on cell-mediated immunity in patients with malignant disease: II. Suppressor cells in patients with cancer. Cancer Immunol Immunother 9:153–158, 1980.
183. Hubbard GW, Wanebo H, Fukuda M, Pace R: Defective suppressor cell activity in cancer patients: A defect in immune regulation. Cancer 47:2177–2184, 1981.
184. Schulof RS, Bochman RS, Garofalo JA, Cirrincione C, Cunningham-Rundles S, Fernandes G, Day NK, Pinsky CM, Incefy GS, Thaler HT, Good RA, Gupta S: Multivariate analysis of T-cell functional defects and circulating serum factors in Hodgkins disease. Cancer 48:964–973, 1981.
185. Bankhurst AD, Kauffman GR, Goodwin JS, Saiki JH, Saiers JH, Williams RC Jr: Suppressor leukocytes in anergic patients with solid tumors. J. Clin Lab Immunol 3:159–163, 1980.

186. Fernandez LA, MacSween JM, Langley GR: Impaired T-cell responses in chronic lymphocytic leukemia: Lack of suppressor cell effect. Clin Immunol Immunopathol 18:168–175, 1981.
187. Catalona WJ, Ratcliff TL, McCool RE: Concanavalin A-inducible suppressor cells in regional lymph nodes of cancer patients. Cancer Res 39:4372–4377, 1979.
188. Herr HW: Suppressor cells in the pelvic lymph nodes regional to bladder cancer. J Surg Oncol 11:289–293, 1979.
189. Gallery CD, Morton DL, Golub SH: Effect of BCG immunotherapy on alloantigen-induced blastogenesis and cytotoxicity of lymph node lymphocytes in patients with melanoma. Surg Forum 30:152–154, 1979.
190. Vose BM, Moore MB: Suppressor cell activity of lymphocytes infiltrating human lung and breast tumours. Int J Cancer 24:579–585, 1979.
191. Uchida A, Micksche M: Concanavalin A-inducible suppressor cells in pleural effusions and peripheral blood of cancer patients. Cancer Immunol Immunother 10:203–210, 1981.
192. Moore M, Vose BM: Extravascular natural cytotoxicity in man: Anti-K562 activity of lymph-node and tumour-infiltrating lymphocytes. Int J Cancer 27:265–272, 1981.

7. Biological Response Modifiers: Current Status and Prospects as Anti-Cancer Agents

RICHARD V. SMALLEY, CEDRIC W. LONG, STEPHEN A. SHERWIN and ROBERT K. OLDHAM

INTRODUCTION

'Biological response modifier' (BRM) is a term recently coined to include the many agents and approaches to the treatment of cancer whose mechanism of action involves modulation of the individual's own biological responses. Although activation of host responses, particularly the host-immune response, has been the goal of therapists for many generations, recent technologic advances have improved our understanding of biological responses making manipulation of them for therapy a practical goal. In particular, tremendous strides have been made in the last three to five years in molecular biology with respect to nucleic acid sequencing and translation, protein sequencing and synthesis, isolation and purification of biological products, mass cell culture, and 'genetic engineering' of eukaryotic genes in bacteria, yeasts and into eukaryotic cells. The advent of these 'new technologies' has led the way to a greater understanding of biologics which may exist in exceedingly minute quantities but have tremendous amplifying powers with respect to their effect on host responses. Historically, 'activity' has been detected in many biological preparations, but the isolation and purification of the active factor has been fraught with many difficulties. The technology is now available for large-scale production of biologics, the isolation and purification of these products, and eventually their production by genetic engineering or by chemical synthesis. Because of these recent advances, biological response modifying therapy is now a reality and our understanding of BRMs will likely be of great value in understanding cancer biology and improving cancer therapeutics in this decade.

The Biological Response Modifiers Program (BRMP) is a comprehensive program of the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), intended to investigate, develop and bring to clinical trials potential therapeutic agents that may alter biological responses important in

R.B. Herberman (ed), Basic and Clinical Tumor Immunology. ISBN-13: 978-1-4613-3875-8
© 1983. Martinus Nijhoff Publishers, Boston.

the biology of cancer growth and metastasis. This program has been described in more detail elsewhere [1]. The program was conceived as a focused approach in the DCT to support further research in BRMs and to rapidly apply these potential leads to the treatment of cancer in man. The classes of agents being investigated in this program include immunoaugmenting, immunomodulating and immunorestorative agents, interferons and interferon inducers, lymphokines, cytokines and growth factors, thymic factors, tumor antigens and modifiers of tumor antigen cell-surface components, antibodies directed against deleterious host substances and cells, antitumor antibodies, antitumor cells, and maturation and differentiation factors. It is recognized that considerable research is underway in each of these areas, but a focused, coordinated approach by the NCI may result in the rapid acquisition of knowledge and a more rapid application of this information to the treatment of cancer.

Since its inception and activation, the program has supported and stimulated a great deal of activity, aimed at defining the role of biological response modification in the treatment of malignant disease. We define and discuss in this chapter, some areas of ongoing activity in the study of biological response modification, in many of which the BRMP has been involved to a greater or lesser degree, discussing the current status of this therapeutic approach, its current role in antitumor therapy and the potential for future development.

ACTIVE SPECIFIC AND NONSPECIFIC IMMUNOTHERAPY

The current era of immunotherapy began with the report in the late 1960's of the clinical efficacy of BCG vaccination in prolonging the duration of response and survival of children with acute lymphatic leukemia [2]. Although these results could not be confirmed, early studies in adult patients with acute myelogenous leukemia also indicated similar benefits from BCG therapy [3-5]. While early reports from other prospectively randomized trials likewise indicated potential benefit, a review of the final reports from approximately 25 such studies performed during the 1970's indicated that nonspecific BCG therapy in patients with acute myelogenous leukemia does not improve the reponse rate and does not increase the duration of response, but does appear to increase survival primarily by prolonging life following the first relapse [6]. The mechanisms of inducing prolonged survival are not known. With perhaps two exceptions, a large number of studies involving the systemic use of BCG and other nonspecific stimulants in patients with a variety of solid tumors have revealed no therapeutic benefit [7]. The exceptions are two controlled, randomized, but as

yet unconfirmed studies, one in patients with non-Hodgkin's lymphoma (NHL) and one in patients with ovarian carcinoma, which have demonstrated improvement in the survival of patients treated with chemoimmunotherapy [8, 9]. Benefit from intralesional BCG therapy, however, has been reproducibly demonstrated in patients with melanoma [10–12]. Approximately 50% of injected lesions will objectively respond and approximately 20% of patients will show an additional simultaneous response in non-injected lesions. These 'remote' responses have been noted in skin and lymph node lesions (most frequently in the region of the injected lesion) in patients with pre-existing delayed hypersensitivity. There has been little evidence indicating these patients have developed systemic tumor immunity by *in vitro* testing and no evidence of visceral disease response reported in these trials.

The chemically-induced hepatocarcinoma of guinea pigs has been extensively studied in an attempt to understand and utilize this form of immunotherapy. Using daily oral nitrosamine, hepatocarcinomas were induced in a number of strain 2 guinea pigs in the late 1960's by Rapp and Zbar [13]. Two of these tumors, line 1 and line 10, have been carried by serial passage *in vivo* in several laboratories in the country. Line 1 is a regressor tumor but following the intradermal or subcutaneous injection of 10^6 line 10 cells, palpable tumors and microscopic nodal invasion develop within 7–14 days. Extensive local invasion follows and the animal dies in about 60 days with massive local tumor, regional node metastases and multiple small pulmonary metastases. I.V. injection of tumor cells (experimental metastases) causes death in 1–2 months from extensive pulmonary metastases. Utilizing vaccines composed of various combinations of BCG organisms, components (extracts) of BCG or other bacteria, live and irradiated tumor cells and mineral oil, Zbar, Rapp and colleagues have, in a series of experiments, induced tumor regression and permanent specific tumor immunity in animals with various stages of disease, providing the original tumor burden is not overwhelming [14–18].

Ribi and his associates have performed a series of studies with the objective of identifying the active ingredients in BCG necessary for the adjuvant effect [19–23]. Following fractionation of the organism and its cell wall, two components, P3 (trehalose dimycolate), a disaccharide esterified with two long-chain fatty acids, and the cell wall skeleton (CWS), composed primarily of polymeric polysaccharide mucopeptide complexes, also esterified with a 90-cabon chain mycolic acid, were identified as essential. Both of these substances act as adjuvants in the induction of delayed hypersensitivity and cell-mediated immunity. When injected together intralesionally, they induce regression of injected lesions and the development of specific systemic tumor immunity. CWS alone will lead to the regression of lesions but its

activity is synergistically enhanced by the addition of P3, which is inactive alone. These agents must be mixed with mineral oil for maximum effect. Phenol extracts of a number of agents including BCG, a variety of salmonella, and *E. coli,* when added to P3 and mineral oil have equivalent activity to CWS+P3 in oil. The active component of these phenol extracts is muramyl dipeptide (MDP). Various combinations of MDP, endotoxin, CWS, and P3 in combination with mineral oil have all proven to be reasonably efficacious. Attempts are now underway to further define and synthesize the ingredients in order to produce a product that will be a pure, reproducibly effective pharmaceutical. Preliminary phase I clinical trials using CWS, P3, and oil combinations have been instituted [24, 25]. While an occasional tumor regression has been seen in patients with melanoma and renal cell carcinoma in these trials, the specific immunologic effect(s) involved is unknown and a great deal has yet to be learned about the effects of these various agents on specific immunological function before the rational manipulation of the host-tumor environment can be initiated.

Hanna and his colleagues have performed a series of studies with the line 10 tumor, identifying a number of factors important to the understanding and use of active specific immunotherapy [26-29]. Using live BCG and irradiated tumor cells, they have investigated a number of factors involved including the source of BCG, the dose of the two agents, the ratio between them, the amount of radiation, and the tumor burden of the animal. Perhaps the most significant aspect of their early studies was the demonstration that persistence of viable tumor cells for several days following vaccination was required for the development of specific tumor immunity. They studied the histologic events occurring post vaccination, demonstrating an initial acute inflammatory response followed, in 4 to 7 days by a chronic inflammatory response. The bacterial components were necessary for the chronic phase and tumor cell viability had to persist into this phase in order to induce systemic tumor-specific immunity. BCG and tumor cells act synergistically, leading to a greater than additive stimulation of inflammatory cells [30-31]. By electron microscopy, the significant role played by the macrophage in the development of tumor-specific immunity in this phase has been identified [32, 33]. Most recently, following the injection of tumor cells i.v., they have demonstrated, both morphologically and by improved survival, the therapeutic effect of intradermal vaccination on pulmonary metastases, i.e. vaccination induces morphologic dissociation and dissolution by a reaction histologically comparable to a delayed hypersensitivity reaction [34]. Significantly, these animals have moderate tumor burdens with macroscopic and microscopic tumor in the lungs *prior* to the initiation of therapy, rejecting the dogma that immunotherapy can *only* work with 10^6 cells or less. Further, the beneficial effect of cytotoxic therapy (cyclophos-

phamide) is enhanced by preceeding its use with a series of vaccinations. Presumably, the vaccinations induce a breakdown of anatomic barriers, allowing access of the cytotoxic agent to the tumor cells [35]. This approach, vaccination *followed* by cytotoxic chemotherapy, is now being tested in the clinic following colon resection in patients with Duke's B, C, and D lesions. Other studies utilizing the line 1 and line 10 system have also demonstrated a nonspecific effect of the immune response on the anatomical structure of the tumor leading to tumor regression and specific tumor immunity [36]. The ability of the inflammatory system to break into anatomic barriers, providing access to tumor cells for potential therapeutic approaches, warrants further study and exploration.

Other studies, using murine syngeneic tumor systems, have demonstrated the induction of tumor specific immunity, mediated by a population of cytolytic T cells, following vaccination with a tumor cell-*C. parvum* mixture [37]. The effect of tumor-specific immunosuppression associated with an increasing tumor cell burden was also demonstrated in this system. It would appear quite likely that with further studies and further dissection of the effects of non-specific augmentation and with the identification of specific bacterial and/or tumor components which have either immunoenhancing or immunosuppressing capabilities, rationale for the use of these materials may be developed.

INTERFERON

The interferons are a family of glycoproteins produced by a number of cells of different origin. Type 1 alpha and beta interferons, produced respectively by induced leukocytes and fibroblasts, show about 30% homology of aminoacids [38]. Type 2 gamma interferon, produced by lymphocytes in response to antigenic or mitogenic stimulation, differs significantly in amino acid composition [39]. The interferons were initially thought to have only antiviral activity but multiple other functions, previously thought related to impurities in the preparations, have now been documented as effects of interferon itself [40]. Documentation of these multiple functions as definitive interferon effects is now possible as highly purified products become available for study and for use in the clinic. In addition to antiviral activity, the interferons have profound effects on the immune system. Relatively, low doses will enhance antibody formation and lymphocyte blastogenesis while higher doses will inhibit both of these functions and inhibit delayed hypersensitivity while enhancing macrophage phagocytosis and cytotoxicity, sensitized lymphocyte cytotoxicity, NK activity, and surface antigen expression [41]. Interferons also prolong and inhibit cell division, having this effect on almost every cell system studied, whether transformed or not [42].

In addition, interferons result in the induction of several intracellular enzyme systems with resultant profound effect on macromolecular activities and protein synthesis [42]. All of these functions have been documented in murine systems but a thorough dose-response effect for many of the above cellular activities has not been thoroughly investigated in either mouse or man. Most of the work to date has been done with alpha interferons. It would appear that beta and gamma may have similar effects on many systems, but relative potency may vary.

Animal tumor models

In a series of experiments, beginning with studies on the effects of interferons on oncogenic virus multiplication, a significant antitumor effect of interferon in many animal models has been demonstrated [43]. When administered prior and immediately subsequent to the inoculation of several oncogenic viruses, including the Friend and Rauscher viruses, interferon can both delay the onset and decrease the incidence of subsequent tumors [44, 45]. Additional studies involving the Friend leukemia virus led to the observation that interferon can effectively inhibit the growth of transplantable tumors and subsequent studies have demonstrated a significant slowing of growth in the Lewis lung and the B_{16} and Cloudman melanoma models [46–48]. In the Lewis lung system, the incidence of pulmonary metastases from subcutaneous implants is decreased. Additionally, interferon administered prophylactically to mice genetically susceptable to the spontaneous development of either lymphoma or mammary carcinoma, will both decrease the incidence and prolong overall survival [49, 50]. It will also prolong survival in the AKR lymphoma model if begun at the time of diagnosis. In this system it is as effective in prolonging survival as any of the cytotoxic chemotherapeutic agents [51]. In only one confirmed instance, however, has interferon been demonstrated to lead to an objective regression in palpable or objectively measurable tumors [52]. Thus, these murine systems suggest that interferon (primarily alpha has been studied) will inhibit or slow the growth of palpable tumors and delay or prevent the onset of clinically overt tumors or metastases. The effect of tumor burden on the efficacy of interferon is significant in that interferon is less effective with larger tumor burdens.

One mechanism of anti-tumor action is almost certainly interferon's antiproliferative effect. Interferon prolongs the cell cycle and inhibits cell multiplication by increasing various phases (differing in different systems) of the cell cycle [41]. The second mechanism of anti-tumor action may be through the profound effects that the interferons have on the immune system. It was demonstrated early in the 1970's by Gresser and his colleagues, that L1210 cells, resistant to interferon's antiproliferative effect *in vitro*, were inhibited

from growing *in vivo*, indicating the presence of an intermediary or host effector cell mechanism [53]. This led to the subsequent demonstration of a profound effect on various components of the immune system and on cell membrane antigenicity. Thus, when administered in the proper dose and schedule, interferons will both increase the expression of surface membrane antigenicity and enhance the effect of various components of the host immune system, providing the potential for cytolysis and tumor regression [54]. While such a cytolytic effect has not been demonstrated in animals with relatively large established burdens, it is presumably the mechanism involved in the prevention of the development of overt pulmonary metastases from a transplanted subcutaneous primary in the Lewis lung carcinoma system. The specific effector cells or components of the immune system responsible have not been identified. It is quite probably that there may be a significant dose-response effect on many of these various functions. It will be of import and interest to dissect out these various possibilities in one or more of the murine systems. Such attempts are underway currently in a variety of human tumor situations.

Human trials

A number of trials during the 1970's using crude alpha interferon preparations obtained from the buffy coats of blood donated to the Red Cross demonstrated an antitumor effect in a variety of human tumors. These studies, performed initially in Scandinavia and subsequently also in the United States, the latter under sponsorship by the American Cancer Society (ACS), utilized a process of obtaining interferon developed by Cantell. Phase II trials in patients with either multiple myeloma or ovarian carcinoma, using an empirically derived dose of between 3 and 9 million units i.m. daily, demonstrated a relatively high degree of antitumor effect in myeloma and a relatively low effect in ovarian carcinoma [55, 56]. An antitumor effect was demonstrable in over 50% of patients with myeloma manifested by both a decrease in myeloma protein and in tumor cell burden in the marrow. This degree of effectiveness has subsequently been confirmed [57]. The incidence of antitumor effect in patients with far advanced ovarian carcinoma has been noted to be significantly less. In two series, two responses were noted out of 20 patients treated [56, 58]. In a non-randomized trial involving a small number of patients evaluating interferon as adjuvant therapy following surgery for osteogenic sarcoma, prolongation of survival and delay of recurrence have been demonstrated [59]. Although a potentially important lead, this trial can not be utilized as evidence for the adjuvant effectiveness of interferon in patients with osteosarcoma because of the small number of patients and lack of randomized controls. It will be important to evaluate the effect of interferon in this disease in a large controlled adjuvant osteo-

sarcoma trial. The initial report of an antitumor effect in previously heavily treated patients with metastatic breast carcinoma has been recently confirmed in patients having received no prior chemotherapy [58, 60]. In this latter ACS sponsored study, previously untreated patients with metastatic breast cancer were treated with 3 to 9 million units of Cantell alpha interferon daily i.m. [60]. Objective tumor regressions were primarily seen in patients with local recurrence but an occasional regression was also noted in patients with visceral metastases. The initial reports by Merigan et al. demonstrating a tumoricidal effect of Cantell alpha interferon in patients with non-Hodgkin's lymphoma, primarily those with nodular poorly differentiated lymphoma, have also been confirmed in subsequent studies [58, 61, 62]. Thus, it would appear that this very impure product, obtained from virally stimulated buffy coat cells and administered in doses of 3 to 9 million units i.m. daily, will induce regressions in patients with B cell tumors, breast carcinoma, and rarely in ovarian carcinoma. The mechanism of action for its effect is not clear.

Attempts to develop a more highly purified and less costly product have led to the production of an extracted material harvested from lymphoblastoid cells grown in culture and of products obtained from recombinant DNA techniques. The Namalwa cell line, originally obtained from a child with Burkitt's lymphoma and containing the Epstein virus genome, produces an alpha-beta interferon mixture when stimulated with the Sendai virus. The supernatant material obtained from these stimulated cultures, following several purification steps, yields a highly purified (85%) alpha interferon pharmaceutical product. When assayed and compared to a reference standard, 1 to 2×10^8 units of antiviral activity are demonstrable per milligram of protein (compared to 0.1×10^6 units of activity/mg protein for material obtained by the Cantell process) [63, 64]. This product consists of two protein peaks by Sephadex G75 on chromatography, each with four bands of interferon activity by SDS polyacrylamide gel electropheresis. Molecular weights range from 17,500 to 22,000. Since at least 12 molecular species have been demonstrated in alpha interferon, this lymphoblastoid material contains over half of the known species. The relative importance of these various species, singly or in combination, in inducing various interferon effects on cellular systems or tumor cells has not been clarified.

Using recombinant DNA techniques some of these molecular species, as well as various hybrids, have been cloned and significant amounts of various molecular species of alpha interferon produced [65, 66]. Studies defining their effects are underway. One of these recombinant molecular species, alpha-2, has been formulated into a pharmaceutical preparation of greater than 95% by two pharmaceutical companies and is undergoing extensive phase I and phase II trials.

The BRMP interferon program

The BRMP has devoted a large amount of its initial time, effort, and resources to the development and study of the interferons [1]. Phase I trials, to obtain both classic phase I data on the maximum tolerated dose (MTD) and the maximum biologically response modifying dose (MBRMD), were initiated. Patients were observed for tumor response and toxicity during these studies. Six trials, three evaluating two different products obtained by the Cantell process and three, evaluating the relatively pure lymphoblastoid (Namalwa cell) product, have been performed.

The three trials involving the two products obtained by the Cantell process evaluated three different dose schedules; a single dose administered i.m., in which dose escalations were performed in different groups of patients [67, 68] a dose administered i.m. daily for 28 days [69], again in which dose escalations were performed in separate groups of patients and a daily dose administered indefinitely until either significant toxicity or tumor progression occurred [70]. Escalations were performed in this latter study in each patient every three weeks. In the two studies utilizing the daily i.m. dose, an MTD of between 18 to 24 million units per m^2 was established. Fever and chills occurred in nearly all patients at all dose levels but were tolerable. Some degree of tachyphylaxis was noted. The dose-limiting toxicity in each study was severe fatigue, anorexia, and weight loss and patients ultimately refused to continue the agent. A mild degree of both hepatic toxicity, manifested by subclinical elevations of SGOT, and myelosuppression manifested by subclinical depression of neutrophil and lymphocyte counts occurred. The study evaluating a single i.m. dose noted that doses as high as 60 million units/m^2 were tolerated without significant difficulty other than mild fatigue, and anorexia. Fever and chills were noted but in no instance was this intolerable. Doses higher than 60 million units per m^2 were difficult because of formulation difficulties.

Regarding the MBRMD, data is currently incomplete but several trends are apparent. A single i.m. dose of 60 million units/m^2 demonstrated a consistent decrease in NK and ADCC activity at 24 hours followed by an increase at 48 hours and a return to baseline at 72 hours. When a dose of 60 million units/m^2 was administered weekly for 5 weeks, a decrease of cytotoxic activity over time was apparent [71]. In the two studies in which the Cantell alpha interferon was administered daily i.m., a decrease in NK activity over time with increasing dose was also observed. No effect on lymphocyte subpopulation numbers was observed at doses between 1 and 50 million units daily i.m., but an increase in the expression of beta-2 microglobulin surface antigen was observed with an increasing expression seen at the higher doses through 36 million units i.m. daily [69]. Data on monocyte function are still incomplete.

No objective partial or complete tumor responses were seen in any of these three studies. A total of approximately 80 patients, all of whom were in reasonably good physical shape and had been previously treated with cytotoxic agents, were entered in these three trials. Minor responses, i.e., objective tumor shrinkage although not greater than 50%, were seen in three patients with breast carcinoma, melanoma and NHL (DPDL). The responses noted in patients with breast carcinoma were observed in those with cutaneous disease. Both responses observed in patients with renal cell carcinoma occurred in pulmonary nodules.

In summary, this series of studies has established that the MTD for these Cantell alpha interferons, administered daily i.m., is between 18 and 24 million units per m^2 per day, the dose-limiting toxicity is fatigue and weight loss, single doses as high as 60 million units/m^2 will decrease NK and ADCC activity at 24 hours, followed by a peak at 48 to 72 hours, and cumulatively administered weekly doses will again lead to a decrease in both NK and ADCC activity over 3 to 4 weeks. No consistently demonstrable immune effect was observed at lower doses. Surface antigenic determinants may be increased by increasing doses while no significant quantitative effect on lymphocyte subpopulations was noted. Minor tumor responses were observed.

Three Phase I studies were performed using the relatively pure product obtained from the lymphoblastoid cell line. One study utilized a two times a day dose scheme, administering the agent on a per-square-meter basis i.m. for 7 days [72]. Escalations were performed in separate groups of patients. A second schedule administered the agent in a single dose following which functional and pharmacokinetic studies were done over a 14 day period [73, 74]. This was then followed by a three times a week schedule for 5 weeks. Escalations again were in different groups of patients. The third study administered the agent intravenously, daily for 5 days using a 6-hour infusion [75]. In this study, weekly escalations of 0.1, 1, 10, 30, 50 million units (Mu) were performed in each patient over a 5-week period. An MTD of 30 Mu/m^2 two times daily (bid) for 7 days, of 30–50 Mu (total dose) daily i.v. for 5 days (following cumulative increases) and 15 Mu/m^2 t.i.w. for 5 weeks were established. As with the Cantell product the subjective criteria of fatigue, anorexia, and weight loss were dose-limiting factors. At dose levels of 30 to 50 Mu, objective toxicities of thrombocytopenia, leukopenia, and increased SGOT levels were noted and also became dose limiting. There was one possible therapy related death in a patient receiving 50 megaunits/m^2 b.i.d. in which GI bleeding developed in association with thrombocytopenia [72]. Although the bleeding was controlled with transfusions of platelets and red cells, the patient died suddenly in her sleep 48 hours subsequent to the discontinuation of interferon and the control of GI

bleeding. Seventy-eight patients were entered on these three trials, 40 on the i.v. trial, 32 on the b.i.d. schedule, and 17 on the t.i.w. trial.

Biologic effects on NK activity, lymphocyte counts, monocyte activity, and bone marrow function were all investigated. In the study in which a single dose was administered followed by a 2-week rest, an increase in NK activity was seen at the end of the first week of treatment at essentially all doses (1 to 15 Mu/m^2) [76]. Initially, 12 to 24 hours following the dose, a decrease in NK activity occurred followed by rebound 72 hours post treatment. This increase was then sustained for 2 to 3 days. Cumulative higher doses, administered on a t.i.w. schedule induced decreased levels of activity. No effect on a variety of monocyte functions was noted but neither cytotoxicity nor cytostatic functions were evaluated by these investigators. Studies involving blood counts and marrow function both *in vivo* and *in vitro* indicate that increasing single doses of the agent decrease polymorphonuclear counts *in vivo* and have an inhibiting effect *in vitro* on both granulopoiesis and erythropoiesis. *In vitro* data from both the IV and the b.i.d. studies are currently incomplete.

Objective partial responses were noted in one patient with melanoma, one patient with renal cell carcinoma, two patients with NHL (NPDL, DH), one patient with Hodgkin's disease and one patient with undifferentiated carcinoma. Of interest is the fact that approximately 15 patients with breast carcinoma were studied and no responses were seen. No dose-response effect correlating with objective response could be discerned.

In summary, it would appear that a maximally tolerable dose of approximately 30 Mu/m^2 administered daily i.v. or b.i.d. i.m. over a short period of time (5–7 days) and a dose of 15 Mu/m^2 administered three times a week over a longer period of time are the maximal clinically tolerable doses. Further, data would suggest that cumulative higher doses of 15–30 Mu/m^2 will decrease NK activity and bone marrow function, i.e., cell proliferation. Infrequently administered lower doses tend to increase NK activity and have virtually no effect on marrow function, i.e., cell proliferation, either *in vivo* or *in vitro*. Thus, it appears possible that a dose-response effect *vis-à-vis* tumor response and the mechanism responsible for such a response can be dissected out in phase II studies. Such studies, in which both high doses, in the range of 15–30 Mu/m^2 administered frequently for several weeks and low doses, 0.5 to 3 Mu/m^2 administered infrequently over the same period of time to patients with potentially responsive tumors are underway under sponsorship by the BRMP in conjunction with Burroughs-Wellcome. These studies, initiated in the fall of 1982, should provide interesting data within the next 12 to 18 months.

Recombinant interferons

Materials containing single molecular species of alpha interferon have been produced by recombinant genetic techniques. These are non-glycosylated protein molecules which, by definition are highly purified (98%) and have approximately $2-4 \times 10^8$ units of interferon activity per milligram of protein [65]. It will be of interest to compare the clinical, biological and toxic effects of these single molecular species to the relatively pure lymphoblastoid product, containing eight molecular species, and the relatively impure Cantell products, presumably containing all molecular species. It is, of course, quite possible and conceivable that different toxic, biological and therapeutic effects reside in these different molecular species. The pharmaceutically available alpha-2 product has been studied in a large number of phase I and phase II trials supported by each of two pharmaceutical houses [77-80]. These two recombinant products differ from each other by only 1 amino acid. Completed phase I trials indicate that toxicity is similar to both the Cantell and lymphoblastoid products in that fever, chills, anorexia, and weight loss are the dominant dose-limiting toxicities. However, doses exceeding 100 Mu have been administered before dose-limiting toxicities are seen [77, 78]. This has been true for either the single dose or cumulative dose schedules. Transaminase increases and marrow suppression are also noted at doses around 130-150 Mu. Phase I trials have utilized both i.m. and i.v. dose schedules. Responses have been seen primarily in patients with myeloma and nodular poorly differentiated lymphoma. Other scattered responses have been seen in a variety of other tumors. In the study performed by the BRMP intramural program, all dose levels were associated with an increased ability of patients' monocytes to inhibit cell growth [81]. Over 85% of analyses, at all dose levels, have demonstrated an increase in monocyte cytostatic activity. Coincidentally, increasing doses of the agent have led to decreased NK activity in about 30% of patients with no change demonstrable in the other 70%. Thus, this study would suggest that increasing doses may have a dual inhibitory effect on cell proliferation, directly as demonstrated by marrow suppression and indirectly through monocyte activity. More studies, evaluating correlations between antitumor responses, monocyte cytotoxicity and/or cytostasis, NK activity and antiproliferative effects are needed.

A large number of phase II trials are underway with these two agents, but the data at the present time is unavailable. One completed phase II trial, in patients with metastatic breast carcinoma, previously heavily treated, has revealed no responses in 19 patients [82]. At least half of these patients had cutaneous and osseous lesions without visceral involvement. There has been only one objective response to the recombinant products of 43 patients with breast cancer entered on phase I or II trials [77, 78, 80, 82]. The lack of responses in patients with breast cancer receiving either the lymphoblastoid

or recombinant products is of interest in light of the earlier reports demonstrating responses with Cantell material. The explanation for this may lie either in the relative impurity of the Cantell material or in the evaluation and selection of patients.

The three alpha interferon products available for clinical trials – the impure Cantell, the relatively pure lymphoblastoid, and the highly pure recombinant – have all demonstrated objective tumor responses in patients with a variety of lymphomas and solid tumors. They have demonstrated similar clinical toxicities, albeit at different dose levels and have all demonstrated biological effect. There is a strong suggestion that higher and cumulative doses of these products will decrease NK activity, will have cell proliferation inhibitory activity and may increase monocyte cytostatic activity. Further analyses of both phase I and phase II data using both high and low dose levels, should provide insight regarding the relationship between a specific antitumor effect and an associated biological effect.

Beta and gamma interferon

These two interferons have been unavailable in large enough quantities for extensive clinical testing. A very few trials have been performed using a beta interferon developed at Roswell Park. A phase I trial, sponsored by the ACS, has established an MTD of 10 Mu/m^2 [83] and a very few phase II trials, also sponsored by the ACS, are currently being conducted. Attempts to produce this material on a large scale have been fraught with technical problems and BRMP-sponsored trials evaluating beta interferon await continued efforts to develop this agent in greater quantities. It is quite problable that the agent may be available for limited phase I and phase II trials shortly. Gamma interferon is soon to become available in large quantities. An extracted material is currently undergoing a phase I trial at two centers [84, 85]. Phase I and Phase II trials using recombinant beta and gamma products should be underway in 1983. Trials with these agents will be of interest to determine whether quantitative differences in biological and cell inhibitory effects, as suggested from animal studies, are observed.

INTERFERON INDUCERS

In an effort to overcome the significant problems of cost and limited availability of clinically usable interferon, an effort was mounted to define, isolate, and purify a number of interferon inducers. Several different agents capable of inducing interferon have been identified but by far the most effective nonviral interferon inducers have been the double-stranded ribonucleic acids [86]. Polyriboinosinic:polyribocytidylic acid (Poly I:C) has proven to be the most effective synthetic double-stranded RNA in murine

systems but this agent was found to be almost totally ineffective as an interferon inducer in primates *in vivo* due to the fact that primate serum contains a relatively high concentration of nucleolytic enzymes that hydrolyze and inactivate poly I:C. Levy et al. demonstrated that by initially forming a hydrophilic complex between poly-L-lysine and carboxymethylcellulose and then combining this with poly I:C, a compound Poly ICLC, soluble in saline and partially resistant to hydrolysis by enzymatic action, was formed [87]. This compound is a potent inducer of interferon in primates, achieving levels of up to 2000 units/ml in monkeys and chimpanzees [88]. Poly IC has been evaluated in several animal tumor systems. It will prolong survival in over 15 animal tumor systems but has achieved cures in only one of these [86]. It is much more effective in relatively slow-growing solid tumors such as sarcomas than it is in the rapidly growing leukemias. Poly ICLC has not been extensively tested in animal systems.

A number of phase I trials with Poly ICLC have been performed in humans [89-93]. It has been administered as a single daily (for 3 to 14 days) dose schedule, in a three times a week schedule [86], and on a weekly basis [94]. All of these administrations have been by 30 minute intravenous infusions. Toxicity, in doses over 6 mg/m^2, has been significant, with fever, rigors, lymphopenia, nausea, and most significantly hypotension being observed. The agent, at doses of 2-5 mg/m^2 induces some degree of fever, lymphopenia, and elevated transaminase levels, but these side effects are tolerable. At these lower dose levels, peak serum levels of interferon of 15-100 units/ml occur 4-24 hours after the injection. When administered daily for several days, some degree of hyporesponsiveness occurs. The interferon induced appears to be alpha interferon. Antitumor responses seen in these phase I trials have included one objective response (a CR) in a child with acute lymphatic leukemia and minor responses in patients with acute leukemia, renal cell carcinoma, and chronic lymphatic leukemia. In phase II trials, some responses have also been observed in patients with myeloma [91]. In a large-scale phase II trial in children with refractory acute lymphatic leukemia, antileukemic effects were seen, i.e., significant decrease in the peripheral blood and bone marrow leukemic cell population, but objective responses did not occur [95]. A current ongoing phase I trial comparing the biological effects of intramuscular versus intravenous poly ICLC has demonstrated that intramuscular administration will lead to an increase in NK cell activity but without achieving demonstrable serum interferon levels. The agent administered i.m. is relatively well tolerated in doses up to 4 mg/m^2 [96].

The future of the interferon inducers and poly ICLC specifically would seem to be dependent upon the results of the interferon trials. Should the recombinant or lymphoblastoid interferon products prove to be effective,

reasonably well tolerated, not imparied by anti-interferon antibody production, and available at a reasonable cost, it seems probable that there would be little need for an agent such as poly ICLC. In the meantime it would be reasonable to perform phase II studies in potentially responsive tumors (i.e. myeloma, renal cell, melanoma) to evaluate its potential effect.

THYMOSIN

During the past few years, the thymus gland has been recognized as a functioning endocrine organ producing factors which play a dominant role in the maturation of the T cell population and maintenance of immune balance [97]. A number of recent studies have demonstrated both an *in vivo* and *in vitro* immune restorative effect in immunodeficient children [98–101]. Further, two studies have demonstrated both an *in vitro* and *in vivo* stimulating effect of thymosin on T cell function in patients with malignancy [102–104]. The latter demonstrated an increase in skin test reactivity and in the number of E rosette positive cells in patients with far advanced malignancy and depressed T cell function, following a weekly injection of thymosin fraction 5 [103]. Additionally, at least two prospectively randomized clinical studies in patients with malignancy have demonstrated an improved disease-free interval and survival [105, 106]. In one, patients with small cell carcinoma of the lung, receiving thymosin (fraction 5) in addition to chemotherapy, had an improved survival [105], while in the second, patients receiving thymosin (fraction 5) in addition to irradiation for squamous cell head and neck cancer had a prolonged disease-free interval [106]. An attempt to confirm and reproduce the results obtained in the small cell carcinoma of the lung study was unsuccessful, however. In a prospectively randomized trial involving patients with limited or extensive disease and treated with cytotoxic chemotherapy, no benefit was demonstrated in terms of response, duration of response or survival in those patients receiving thymosin [107]. In the cyclophosphamide-sensitive plasma cell tumor in mice, the addition of low doses of thymosin fraction 5 one week following a dose of cyclophosphamide demonstrated significantly improved survival when compared with cyclophosphamide administered alone or cyclophosphamide plus thymosin administered in higher doses [108].

Two thymosin products are available for clinical study. Fraction 5, the material used in all the above cited studies, is extracted from the thymus gland of fetal calves and contains several polypeptides with molecular weights ranging from 1 to 15 thousand [109]. It is conceivable that both stimulatory and suppressive functions may reside in this mixture [108]. More recently, several pure fractions have been isolated and synthesized.

One of these, alpha-1, a polypeptide with lymphokine function several times more potent than fraction 5 is available as a pharmaceutical preparation [109]. Six BRMP-sponsored phase I trials, four evaluating fraction 5 and two evaluating alpha-1, have been performed. Fraction 5 was evaluated as a single dose [110], on a t.i.w. schedule administered for two weeks [111], on a daily for 5 day schedule repeated weekly for 3 weeks [112], and in a daily for seven day schedule repeated monthly [113]. All administrations were either subcutaneous or intramuscular. Doses were escalated from 0.6 to 960 mg/m^2, evaluating several dose levels in between. These studies have revealed this material to be non-toxic within this dose range with the exception of a 10–15% incidence of allergic reactions when administered daily for 7 days every 4 weeks. All of these allergic reactions occurred in patients receiving either 480 or 960 mg/m^2, two were of the anaphylactoid type, and all six responded to appropriate therapy. Formulation difficulties prevented administering doses higher than 960 mg/m^2. Pharmaokinetic data revealed a peak alpha-1 level 4 hours following administration. Serum levels up to 12 ng/ml were noted, levels correlating with dose. Only doses above 60 mg/m^2 produced detectable serum levels. Biological studies evaluating quantitative T cell subsets, using both E-rosette and T cell differentiation antigen techniques revealed no changes in total lymphocyte, T cell levels or helper-suppressor cell ratios. Ia antigen determination, blastogenesis response (both MLC and mitogen stimulation) and delayed hypersensitivity (skin testing) likewise revealed little immunostimulatory or biologically response modifying effect. Antitumor responses (one a complete response) were seen in three patients with renal cell carcinoma [112]. All had pulmonary metastases and all received either 60 or 150 mg/m^2 daily for 5 days, repeated weekly for 3 weeks.

Thus, thymosin fraction 5 is a relatively nontoxic agent although allergic phenomena may occur. No lymphocyte immunomodulatory capabilities were demonstrated in patients with widespread malignancy, all of whom had demonstrably depressed function prior to therapy. Its effect as an antitumor agent, either direct or indirect, in patients with clinically overt tumor burden appears to be nil in breast carcinoma (16 patients), lung carcinoma (33 patients) and melanoma (15 patients) although a variety of doses were used in this Phase I study. In patients with pulmonary metastases from renal cell carcinoma objective responses (mechanism unknown) may be anticipated.

Thymosin alpha-1 has been evaluated as a single dose i.m. [119] and on a daily for 7 day schedule every 4 weeks [113]. Doses ranged from 0.6 mg/m^2 to 9.6 mg/m^2. The agent is nontoxic at these dose levels. Again, higher doses could not be administered because of formulation problems. A biological response, evaluating the same T cell functions as in the studies with fraction

5, was not noted. Therapeutically, no responses were seen in over 40 patients with overt tumor burdens. An additional study is evaluating the adjuvant use of alpha-1 in patients following radiation therapy for Stage I–III non-oat cell lung cancer [114]. In this ongoing study in which two dose schedules – a twice a week administration and a daily dose for 14 days followed by twice a week maintenance, (all patients receiving 0.9 mg/m^2 per dose) are compared with a placebo, there is a suggestion that patients receiving alpha-1 have a prolonged disease-free interval. The data are preliminary, the numbers of patients small, and the follow-up time very short. However, based on the animal model study, therapeutic benefits may only be seen in patients with a low tumor burden. Additional evaluation in a larger study is warranted.

These phase I studies have not been able to confirm the previously reported immunostimulatory capabilities of thymosin fraction 5 in patients with overt malignancy and depressed immune function. They have also not been able to demonstrate any immunorestorative or stimulatory function for the alpha-1 fraction. Therapeutic benefits have been noted, however, and phase II or phase III trials, evaluating these agents in selected patients with limited tumor burdens, are indicated.

MONOCLONAL ANTIBODY

Previously, multiple trials using xenogeneic antitumor immune globulin have suggested a potential for therapeutic benefits for passive serotherapy [115]. The problems of obtaining large quantities of a relatively pure antitumor antibody, however, have significantly dampened enthusiasm for this approach. The development of the hybridoma technique for production of monoclonal antibody resulting in widely available murine monoclonal antibodies reactive to a large number of tumor-associated and differentiation antigens and the real potential of human-human hybridomas in the near future providing human monoclonal antibodies has generated significant interest in this field.

At least three transplanted murine models, the AKR spontaneous lymphoma, which transplants as a subcutaneous tumor followed by a leukemic phase, the spontaneous A strain leukemia and the chemically induced lymphoma, have been used to evaluate the therapeutic use of murine monoclonal antibody [116–118]. In each of these systems, an antibody against one of the thymus differentiation antigens was used. In the study using the AKR system, both tumor and host were identical antigenically while in the other two models, they differed by one allele. Both studies demonstrated similar principles: (1) that antibody therapy did have a definite antitumor

effect; (2) that there was a dose response demonstrable with a plateau effect being reached; (3) that the IgG_2 subclass of antibodies was more effective than either other IgG subclasses or IgM antibody; and (4) that antibody, in order to be effective, had to be given relatively soon after the initial transplantation of the tumor. Specifically, a dose of tumor cells two logs higher than the 50% minimal lethal dose was administered and antibody therapy, to be effective (both cures and prolonged survival), had to be administered within 48 hours of tumor cell injection. Thus, these studies demonstrated that a pure, high titer, IgG_2 allogeneic antibody, given at a time when the tumor burden is relatively small, can have a significant antitumor effect and will cure a significant number of animals.

A number of preliminary studies in humans using a monoclonal antibody produced against T cell differentiation antigens have been performed. Similar antibodies, all produced in different laboratories but each recognizing a mature T cell differentiation antigen with a molecular weight of 65,000, have been produced [119–122]. T101, developed by Royston et al., and L17F12, developed by Levy et al., both IgG_2 antibodies, have each been administered to a small number of patients with either adult T cell ALL or one of the varieties of cutaneous T cell lymphoma [123–125]. These studies have revealed that murine antibodies, when administered in a dose under 20 mg and by a relatively slow i.v. infusion, i.e., 60 to 120 minutes, do not induce significant acute toxicity and will induce an immediate, albeit very brief, antitumor effect. Antibody therapy leads to a rapid decrease in circulating leukemic cells (within 1–2 hours) followed by a rapid rebound to pretreatment levels within 24 hours. It has also been demonstrated that this antigen will modulate, that patients can tolerate multiple doses without significant toxicity, and that a host anti-mouse immune response may develop (in these immunodeficient patients) although infrequently. Such host antimurine response has not been associated with significant clinical toxicity although it does appear to be associated with decreased antitumor efficacy.

The doses and the schedules used in these early exploratory trials have been rather arbitrarily and empirically chosen. The BRMP is supporting three phase I studies with T101 antibody through a contract mechanism and a fourth phase I study, submitted in response to an RFA, using Leu-1 antibody. An intramural phase I dose escalating study with T101 is also underway. In addition to an organized dose escalation approach which will seek to determine a maximally tolerated dose based on clinical toxicity, these studies will determine the pharmacokinetics of murine antibody, tumor antigen and immune complexes in the circulation, evaluate the kinetics of antigenic modulation, evaluate the development of host anti-murine immunologic response, evaluate the effects and toxicity of multiple doses given in

a variety of schedules, ultimately evaluate the toxicity and the effects of a combination of several different murine anti-T cell antibodies and evaluate the toxicity and effect of these antibodies on normal T cell function.

Studies in other hematologic malignancies are also underway. All B cells carry a unique idiotype immunoglobulin coating their cell surface. Therefore, all B cell malignancies of clonal origin carry a tumor marker, in this instance a tumor-specific antigen, perhaps the only pure example of a tumor-specific antigen [126, 127]. This immunoglobulin, exposing the variable region of its F(ab) components, becomes an excellent target for tumor specific monoclonal antibody therapy. This unique idiotype is present on every malignant lymphoma cell and for all practical purposes, is not present on any other cell within that individual. Several investigators have exploited this fact, using polyclonal antibody in therapeutic studies in animal models. With the L2C (a B cell malignancy) guinea pig model, Stevenson et al. developed techniques for producing xenogeneic polyclonal anti-idiotype tumor specific antibody developed against the tumor specific idiotype [128]. They subsequently used these techniques for producing antibodies to CLL cells in man [129]. Recently, Lanier et al., using the CH1 and CH2 B cell lymphoma which developed spontaneously following antigenic stimulation, demonstrated significant antitumor effect (increased survival and cures) using a xenogeneic polyclonal anti-idiotype antibody raised in rabbits [130]. The following principles were demonstrated: (1) an IgG molecule (subclass unknown) was the effective isotype; (2) a dose response effect is demonstrable but in this instance increasing the dose leads to the disappearance of therapeutic effect; (3) antibody therapy must be given with a relatively low tumor burden to be effective.

In a human study parallelling these animal models, Miller and Levy produced a murine monoclonal anti-idiotype antibody reactive against the tumor cells of a patient with a poorly differentiated diffuse lymphoma [131]. This patient, treated with several doses of antibody, entered a complete remission which has continued unmaintained for over 12 months. There are significant technical problems inherent in the development and production of anti-idiotype monoclonal antibodies. Since it is both costly and time consuming [132], every patient with a B cell lymphoma will not be able to benefit from this approach at the present time. With further experience, improvements in the technological approach can be anticipated.

There is significantly less experience with monoclonal antibody in solid tumors of either animals or humans. No detailed effective studies have been done in any animal system although preliminary studies have been performed in the line 10 hepatocarcinoma guinea pig model [133]. Koprowski et al., at the Wistar Institute, have developed several monoclonal antibodies reactive against human melanoma and colon tumor cells [134–136]. They

have demonstrated an antitumor effect with these antibodies *in vitro* using both an ADCC monocyte effector cell system and the clonogenic assay system. They have also demonstrated an antitumor effect *in vivo* using tumor transplants in nude mice and *ex vivo* using an explant system in which tumor and blood supply are removed en block and treated with monoclonal antibody administrated by pump [137]. Therapeutically effective antibodies using all three assays (ADCC, clonogenic assay and nude mice systems) belong to the IgG_{2a} class. They have demonstrated the need for presence of effector cells with Fc receptors (presumably macrophages) and an intact immunoglobulin molecule for effect in either the *in vitro* or *in vivo* systems [138]. This corroborates previously reported work with polyclonal antibody which indicated that both macrophages and IgG_{2a} class antibody were needed for demonstrable antitumor effect [139]. It also supports the effectiveness of IgG_2 antibodies demonstrated in the various animal model systems. Preliminary experience with the *in vivo* intravenous administration of 17-1A, an IgG_{2a} murine monoclonal antibody which recognizes a tumor-associated antigen present on several human GI malignancies has been reported [140]. An organized dose exploratory phase I trial with this antibody is now underway.

Several years ago an antigen was described which appeared to be common and specific to the acute lymphatic leukemic tumor cell of childhood [141]. It is now recognized that this antigen is a differentiation antigen of lymphocytes, is present on the tumor cells of about 80% of children with null cell ALL, is a member of a family of glycoprotein antigens and is also present on tumor cells in 40% of patients with blast crises of CML and on tumor cells of about 10% of patients with T cell ALL tumors [142, 143]. A murine monoclonal antibody, J5, reactive with CALLA (common acute lymphatic leukemia antigen) has been developed by the group at Sidney Farber Institute and has been utilized in a small number of exploratory *in vivo* trials [144]. Findings similar to the studies of Royston et al., and Levy et al., using either T101 or Leu-1, i.e., a brief antitumor response with a rapid decrease in circulating leukemic cells followed within 24 hours by a rapid return to pretreatment levels, the lack of significant toxicity, the ultimate development of an anti-mouse response by the host, and antigenic modulation, have all been demonstrated. This group has directed the second stage of their studies to evaluating the effect of monoclonal antibody treatment, *in vitro*, on samples of autologous bone marrow obtained subsequent to heavy doses of cytotoxic chemotherapy [145]. Using this IgG_2 antibody, which is lytic *in vitro* with complement, they are attempting to remove all leukemic cells from the marrow samples prior to reinfusion. To date this approach has been tried in ten patients following initial relapse. All receive autologous marrow following reinduction, intensification and ablative cytotoxic chemo-

therapy. Nine of these patients continue in unmaintained complete response three to 23 months following treatment [146].

Future studies will exploit the ability to conjugate antibody to a variety of toxic substances. Although the techniques are difficult and require further refinement, both human and murine monoclonal antibodies may be conjugated with a variety of potential toxic agents and administered either *in vivo* or *in vitro* for therapeutic effect. Thus, cytotoxic agents such as adriamycin and daunomycin, the toxic component from either the diphtheria or the ricin molecule, or radioisotopes may be prepared and conjugated to antibodies [147-150]. Potentially a very interesting isotope to be conjugated and investigated is boron-10, an alpha emitter with a radiation range of about 10 microns [149]. In order for the toxic conjugates (other than the radioactive one) to be most effective, internalization, perhaps through antigenic modulation of the toxic molecule, needs to occur. To date, this appears to be a fairly common occurrence. Antigen specific antibodies, other than IgG_{2a} subclass, without inherent therapeutic efficacy, could be utilized as carriers for these toxic molecules. Preliminary trials with both *in vitro* and *in vivo* models are underway.

The detrimental effect on host function and survival, caused by tumor-cell-specific suppressor cell activity (both macrophage and lymphocyte) in both animals and patients with malignancy has been well demonstrated in a number of circumstances [151-157]. A subset of T cells with suppressive and cytotoxic ability can be distinguished from helper cells by their distinct differentiation antigens. Both OKT_5 or T_8 in the system of differentiation T cell antibodies developed by Reinherz et al., and Leu 2A in the system developed by Evans et al., recognize suppressor T cells [157, 158]. The Leu 2A antibody, an IgG_1, is being used in an exploratory phase I dose escalation study sponsored by the BRMP. This study well define a tolerable and biologically effective dose, defined by the effect on suppressor cell function, with the ultimate goal of potentially administering such an antibody to depress specific antitumor-associated suppressor cell activity.

The potential for monoclonal antibody therapy appears to be significant. The use of antibody in animal tumor models and in man has been recently reviewed and the results summarized [159, 160]. This approach appears to offer the best possibilities of *tumor specific* therapy for the next few years.

LYMPHOKINES AND CYTOKINES

Many of the biologicals which will be tested as BRMs are cell products of lymphocytes (lymphokines) or of cells in general (cytokines). The lympho-

kines have a specific ability to regulate certain components of the immune response which may be useful in altering the growth and metastasis of cancer in man. There are early indications of the specific ability of lymphokines to alter immune responses in ways that may be beneficial in the host-tumor interaction. For example, it is possible that certain lymphokines may augment the ability of T-cells to respond to tumor-associated antigens and others may induce higher responsiveness with respect to B-cell activity in cancer patients. Additionally, lymphokines which decrease suppressive functions of the immune system may be useful in enhancing immune responses through a lessening of the suppressive effects of suppressive factors or suppressor cells in patients with cancer. Another specific use of lymphokines may be in the pharmacological regulation of tumors of the lymphoid system. While many of these tumors are considered to be unresponsive to normal growth controlling mechanisms mediated by lymphokines, it is possible that large quantities of pure lymphokines administered pharmacologically or the use of certain molecular analogues of these naturally occurring lymphokines may be useful in the treatment of lymphoid malignancies. A further use of lymphokines may be to manipulate the immune response *in vitro* to produce products which may subsequently be used therapeutically *in vivo.*

Interleukin 1 (IL1), originally known as lymphocyte activating factor, is a macrophage derived cytokine, identified originally as a result of its non-specific enhancing effect on murine thymocyte proliferation [161]. It has subsequently been shown to enhance, *in vitro*, the production of anti sheep erythrocyte plaque forming cells and to enhance the generation of alloantigen-specific cytotoxic T-cells [162, 163]. Both IL1 and viable macrophages are necessary for the initial step in the activation of specific T cells in the process of the *in vivo* production of effector T cells.

IL-1, in its purest form, stimulates the production of interleukin 2 (IL-2), also known as T cell growth factor (TCGF) [164]. The assistance of macrophages is also required in this step. IL-2 will stimulate and maintain *in vitro*, through several passages although not perpetually, the growth of T-cells once they are specifically activated by antigenic stimulation [165]. IL-2 is produced by nearly all malignant T cell lines *in vitro* and will, under appropriate conditions, maintain growth of these cells indefinitely [166]. Receptors for IL-2 have been demonstrated on both activated and most malignant T-cell populations. An oncogenic virus, the human T-cell leukemia virus, has been recently described and isolated from patients with adult T-cell leukemia of Japan. This virus codes for the production of such a receptor, thereby providing the means by which these cells may proliferate indefinitely [167].

Murine IL-1 has been purified to homogeneity and human IL-1 is being

purified [168, 169]. Human IL-2 also has been purified to homogeneity and anti IL-2 monoclonal antibodies have been produced [170, 171]. The availability of these materials will provide the tools to more accurately and completely dissect out the control system for T-cell activation and provide means to obtain knowledge needed to intelligently manipulate the immune system in favor of the host.

Pharmacologic doses of these lymphokines can conceivably be utilized to alter the maturation and kinetic capabilities of various T-cell malignancies. Further, by selective activation and subsequent cloning *in vitro*, T-cells lines with specific cytotoxic capabilities can be obtained and utilized in autologous or allogeneic adoptive immunotherapy. (See Fefer, A. – this volume.)

The role of and place for many other cytokines is currently undergoing extensive investigation. At least four subclasses of colony stimulating factors exist, one of which stimulates macrophage production exclusively [172]. Interleukin-3 has recently been described and appears to have a role in a very early stage of T-cell differentiation and in the differentiation of T-helper cells [173]. T-cell replacing factor appears to influence the terminal differentiation of the proliferating B-cells into antibody secreting cells [174]. Using an antigenically defined subset of murine splenic B lymphocytes, representing 50% of the mature B cell pool, a T-cell derived factor designated B-cell growth factor, has been described which fulfills partially the requirements for DNA synthesis [175].

Further refinement, definition, and purification are needed before any of these factors are ready for use in the manipulation *in vitro* or *in vivo* of the immune system in man. IL-1, IL-2, CSF and macrophage activating factor (MAF) would appear to be the closest to entering clinical trials. At a recent retreat held in Frederick in August, 1982, a panel of BRMP consultants discussed approaches needed in this area to promote the development of *in vivo* lymphokine use. There was general agreement for the need to develop quality controlled standards for specific lymphokine preparations, similar to the interferon standards now available through the auspices of the NIAID. Appropriate and standard bioassays also need to be defined so that generally agreed upon measures of activity for specific preparations are available. Once these are in place and available, clinical trials utilizing preparations of defined activity and high purity can be undertaken. A limited number of clinical trials using crude materials containing a variety of lymphokines have been performed [176, 177]. While these are of interest, further trials await the availability of relatively pure material.

During the past year several immunomodulating agents have been introduced into a BRMP screen designed to evaluate therapeutic potential and to make correlations with specific immunologic functions. This group of biologic response modifiers represents a heterogeneous group of agents that may either stimulate immune reactivity in an immunodepressed host or alternatively increase defense mechanisms above the normal levels of immune reactivity. Many of these agents have been isolated from viruses, bacteria and fungi. In some cases immunomodulating agents consist of crude extracts while others are chemically well defined.

The BRMP and the Cancer Treatment and Metastasis Laboratory of the Frederick Cancer Research Facility have conjointly established an *in vitro* and *in vivo* screen in order to evaluate agents prior to their use in the clinic [178]. This screen is and will be undergoing evolutionary changes in the future but currently evaluates the effects of agents on T cell, B cell, NK cell and macrophage function *in vitro* following *in vivo* or *in vitro* stimulation and also on several *in vivo* tumor systems designed to study the effects on both development of metastases, presence of suppressor cells, and overall survival. To date, MVE-2, azimexon and MDP have completed their evaluations and either have or will shortly undergo clinical testing. Tuftsin, NED-137, Lentinan and Picibanil have each recently entered the screen.

MVE-2

MVE-2 is a divinylether-maleic anhydride copolymer with a molecular weight of 15,500. Several MVE polymers have been examined for biological activity and toxicity [179–184]. The lower molecular weight fractions are less toxic and have been reported to activate macrophages for tumor cytotoxicity *in vitro* and for antitumor activity *in vivo*. MVE-2 has been studied for primary *in vivo* antitumor activity in several animal models and has shown significant antitumor activity against both transplanted and spontaneous mammary tumors [179] and against the P-815 mastocytoma system in mice [180]. MVE-2 has also been shown to be effective when used in combination with BCNU and L1210 vaccine in mice [181–184]. It has been shown to activate macrophages following both *in vitro* and *in vivo* stimulation and this may make an important contribution to the antitumor effect. Thus, macrophages from CD_2F mice cultured with MBL-2 target cell *in vitro* in the presence of MVE-2 are cytotoxic and macrophages from CD_2F mice injected with MVE-2 are cytotoxic for M109 cells, upon co-activation [181]. Macrophages from Balb/c mice treated with MVE-2 also showed cytotoxicity for M109 cells *in vitro*. Studies have also shown an increase in vascular clearance of SRBC by the RES of mice given MVE-2 [180, 185].

Recently, MVE-2 has been demonstrated to enhance natural killer cell (NK) activity and induce interferon following administration to mice [186–188]. This agent has been studied in the *in vitro* and *in vivo* BRMP screen and has undergone phase I testing in man. In the screen it has been noted to increase macrophage activity *in vitro* and to prolong survival when administered *in vivo* to animals with low tumor burden. It was not demonstrably immunostimulatory in man in phase I testing and did not induce objective tumor responses in heavily pretreated patients [189–192].

Azimexon

Azimexon (BM 12.531), chemically, is 2-[2-cyanaziridinyl-(1)]-2-[2-carbamoylaziridinyl-(1)]-propane and is a representative of the 2-cyanaziridines developed by Boehringer Mannheim [193]. These agents possess neither alkylating nor directly cytostatic activity *in vitro* and *in vivo* and as a result toxicity is very low afeter parenteral or oral administration [193, 184]. Antitumor activity of azimexon has been reported against the Meth A sarcoma in mice, Friend virus induced transplantable leukemia in mice, Walker carcinosarcoma 256 in rats, DS carcinosarcoma in rats, Ehrlich ascites carcinoma in mice, M109 alveolar carcinoma in mice, L1210 leukemia in mice, AKR leukemia in mice, Lewis lung carcinoma in mice, and fibrosarcoma in mice [193–195]. In one study spontaneous AKR leukemia cells and various immunogenic and nonimmunogenic AKR clones were injected into young AKR mice. Azimexon, administered at 25 mg/kg (optimum dose) i.v. six times during a four day study beginning 1 day after tumor inoculation, showed a significant reduction (50–60%) in the number of nonimmunogenic AKR clones. A similar effect was found on the growth of Lewis lung tumors and in the mouse fibrosarcoma system. In all systems there was good agreement in the dose-response relationship between azimexon and tumor reduction. A good correlation was obtained between antitumor effects and an increase in cytotoxic 'autoreactive' cells. Autoreactive cells are similar to NK cells with respect to receptors, but differ in being cytotoxic for normal embryonic cells. Cytotoxic autoreactive cell activity induced in AKR mice or C57Bl/6 mice against syngeneic lymphoid spleen cells was examined in a chromium release assay with respect to dose and number of injections. The clearest increase was found with 25 mg/kg administered six times, during a 4 day period – in good agreement with *in vivo* action against tumors. Injection of greater than 25 mg/kg resulted in an increase in suppressor cell activity, manifested as a reduction of cytotoxic autoreactive cell activity against Lewis lung tumor cells or AKR leukemia cells. Azimexon has also been found to cause activation of peritoneal macrophages against AKR leukemia cells [193]. Macrophages prepared from animals treated with 25 mg/kg of azimexon 7 to 10 days before isola-

tion produce inhibition of tumor growth *in vitro*. Azimexon has been found to reduce the cytotoxicity produced by cyclophosphamide and x-rays resulting in a therapeutic effect [196].

MDP

N-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) is the minimal adjuvant-active structure capable of replacing whole mycobacterial cells in Freunds adjuvant for the induction of humoral antibodies or delayed hypersensitivity. The primary action of MDP appears to be on the macrophage with liberation of monokines, leading to activation of B cells and T-cells. MDP has been shown to increase the proliferative response of CBA/Ca spleen cells to varying numbers of DBA/2 stimulator cells in the mixed lymphocyte culture system [197]. The proliferative response was enhanced most at low antigen concentrations and MDP exerted a mitogenic response in the absence of antigen. The proliferative response *in vivo* was measured in a host vs graft reaction where MDP enhanced the local proliferative response and, as with the *in vitro* test, produced an increase in background proliferation in the absence of antigenic challenge [197]. The cytotoxic T-cell response to MDP was studied by co-culturing C57 responder cells with irradiated DBA/2 stimulator cells. On day 4 to 5 the cytotoxic activity against fresh P-815 mastocytoma target cells was measured. MDP did not increase cytotoxicity in the absence of antigen, but did show enhancement at low to optimal antigen doses [198]. This allogeneic system was also used to generate cytotoxic T-cells *in vivo*; C57 peritoneal exudate cells were tested for lytic activity 10 to 11 days after inoculation with P-815 mastocytoma cells. MDP was given i.v. on days 1–3 after tumor challenge. MDP produced about an eight fold increase in lytic activity of spleen cells for P815 cells. It has been found that macrophage monolayers from DBA/2 mice treated with MDP for 24 hours become cytotoxic for P-815 cells and peritoneal macrophages from DBA/2 mice treated three times with MDP show a dose dependent cytotoxicity. Macrophages for MSV-induced tumors and several macrophage cell lines have been shown to possess cytolytic activity against target tumor cells that can be augmented by addition of MDP to the cytotoxicity assay [199]. MDP appears to act directly on the macrophage without participation of other cell types since the effect of MDP was shown with macrophage cell lines. Adherent peritoneal cells from mice injected with MDP show increased secretion of a monokine, thymocytic mitogenic protein (TMP) and are cytostatic *in vitro* for P-815 mastocytoma cells [198]. Other effects attributed to MDP include stimulation of the reticuloendothelial system of mice by increasing phagocytic function [200], an inhibition of macrophage migration [201], and stimulation of prostaglandin and collagenase production by macrophages [202].

An important study has demonstrated the potential usefulness of liposomes as a carrier vehicle for MDP [203]. It was found that MDP encapsulated in liposomes rendered mouse or rat alveolar macrophages tumoricidal *in vitro* at less than 1/1000 the concentration required for free MDP. The significant potentiation of the tumoricidal activity of alveolar macrophages obtained *in vitro* by inclusion of MDP in liposomes has been used by Fidler and coworkers to study the eradication of established spontaneous pulmonary and lymph node metastasis *in vivo* by multiple systemic administration of MDP in liposomes [203]. In these experiments 74% of treated mice were free of visible metastasis. In other experiments 60% of mice treated with MDP containing liposomes were free of tumor 120 days after the last injection of liposomes or 110 days after control mice injected with MDP alone or liposomes without MDP had died. Recently, the same laboratory has compared efficacy of hydrophilic and lipophilic MDP entrapped in liposomes [204]. Liposomes containing lipophilic MDP were found superior in activating mouse alveolar macrophages to higher and more sustained levels of cytotoxicity [204].

Tuftsin

Tuftsin is a naturally occuring tetrapeptide (Thr-lys-Pro-Arg) found normally in human and animal plasma while covalently linked to a gamma globulin fraction [205]. It is enzymatically released from the protein carrier and plays a role in activation of several functions of polymorphonuclear leukocytes, monocytes, and macrophages at physiological concentrations. A toxicologic study has been carried out in mice and the acute LD_{50} was found to be 2 gm/kg [206]. Tuftsin has been shown to possess antitumor activity against a 3-methylcholanthrine induced transplantable fibrosarcoma in C_3H mice [206]. In this study, Carrageenan, an inhibitor of macrophage activity was found to suppress antitumor activity when given following Tuftsin administration. Tuftsin has also been shown to possess antitumor activity against L1210 tumor cells in DBA mice and against cultured Cloudman (S-91) melanoma cells in DBA mice [207]. Because Tuftsin is subject to enzymatic degradation *in vivo*, several analogs have been synthesized and analyzed with increased *in vivo* half life and efficacy [208]. The octapeptide tuftsinyltuftsin has been examined in the L1210/DBA system and shows an increased antitumor effect [208]. Several studies suggest that activation of macrophages may be involved in the anti-tumor effect of Tuftsin. Thus, macrophages from DBA mice treated with Tuftsin show increased cytotoxicity against L1210 tumor cells *in vitro* [209] and macrophages from DBA mice exposed to Tuftsin *in vitro* show enhanced cytotoxicity against cultured L1210 cells. Recently, it has been shown that *in vitro* treatment of mouse splenic cells with synthetic Tuftsin enhances natural killer cell toxicity against the T-cell lymphoma Yac-1 [210].

Lentinan

Lentinan is a purified polysaccharide obtained from extracts of the edible mushroom *Lentinus* edodes [211]. Chemically Lentinan is a B-(1,3)-glucan with some B-(1-6)-glucosidic side chains and has a molecular weight of about 500,000 daltons with glucose as the sugar component. Toxicity tests have been performed in various animals [212]. Acute and subacute toxicities have been examined following IV injection into mice, rats, dogs and monkeys. In all species tested, the LD_{50} was greater than 100 mg/kg. Lentinan does not show any direct cytotoxicity against tumor cells and its antitumor action is host mediated. Lentinan can cause complete regression of sarcoma - 180 transplanted into ICR mice when administered at 1 mg/kg for 10 days [213, 214]. Larger doses are ineffective. This phenomenon of 'optimal dose' is also evident in various immunological responses. The regressive activity of Lentinan against sarcoma - 180 is strain dependent; a/Ph(A/J), C57B16, C_3H/HeN, DBA/2 and SWM/MS mice are strong responders to Lentinan [215-217]. Lentinan also inhibits the growth of Lewis lung and EL-4 lymphoma transplanted in C57B1/6, MM46 and MM102 carcinoma and SR-C_3H/He sarcoma transplanted in C_3H/HeN mice [211]. Lentinan is also active in inhibiting the growth of adenovirus type 12 induced tumors in C_3He mice and is very effective against the methylcholanthrene-induced primary tumor in C_3H/HeN mice in combination with cyclophosphamide [211, 218].

Evidence has been presented that Lentinan is a T cell adjuvant. Antitumor activity of Lentinan is not evident in neonatal thymectomized mice and is lost by administration of anti-lymphocyte serum and also by whole-body irradiation within 3 days of tumor transplantation [219-222]. Further evidence for T-cell involvement in Lentinan action has been provided by studies showing that antibody production in CBA/J mice against sheep erythrocytes occurs only in presence of T-cells [223] and in a second study showing that Lentinan enhancement of antibody dependent cell mediated cytotoxicity is mediated through helper T-lymphocytes [224]. Lentinan also has the characteristics of a macrophage activator. *In vivo* administration of Lentinan enhances the cytotoxic and cytostatic activities of peritoneal exudate cell against various tumor cells *in vivo* [222, 225]. Evidence in support of this comes from the fact that the anti-tumor activity of Lentinan is blocked by anti-macrophage agents such as carrageenan or silica. Lentinan does not enhance the phagocytic and cytotoxic activity of macrophages *in vitro.* Recent studies have shown that Lentinan can augment generation of antigen specific cytotoxic T lymphocytes against alloantigens and haptenated syngeneic cells *in vivo* and *in vitro* via soluble factors secreted by Lentinan stimulated macrophages [226, 227].

Lentinan has also been examined for NK augmenting activity in CBA,

C_3H/He and Balb/C mice [228–230]. Intraperitoneal injection of Lentinan results in a rapid and significant increase in NK activity with the increases being higher in CBA than Balb/C mice. Lentinan administration results in modest increase in IFN activity which may be sufficient to enhance NK activity. Lentinan did not activate NK cells when incubated *in vitro*. The doses required to activate NK activity in C_3H/He mice were higher than those required to generate an anti-tumor response. Lentinan has also been shown to increase secretion of CSF from splenic T-cells, and LAF from peritoneal macrophages [230].

NED-137

NED-137 is one of a family of low molecular weight synthetic polymers developed by Monsanto. It consists of a group of small molecules with a mean weight of 800. The only animal model system that has been used to evaluate NED-137 is the transplantable MCA induced bladder tumor in Fischer rats [231–233]. In this system 50% of animals given NED-137 seven days after subcutaneous tumor implantation were still alive after 6 weeks and free of metastasis while control animals died from local tumor and metastasis at a mean of 23 days. A study was also carried out on the effect of NED-137 on tumor recurrence and the development of metastasis following surgical removal of primary tumor. Animals treated with NED-137 at the time of excision showed no recurrences 60 days later and were free of metastasis while animals not receiving NED-137 died within 5 weeks. In the same study animals receiving NED-137 at 6 weekly intervals following excision remained free of tumor for more than 6 months. NED-137 has not been found to have any *in vitro* effects on tumor cells in assays for ADCC and lymphocyte mediated cytotoxicity and does not increase numbers or activity of peritoneal macrophages. NED-137 has been shown to promote an increase in differentiation of B cells to antibody secreting cells in response to SRBC immunization and can restore antibody production against SRBC to normal levels when administered to T-cell depleted rats following thymectomy or irradiation and syngeneic bone marrow transplantation.

Picibanil (OK-432)

Picibanil (OK-432) is a cell suspension prepared by treating a low virulence strain of *streptococcus hemolyticus* with penicillin followed by heating at 45 °C. Picibanil has been shown to have antitumor activity in several tumor systems [234–237]. Activity has been found against various strains of rat ascites, hepatoma ascites, solid forms of Ehrlich carcinoma of mice, Yoshida sarcoma cell lines, spontaneous mammary tumors and the spontaneous tumor system of AKR mice. Picibanil has been reported to have a direct inhibitory effect on malignant cells with little effect on normal host

cells [237] by a number of *in vitro* studies with Yoshida sarcoma or rat ascites hepatoma cells and Ehrlich ascites cells [235]. More recently, Picibanil has been shown to induce interferon in mice and patients with oral squamous cell carcinoma [238, 239]. In mice the interferon induced was the gamma type (immune) and possible involvement of thymus derived (T) lymphocytes and macrophages was inferred from experiments with thymus defective nude mice. Evidence has also been presented that Picibanil enhances the natural killer (NK) cell activity when administered to $C_{57BL/6}$ mice [240] and further augments NK cell activity when administered *in vivo* to cancer patients and *in vitro* on peripheral blood lymphocytes from both normal and cancer patients [214–243].

BRMP preliminary screen results and projected trials

Of these seven agents, three, aximexon, MDP and MVE-2, have completed *in vitro* testing in the BRMP screen [244]. Azimexon has been noted to increase both T-cell and B-cell function but not macrophage or NK activity. MDP has the capability to boost T-cell, B-cell and macrophage function but has no effect on NK activity. MVE-2 will boost B-cell and macrophage activity. All are undergoing testing *in vivo* therapy animal models. NED-137, Picibanil (OK-432), Lentinan and Tuftsin will all be tested in the screen by early 1983. MVE-2 has undergone phase I testing while Azimexon should enter phase I clinical trials in early 1983 and MDP in liposomes in late 1983. A decision regarding phase I testing of the other agents will be made following availability of full screen data. The interferons and thymosins have also been tested in certain components of the screen. Multiple other bacterial extracts, immuno-stimulators and immunomodulators await screen evaluation. Once all data are available for correlation from the various aspects of screen testing and from phase I clinical testing on a number (10–20) of agents, predictive efficacy for various screen components can be determined. In this way, screen design will undergo constant revision until a efficient predictive model is in place. Multiple agents can then be tested rapidly and decisions made based on results regarding phase I clinical testing.

SUMMARY

Biological response modifier therapy remains in its infancy. However, significant advances have been made in the application of this approach. The interferons have induced clinical antitumor responses, primarily in the lymphomas but also in various solid tumors. A number of responses have been noted with other agents in patients with renal cell carcinoma, a tumor

unresponsive to other modalities. Specific application of these agents awaits further data regarding the effect of dose and schedule on cell proliferation and immune parameters. The use of monoclonal antibody therapy is undergoing phase I trials and the rational use of antibody-toxin conjugates awaits these phase I data and further animal model work with conjugates. The availability in the near future of purified lymphokine/cytokine preparations will stimulate a number of phase I/II trials with these agents with the potential of manipulating the host immune response in favor of the host. Lastly, the availability of a large number of immunomodulatory agents and the ability to define their specific role in manipulating the host defense system also offers the real possibility of providing new therapeutic agents.

REFERENCES

1. Oldham RK: Biological Response Modifiers Program. J Biol Resp Mod 1:81–100, 1982.
2. Mathe G, Amiel JL, Schwarzenberg L, Schneider M, Cattan A, Schlumberger JR, Hayat M, Vassal F: Active immunotherapy for acute lymphoblastic leukemia. Lancet I:697–699, 1969.
3. Powles RL, Crowther D, Bateman CJT, Beard MEJ, McElwain TJ, Russel J, Lister TA, Whitehouse JMA, Wrigley PFM, Pike M, Alexander P, Hamilton Fairley G: Immunotherapy for acute myelogenous leukaemia. Br J Cancer 28:365–376, 1973.
4. Gutterman JU, Hersh EM, Rodriguez V, McCredie KB, Mavligit G, Reed R, Burgess MA, Smith T, Gehan E, Bodey GP, Freireich EJ: Chemoimmunotherapy of adult acute leukaemia: Prolongation of remission in myeloblastic leukaemia with B.C.G. Lancet 2:1405–1409, 1974.
5. Vogler WR, Chang YK: Prolonging remission in myeloblastic leukaemia by Tice-Strain Bacillus Calmette-Guerin. Lancet 2:128–131, 1974.
6. Foon KA, Smalley RV, Gale RP: The role of immunotherapy in acute myelogenous leukemia Arch Int Med. In press, 1983.
7. Terry WD, Rosenberg SA (eds), Immunotherapy of human cancer. New York: Excerpta Medica, 1982.
8. Jones SE: Chemoimmunotherapy of malignant lymphoma. In: Immunotherapy of human cancer, Terry WD, Rosenberg SA (eds). New York: Excerpta Medica, 1982, pp 55–60.
9. Albert DS, Mason NL, O'Toole R, Neff J, Hilgers R, Carlin D, Moon TE: A randomized trial of doxorubicin and cyclophosphamide plus BCG versus doxorubicin and cyclophosphamide therapy of advanced ovarian cancer. In: Immunotherapy of human cancer, Terry WD, Rosenberg SA (eds). New York: Excerpta Medica, 1982, pp 343–350.
10. Morton DL, Eilber FR, Malmgren RA: Immunological factors which influence response to immunotherapy in malignant melanoma. Surgery 68:158–164, 1970.
11. Nathanson L, Schoenfeld D, Regelson W, Colsky J, Mittelman A: Prospective comparison of intralesional and multipuncture BCG in recurrent intradermal melanoma. Cancer 43:1630–1635, 1979.
12. Mastrangelo MJ, Bellet RE, Laucius JF, Berkelhammer J: In: Oncologic medicine, AJ Sutnick and PF Engstrom (eds). Baltimore: University Park Press, 1976, pp 71–93.
13. Rapp HJ, Churchill Jr. WH, Kronman BS, Rolley RT, Hammond WG, Borsos T: Antigenicity of a new diethylnitrosamine-induced transplantable guinea pig hepatoma: Pathology and formulation of ascites variant. JNCI 41:1–11, 1968.

14. Zbar B, Canti G, Ashley MP, Rapp HJ, Hunter JT, Ribi E: Eradication by immunization with mycobacterial vaccines and tumor cells of microscopic metastases remaining after surgery. Ca Res 39:1597–1603, 1979.
15. Hunter JT, Ashley MP, Rapp HJ, Sukumar S, Zbar B: Pulmonary Metastases in guinea pigs as a consequence of dermal implantation of line-10 tumor cells. Ca Imm Immunother 11:39–44, 1981.
16. Hunter JT, Ashley MP, Sukumar S, Sugimoto T, Zbar B, Rapp HJ, Yarkoni E: Treatment by limited surgery and specific immunization of guinea pigs with stage II experimental cancer. J Exp Med 154:253–261, 1981.
17. Yarkoni E, Rapp HJ: Regression by active specific immunotherapy of established dermal tumor transplants and lymph node metastases in guinea pigs. Inf and Imm 31:514–516, 1981.
18. Yarkoni E, Ashley MP, Zbar B, Sugimoto T, Rapp HJ: Eradication by active specific immunotherapy of established tumor transplants and microscopic lymph node metastases. Ca Res 42:2544–2546, 1982.
19. Ribi E, Cantrell J, Schwartzman S, Parker R: BCG cell wall skeleton, P3, MDP and other microbial components - Structure activity studies in animal models. In: Augmenting agents in cancer therapy, Hersch EM, Chirigos MA, Mastrangelo MJ (eds). New York: Raven Press, 1981, pp 15–32.
20. McLaughlin CA, Strain SM, Bickel WD, Goren MB, Azuma I, Milner K, Cantrell JL, Ribi E: Regression of line-10 hepatocellular carcinomas following treatment with water-soluble, microbial extracts combined with trehalose or arabinose mycolates. Ca Imm Immunother 4:61–68, 1978.
21. Cantrell JL, Ribi E, McLaughlin CA: Passive transfer of tumor immunity with spleen cells from guinea pigs cured of hepatocarcinoma by nonspecific immunotherapy. Ca Imm Immunother 4:21–28, 1978.
22. McLaughlin CA, Bickel WD, Kyle JS, Ribi E: Synergistic tumor regressive activity observed following treatment of line-10 hepatocellular carcinomas with deproteinized BCG cell walls and mutant salmonella typhimurium glycolipid. Ca Imm Immunother 5:45–52, 1978.
23. Kelly MT, McLaughlin CA, Ribi EE: Eradication of microscopic lymph node metastases of the guinea lig line-10 tumor after intradermal injection of endotoxin plus mycobacterial components. Ca Imm Immunother 4:29–32, 1978.
24. Vosika GJ, Schmidtke J, Goldman A, Ribi E, Parker R, Gray GR: Local and systemic immunotherapy of human cancer with mycobacterial cell wall skeleton and trehalose dimycolate (P_3). In: Augmenting agents in cancer therapy, Hersh EM, Chirigos MA, Mastrangelo M (eds). New York: Raven Press, 1981, pp 33–51.
25. Richman SP, Gutterman JU, Hersh EM, Ribi EE: Phase I-II study of intratumor immunotherapy with BCG cell wall skeleton plus P_3. Ca Imm Immunother 5:41–44, 1978.
26. Peters LC, Brandhorst JS, Hanna, Jr. MG: Preparation of immunotherapeutic autologous tumor cell vaccines from solid tumors. Ca Res 39:1353–1360, 1979.
27. Hanna, Jr. MG, Brandhorst JS, Peters LC: Active specific immunotherapy of residual micrometastasis. Ca Imm Immunother 7:165–173, 1979.
28. Peters LC, Hanna, Jr. MG: The specific immunotherapy of established micrometastasis: Effect of cryopreservation procedures on tumor cell immunogenicity in guinea pigs. JNCI 64:1521–1525, 1980.
29. Hoover Jr. HC, Peters LC, Brandhorst JS, Hanna Jr. MG: Therapy of spontaneous metastases with an autologous tumor vaccine in a guinea pig model. J Surg Res 30:409–415, 1981.
30. Key ME, Hanna Jr. MG: Mechanism of action of BCG-tumor cell vaccines in the gener-

ation of systemic tumor immunity: I. Synergism between BCG and line 10 tumor cells in the induction of an inflammatory response. JNCI 67:853–861, 1981.

31. Key ME, Hanna Jr. MG: Mechanism of action of BCG-tumor cell vaccines in the generation of systemic tumor immunity: II. Influence of the local inflammatory response on immune reactivity. JNCI 67:863–869, 1981.
32. Hanna Jr. MG, Bucana C: Active specific immunotherapy of residual micrometastasis: The acute and chronic inflammatory response in induction of tumor immunity by BCG-tumor cell immunization. J Ret Soc 26:439–452, 1979.
33. Key ME, Hoyer, L, Bucana C, Hanna Jr. MG: Mechanisms of macrophage-mediated tumor cytolysis. In: Mechanisms of cell mediated cytotoxicity, Clark WR, Goldstein P (eds). New York: Plenum, 1982, pp 265–310.
34. Hanna Jr. MG, Peters LC: Morphological and functional aspects of active specific immunotherapy of established pulmonary metastases in guinea pigs. Ca Res 41:4001–4009, 1981.
35. Hanna Jr. MG, Key ME: Immunotherpay of metastases enhances subsequent chemotherapy. Science, 217:367–369, 1982.
36. Galli SJ, Bast Jr. RC, Bast BS, Isomura T, Zbar B, Rapp HJ, Dvorak HF: Bystander suppression of tumor growth: Evidence that specific targets and bystanders are damaged by injury to a common microvasculature. J Imm 129:890–899, 1982.
37. Dye ES, North RJ, Mills CD: Mechanisms of anti-tumor action of corynebacterium parvum. J Exp Med 154:609–620, 1981.
38. Taniguchi T, Mantei N, Schwarzstein M, Nagata S, Muramatsu M, Weissmann C: Human leukocyte and fibroblast interferons are structurally related. Nature 285:547–549, 1980.
39. Yip YK, Pang RHL, Urban C, Vilcek J: Partial purification of human gamma (immune) interferon. Proc NAS (USA) 78:1601–1605, 1981.
40. De Mayer-Guignard J, Tovey MG, Gresser I, De Maeyer E: Purification of mouse interferon by sequential affinity chromatography on poly(U) – and antibody – agarose columns. Nature 271:622–625, 1978.
41. Taylor-Papadimitriou J: Effects of interferons on cell growth and function. In: Interferon 2, Gressor I (ed). London: Academic Press, 1980, pp 13–46.
42. Borden EC, Ball LA: Interferon: Biochemical cell growth inhibitory and immunological effects. In: Progress in hematology, Vol XII, Brown, ER (ed). New York: Grune & Stratton, 1981, pp 299–339.
43. Gresser I, Tovey MG: Antitumor effects of interferon. Biochem. & Biophys Acta 516:231–247, 1978.
44. Gresser I, Coppey J, Brouty-Boye DF, Falcoff E: Interferon and murine leukemia: III. Efficacy of interferon preparation administered after inoculation friend virus. Nature 215:174–175, 1967.
45. Gresser I, Bergman L, DeThe G, Broutz-Boye DF, Coppey J, Falcoff E: Interferon and murine leukemia: V. Effect of interferon preparations on the evolution of Rauscher disease in mice. JNCI 41:505–509, 1968.
46. Rossi GB, Marchegiani M, Matarese GP, Gresser I: Brief communication: Inhibitory effect of interferon on multiplication of friend leukemia cells in vivo. JNCI, 54:993–996, 1975.
47. Bart RS, Porzio NR, Kopf AW, Vilcek JT, Cheng EH, Farcet Y: Inhibition of growth of B16 murine malignant melanoma by exogenous interferon. Ca Res 40:614–619, 1980.
48. Ratner L, Nordlund JJ, Lengyel P: Interferon as an inhibitor of cell growth: Studies with mouse melanoma cells. Proc Soc Exp Bio Med 163:267–272, 1980.
49. Gresser I, Coppey J, Bourali C: Interferon and murine leukemia: IV. Effect of interferon preparations on the lymphoid leukemia of AKR mice. JNCI 43:1083–1089, 1969.

50. Came PE, Moore DH: Inhibition of spontaneous mammary carcinoma of mice by treatment with interferon and poly I: C. Proc Soc Exp Biol Med 137:304–305, 1971.
51. Gresser I, Maury C, Tovey M: Interferon and murine leukemia. VII. Therapeutic effect of interferon preparations after diagnosis of lymphoma in AKR mice. Int J Cancer 17:647–651, 1976.
52. Kassel RL, Pascal RR, Vas A: Interferon-mediated oncolysis in spontaneous murine leukemia. JNCI 48:1155–1159, 1972.
53. Gresser I, Maury C, Brouty-Boye D: Mechanism of the antitumor effect of interferon in mice. Nature 239:167–168, 1972.
54. Gresser I: Commentary: On the varied biologic effects of interferon. Cell Imm 34:406–415, 1977.
55. Mellstedt H, Bjorkholm M, Johansson B, Ahre A, Holm G, Strander H: Interferon therapy in myelomatosis. Lancet 1:245–247, 1979.
56. Einhorn N, Cantell K, Einhorn S, Strander H: Human leukocyte interferon therapy for advanced ovarian carcinoma. Am J Clin Oncol (CCT) 5:167–172, 1982.
57. Gutterman JU, Blumenschein GR, Alexanian R, Yap HY, Buzdar AU, Cabanillas F, Hortobagyi GN, Hersh EM, Rasmussen SL, Harmon M, Kramer M: Leukocyte interferon-induced tumor regression in human metastatic breast cancer, multiple myeloma, and malignant lymphoma. Ann Int Med 93:399–406, 1980.
58. Freedman R, Wharton JT, Rutledge F: Human leukocyte interferon (IFN alpha) in patients with epithelial ovarian carcinoma: (A preliminary report). Proc AACR 22:372, 1981.
59. Einhorn S, Strander H: Interferon therapy for neoplastic diseases in man: *In vitro* and *in vivo* studies. Adv Exp Med Biol 110:159–174, 1978.
60. Borden EC, Holland JF, Dao TL, Gutterman JU, Wiener L, Chang YC, Patel J: Leukocyte-derived interferon (alpha) in human breast carcinoma. Ann Int Med 97:1–10, 1982.
61. Louie AC, Gallagher JG, Skora K, Levy R, Rosenberg SA, Merigan TC: Follow-up observations on the effect of human leukocyte interferon in non-Hodkin's lymphoma. Blood 58:712–718, 1981.
62. Merigan TC, Sikora K, Breeden JH, Levy R, Rosenberg SA: Preliminary observations on the effect of human leukocyte interferon in non-Hodgkin's lymphoma. NEJM 299:1449–1453, 1978.
63. Finter NB, Fantes KH: The purity and safety of interferons prepared for clinical use: The case of lymphoblastoid interferon. In: Interferon 2 Gresser I (ed). London: Academic Press, 1980, pp 65–80.
64. Fantes KH, Allen G: Specific activity of pure human interferons and a non-biological method for estimating the purity of highly purified interferon preparations. J Interferon Res 1:465–472, 1981.
65. Goeddel DV, Yelverton E, Ullrich A, Heyneker HL, Miozzari G, Holmes W, Seeburg PH, Dull T, May L, Stebbing N, Crea R, Maeda S, McCandliss R, Sloma A, Tabor JM, Gross M, Familletti PC, Pestka S: Human leukocyte interferon produced by *E. coli* is biologically active. Nature 287:411–416, 1980.
66. Streuli M, Nagata S, Weissmann C: At least three human type alpha interferons: Structure of alpha-2. Science 209:1343–1347, 1980.
67. Neefe JR, Streevalsan T, Bash JA: Phase I trial of immunomodulatory activities of human leukocyte interferon in advanced cancer patients. Proc AACR 23:245, 1982.
68. Bash JA, Woody JN, Neefe JR: Phase I trial of immunomodulating activities of human leukemic interferon in advanced cancer patients. In: NK cells and other natural effector cells, Herberman RB (ed). New York: Academic Press, 1982, pp 1273–1278.
69. Merigan TC: Human interferon as a therapeutic in cancer. Proc 13th Annual Int Ca Cong, Seattle, p 82, 1982.

70. Frei E, Personal communication.
71. Bash JA, Teitelbaum D, Neefe JR: Immunomodulatory activities of human leukocyte interferon in advanced cancer patients. J Biol Resp Mod. In press, 1983.
72. Sarna G, Figlin R, Bryson Y, Mauritzon N, Cline M: Phase I trial of human lymphoblastoid interferon (HuLyINF) administered intermittently. Proc ASCO 1:39, 1982.
73. Laszlo J, Huang A, Brenckman W, Koren H, Cianciolo G, Buckley Jr CE, Cox E, Cashdollar W, Metzgar R, Lucas Jr VS, Jeffs C, Dorenkamp S: Immunopharmacology of human lymphoblastoid interferon (IF) in man. Proc AACR 23:245, 1982.
74. Laszlo J, Huang A, Brenckman W, Koren H, Cianciolo G, Buckley III CE, Cox E, Cashdollar W, Metzgar R: Immune modulation and toxicity produced in a phase I study of human lymphoblastoid interferon (IF). Proc 13th Annual Int Ca Cong, Seattle, WA, p 235, 1982.
75. Knost J, Sherwin S, Foon K, Abrams P, Ochs J, Williams R, Maluish A, Oldham R: A phase I trial of lymphoblastoid interferon using a 6 hour intravenous infusion. Proc ASCO, 1:35, 1982.
76. Koren H, Brandt C, Laszlo J: Modulation of natural killer (NK) cell activity induced by human lymphoblastoid interferon (IFN) In vivo and *in vitro*. Proc 13th Annual Int Ca Cong, Seattle, WA, p 377, 1982.
77. Gutterman J, Fein S, Quesada J, Horning S, Levine J, Alexanian R, Bernhardt L, Kramer M, Spiegel H, Colburn W, Trown P, Merigan T, Dziewanowska Z: Recombinznt leukocyte A interferon: Pharmacokinetics, single-dose tolerance, and biologic effects in cancer patients. Ann Int Med 96:549–556, 1982.
78. Sherwin S, Knost J, Fein S, Abrams P. Foon K, Ochs J, Schoenberger C, Maluish A, Oldham R: A multiple dose phase I trial of recombinant leukocyte A interferon on a 3 times weekly schedule. JAMA 248:2461–2466, 1982.
79. Fein S, Bernhardt L, Grossbard E, Franco S, Bigley J, Dziewanowska Z: The Biologic activity of recombinant leukocyte A interferon. Proc 13th Annual Int Ca Cong, Seattle, WA, p 173, 1982.
80. Rudnick S, Schering Corporation: Personal Communication.
81. Maluish A, Ortaldo J, Sherwin S, Leavitt R, Strong D, Fein S, Wiernik P, Oldham R, Herberman R: Immunological monitoring of patients receiving recombinant leukocyte A interferon. Proc ASCO, 1:39, 1982.
82. Sherwin S, Mayer D, Ochs J, Abrams P, Knost J, Foon K, Fein S, Oldham R: Recombinant leukocyte A interferon in advanced breast cancer: Results of a preliminary phase II efficacy trial. Ann Int Med 98:598–602, 1983.
83. Hawkins M, Krown S, Borden E, Real F, Krim M, Cunningham-Rundles S, Oettgen H, Fox R, Stock C, Rauscher F: American Cancer Society phase I trial of human interferon beta (IFN-β). Proc AACR, 23:246, 1982.
84. Sherwin S, Oldham R: BRMP, NCI, Frederick, MD.
85. Gutterman J: MD Anderson Hospital and Tumor Institute, Houston, Tx.
86. Levy H, Stephen E, Harrington D, Engel K, Riley F, Lvovsky E: Polynucleotides in the treatment of disease. In: Augmenting agents in cancer therapy, Hersh E, Chirigos M, Mastrangelo M (eds). New York: Raven Press, 1981, pp 135–150.
87. Levy H, Bear G, Baron S, Buckler C, Gibbs C, Iadarola M, London W, Rice J: A modified polyriboinosinic-polyribocytidylic acid complex that induces interferon in primates. J Inf Dis 132:434–439, 1975.
88. Lampson G, Field A, Tytell A, Hilleman M: Poly I:C/Poly-L-Lysine: Potent inducer of interferons in primates. J Interferon Res 1:539–549, 1981.
89. Levine A, Sivulich M. Wiernik P, Levy H; Initial Clinical trials in cancer patients of polyriboinosinic-polyribocytidylic acid stabilized with poly-L-lysine, in carboxymethylcellulose [Poly(ICLC)], a highly effective interferon inducer. Ca Res 39:1645–1650, 1979.

90. Levine A, Levy H: Phase I/II trials of poly IC stabilized with poly-L-lysine. Ca Trt Rep 62:1907–1913, 1978.
91. Levine A, Durie B, Lampkin B, Leventhal B, Levy H: Poly (ICLC): Interferon induction, toxicity, and clinical efficacy in leukemia, lymphoma, solid tumors, Myeloma, and laryngeal papillomatosis. In: Augmenting agents in cancer therapy, Hersh E, Chirigos M, Mastrangelo M (eds). New York: Raven Press, 1981, p 151–164.
92. Krown S, Kerr D, Stewart II W, Pollack M, Cunningham-Rundles S, Hirshaut Y, Pinsky C, Levy H, Oettgen H: Phase I trial of poly ICLC in patients with advanced cancer. In: Augmenting agents in cancer therapy, Hersh E, Chirigos M, Mastrangelo M (eds). New York: Raven Press, 1981, pp 165–176.
93. Champney K, Levine D, Levy H, Lerner M; Modified polyriboinosinic-polyribocytidylic acid complex: Sustained interferonemia and its physiological associates in humans. Inf and Imm 25:831–837, 1979.
94. Engel WK, Cuneo RA, Levy HB: Polyinosinic-polycytidylic acid treatment of neuropathy. Lancet 1:503–504, 1978.
95. Lampkin B, Levine AS, Levy H, Krivit W, Hammond D: Phase II trial of poly (ICLC) (An interferon (IF) inducer) in the treatment of children with acute leukemia (AL). Proc ASCO 1:123, 1982.
96. Herberman RB, Reid J, Maluish A: Personal communication.
97. Thurman G, Marshall, G, Low T, Goldstein A: Recent developments in the classification and bioassay of thymosin polypeptides. In:Cell biology and immunology of leukocyte function, Quastrei MR (ed). New York: Academic Press, 1979, pp 189–199.
98. Wara D, Goldstein A, Doyle N, Ammann A: Thymosin activity in patients with cellular immunodeficiency. NEJM 292:70–74, 1975.
99. Goldstein A, Wara D, Amman A, Sakai H, Harris N, Thurman G, Hooper J, Cohen G, Goldman A, Constanzi J, McDaniel M: First clinical trial with thymosin: Reconstitution of T-cell in patients with cellular immunodeficiency diseases. Trans Proc 7:681–686, 1975.
100. Wara D, Ammann A: Thymosin treatment of children with primary immunodeficiency disease. Trans Proc 10:203–209, 1978.
101. Wara D, Barrett D, Ammann A, Cowan M: *In vitro* and *in vivo* enhancement of mixed lymphocyte culture reactivity by thymosin in patients with primary immunodeficiency disease. J Pediat 97:66–71, 1980.
102. Costanzi J, Gagliano R, Loukas D, Delaney F, Sakai H, Harris N, Thurman G, Goldstein A: The effect of thymosin on patients with disseminated malignancies: A phase I study. Cancer 40:14–1977.
103. Costanzi J, Harris N, Goldstein A: Thymosin in patients with disseminated solid tumors: Phase I and II results. In: Immune modulation and control of neoplasia by adjuvant therapy, Chirigos M (ed). New York: Raven Press, 1978, pp 373-380.
104. Schafer L, Gutterman J, Hersh E, Mavligit G, Dandridge K, Cohen G, Goldstein A: Partial Restoration by *in vivo* thymosin of E-rossettes and delayed-type-hypersensitivity reactions in immunodeficient cancer patients. Ca Imm Immunother 1:259, 1976.
105. Cohen M, Chretien P, Ihde D, Fossieck Jr. BE, Makuck R, Bunn Jr PA, Johnston AV, Shackney SE, Matthews MJ, Zpson SD, Kenady DE, Minna JD: Thymosin fraction V and intensive combination chemotherapy. JAMA 241:1813–1815, 1979.
106. Wara W, Neely M, Ammann A, Wara D: Biologic modification of immunologic parameters in head and neck cancer patients with thymosin fraction V. In: Lymphokines and thymic hormones: Their potential utilization in cancer therapeutics, Goldstein, A, Chirigos, M (eds). New York: Raven Press, 1981, pp 257-262.
107. Pinsky C, Wittes R: Personal communication.

108. Zatz M, Moshe G, Seals C, Goldstein A: Effects of combined cyclophosphamide and thymosin treatment on tumor growth and host survival in mice bearing a syngeneic tumor. In: Lymphokines and thymic hormones: Their potential utilization in cancer therapeutics, Goldstein A, Chirigos M (eds). New York: Raven Press, 1981, pp 249–256.
109. Low T, Thurman G, McAdoo M, McClure J, Rossio J, Naylor P. Goldstein A: The chemistry and biology of thymosin: I. Isolation, characterization and biological activities of thymosin α1 and polypeptide β1 from calf thymus. J Biol Chem, 254:981–985, 1979.
110. Dillman R, Beauregard J, Zavanelli M, Halliburton B, Royston I: Phase I trials of thymosin fraction 5 (TF5) and thymosin alpha-1 (TA1) and assessment of immunologic response. Proc ASCO 1:42, 1982.
111. Fabrega R, Pinsky C, Braun Jr D, Chung T, Krown S, Oettgen H: Phase I trial of thymosin fraction 5 in cancer patients. Proc AACR 23:126, 1982.
112. Wara W, Wara D, Neely M, Carter S: Phase I trial of thymosin fraction V in advanced malignancies. Preliminary NCOG Report. Presented at the Intern'tl Symp on Immunomodulators, Montpelier, France, 1982.
113. Fefer A, Wright P, Goldberg R: Personal communication.
114. Schulof R, McClure J, Goldstein A: Thymosin αl following mediastinal irradiation: An immunorestorative trial in patients with localized non-small cell lung cancer. Proc ASCO 1:34, 1982.
115. Rosenberg SA, Terry WD: Passive immunotherapy of cancer in animals and man. Adv Ca Res, 25:323–388, 1977.
116. Bernstein ID, Tam MR, Nowinski RC: Mouse leukemia: Therapy with monoclonal antibodies against a thymus differentiation antigen. Science, 207:68–69, 1980.
117. Bernstein ID, Nowinski RC: Monoclonal antibody treatment of transplanted and spontaneous murine leukemia. In: Hybridomas in cancer diagnosis and treatment, Mitchell MS, Oettgen HF (eds). New York: Raven Press, 1982, pp 97–112.
118. Kirch ME, Hammerling U: Immunotherapy of murine leukemias by monoclonal antibody: I. Effect of passively administered antibody on growth of transplanted tumor cells. J Imm 127:805–810, 1981.
119. Royston I, Majda J, Baird SM, Meserve BL, Griffiths JC: Monoclonal antibody specific for human T lymphocytes: Identification of normal and malignant T cells. Blood 54 (Suppl 1):106A, 1979.
120. Engleman EG, Warnke R, Rox RI, Dilley J, Benike CJ, Levy R: Studies of a human T lymphocyte antigen recognized by a monoclonal antibody. Proc NAS (USA) 78:1791–1795, 1981.
121. Wang CY, Good RA, Ammirati P, Dymbort G, Evans RL: Identification of p69, 71 complex expressed on human T cells sharing determinants with B-type chronic lymphatic leukemic cells. J Exp Med 151:1539–1544, 1980.
122. Martin PJ, Hansen JA, Siadak AW, Nowinski RC: Monoclonal antibodies recognizing normal human T lymphocytes and malignant human B lymphocytes: A comparative study. J Imm 127:1920–1923, 1981.
123. Dillman RO, Shawler DL, Sobol RE, Collins HA, Beauregard JC, Wormsley SB, Royston I: Murine monoclonal antibody therapy in two patients with chronic lymphocytic leukemia. Blood 59:1036–1045, 1982.
124. Miller RA, Levy R: Response of cutaneous T cell lymphoma to therapy with hybridoma monoclonal antibody. Lancet 2:226–230, 1981.
125. Miller RA, Maloney DG, McKillop J, Levy R: In vivo effects of murine hybridoma monoclonal antibody in a patient with T-cell leukemia. Blood 58:78–86, 1981.
126. Lynch RG, Rohrer JW, Odermatt B, Gebel HM, Autry JR, Hoover RG: Immunoregulation of murine myeloma cell growth and differentiation: A monoclonal model of B cell differentiation. Imm Rev 48:45–80, 1979.

127. Geha RS: Current concepts in immunology: Regulation of the immune response by anti-idiotypic interactions. NEJM 305:25–28, 1981.
128. Stevenson GT, Stevenson FK: Antibody to a molecularly-defined antigen confined to a tumour cell surface. Nature 254:714–716, 1975.
129. Hough DW, Eady RP, Hamblin TJ, Stevenson RF, Stevenson GT: Anti-idiotype sera raised against surface immunoglobulin of human neoplastic lymphocytes. J Exp Med, 144:960–969, 1976.
130. Lanier LL, Babcock GF, Raybourne RB, Arnold LW, Warner NL, Haughton G: Mechanism of B cell lymphoma with passive xenogeneic anti-idiotype serum. J Imm 125:1730–1736, 1980.
131. Miller RA, Maloney DG, Warnke R, Levy R: Treatment of B-cell lymphoma with monoclonal anti-idiotype antibody. NEJM 306:517–523, 1982.
132. Lynch R: Immunoregulation of malignant lymphoma. NEJM 306:543–544, 1982.
133. Oldham RK, Bernhard MI, Key M, Peters LC, Brandhorst J, Hanna MG, Foon KA: Monoclonal antibody serotherapy of solid tumors: A guinea pig model II. In vivo studies. Proc 13th Annual Int Ca Cong, Seattle, WA, pp. 585, 1982.
134. Herlyn D, Herlyn M, Steplewski Z, Koprowski H: Monoclonal antibodies in cell-mediated cytotoxicity against human melanoma and colorectal carcinoma. Eur J Imm 9:657–659, 1979.
135. Herlyn D, Steplewski Z, Merlyn M, Koprowski H: Inhibition of growth of colorectal carcinoma in nude mice by -onoclonal antibody. Ca Res 40: 717–721, 1980.
136. Herlyn D, Koprowski H: Monoclonal anticolon carcinoma antibodies in complement-dependent cytotoxicity. Int J Ca 27: 27:769–774, 1981.
137. Sears HF, Herlyn M: Ex vivo perfusion of tumor-containing colon with monoclonal antibody. J. Surg Res 31:145–50, 1981.
138. Herlyn D, Koprowski H: $IgG2_a$ monoclonal antibodies inhibit human tumor growth through interaction with effector cells. Proc NAS(USA) 79:4751–4765, 1982.
139. Langlois AJ, Matthews T, Roloson GJ, Thiel HJ, Colling JJ, Bolognesi DP: Immunologic control of the ascites form of murine adenocarcinoma 755 V: Antibody-directed macrophages mediate tumor cell destruction. J Imm 126:2337–2341, 1981.
140. Sears HF, Mattis J, Herlyn D, Hayry P, Atkinson B, Ernst C, Steplewski Z, Koprowski H: Phase-I clinical trial of monoclonal antibody in treatment of gastrointestinal tumours. Lancet 1:762–765, 1982.
141. Greaves MF, Brown G, Rapson NT, Lister TA: Antisera to acute lymphoblastic leukemia cells. Clin Imm Immunopath 4:67–76, 1975.
142. Ritz J, Nadler LM, Bahn AK, Notis-McConarty J, Pesando JM, Schlossman SF: Expression of common acute lymphoblastic leukemia antigen (CALLA) by lymphomas of B-cell and T-cell lineage. Blood 58:648–652, 1981.
143. Pesando JM, Ritz J, Levine H, Terhorst C, Lazarus H, Schlossman SF: Human leukemia – Associzted antigen: Relation to a family of surface glycoproteins. J Imm 124:2794–2799, 1980.
144. Ritz J, Pesando JM, Sallan SE, Calvell LA, Notis-McConarty J, Rosenthal P, Schlossman SF. Serotherapy of acute lymphoblastic leukemia with monoclonal antibody. Blood 58:141–152, 1981.
145. Ritz J, Sallan SE, Bast RC, Lipton JM, Clavell LA, Feeney M, Hercend T, Nathan DG, Schlossman SF: Autologous bone marrow transplantation in CALLA positive acute lymphoblastic leukemia following in vitro treatment with J5 monoclonal antibody and complement. Lancet 2:60–63, 1982.
146. Ritz J, Sallan SE, Bast RC, Lipton JM, Nathan DG, Schlossman SF: Utilization of monoclonal antibodies in the diagnosis and treatment of acute lymphoblastic leukemia. Proc 13th Annual Int Ca Cong, Seattle, WA p 9, 1982.

147. Neville DM, Youle RJ: Monoclonal antibody-ricin or ricin A chain hybrids: Kinetic analysis of cell killing for tumor therapy. Imm Rev 62:135–151, 1981.
148. Gilliland DG, Steplewski Z, Collier RF, Mitchell KF, Chang TH, Koprowski H: Antibody-directed cytotoxic agents: Use of monoclonal antibody to direct the action to toxin A chains to colorectal carcinoma cells. Proc NAS(USA) 77:4539–4543, 1980.
149. Mizusawa E, Dahlman HL, Bennett SJ, Goldenberg DM, Hawthorne, MF: Neutron-capture therapy of human cancer: In vitro results on the preparation of boron-labeled antibodies to carcinoembryonic antigen. Proc NAS(USA) 79:3011–3014, 1982.
150. Hurwitz E, Maron R, Bernstein A, Wilchek M, Sila M, Arnon R: The effect in vivo of chemotherapeutic drug-antibody conjugates in two murine experimental tumor systems. Int J Ca 21:747–755, 1978.
151. Jerrels TR, Dean JH, Richardson GL, McCoy JL, Herberman RB: Role of suppressor cells in depression of in vitro lymphoproliferative responses of lung cancer and breast cancer patients. JNCI 61:1001–1009, 1978.
152. Broder S, Waldmann TA: The suppressor-cell network in cancer. NEJM 299: 1281–1284, 1978.
153. Broder S, Waldmann TA: The suppressor-cell network in cancer. NEJM 299: 1335–1341, 1978.
154. Kripke ML: Immunologic aspects of UV carcinogenesis. In: Trends in photobiology, Helene C, Charlier M, Montensy-Garostier T, Laustriat G (eds). New York: Plenum, 1982, pp 235–242.
155. Perry LL, Greene MI: T cell subset interactions in the regulation of syngeneic tumor immunity. Fed Proc 40:39–41, 1981.
156. Enker WE, Jacobitz JL: *In vivo* splenic irradiation eradicates suppressor T cells causing the regression and inhibition of established tumor. Int J Ca 25:819–825, 1980.
157. Reinherz EL, Kung PC, Goldstein G, Schlossman SF: Separation of functional subsets of human T cells by a monocloncal antibody. Proc NAS(USA) 76:4061-4065, 1979.
158. Evans RL, Wall DW, Platsoucar CD, Segal FP, Fiking SM, Testa CM, Good RA: Thymus dependent membrane antigens in man: Inhibition of cell-mediated lympholysis by monoclonal antibodies to T_{H2} antigen. Proc NAS(USA) 78:544–548, 1981.
159. Foon K, Bernhard M, Oldham R: Monoclonal antibody therapy: Assessment by animal tumor models. J Biol Resp Mod. In press, 1982.
160. Oldham R: Monoclonal antibodies as anticancer agents. In: Proc Symp BRM in Hum Oncol and Immun, Friedman H. New York: Plenum Press. In press, 1983.
161. Gery I, Gershon RK, Waksman BH: Potentiation of the T-lymphocytes response to mitogens: The responding cell. J Exp Med 136:128–138, 1972.
162. Mizel SB, Ben-Zvi A: Studies on the role of lymphocyte-activating factor (interleukin 1) in antigen-induced lymph node lymphocyte proliferation. Cell Imm 54:382–389, 1980.
163. Farrar WL, Mizel SB, Farrar JJ: Participation of lymphocyte activating factor (interleukin 1) in the induction of cytotoxic T cell responses. J Imm 124:1371–1377, 1980.
164. Oppenheim JJ, Stadler BM, Siragania RP, Mage M, Mathieson B: Lymphokines: Their role in lymphocyte responses – Properties of interleukin 1. Fed Proc, 41:257–262, 1982.
165. Ruscetti FW, Gallo RC: Human T-lymphocytes growth factor: Regulation of growth and function of T lymphocytes. Blood, 57:379–394, 1981.
166. Mier J, Gallo RC: The characterization of human T-cell growth factor and its role in the proliferation of malignant T-cells. In: Lymphokines and thymic hormones: Their potential utilization in cancer therapeutics, Goldstein A and Chirigos A (eds). New Yrok: Raven Press, 1981, pp 33–48.
167. Gallo RC: Control of T-cell proliferation. Proc 13th Annual Ca Cong, Seattle, WA, p 431, 1982.

168. Mizel SB: Purification to homogeneity of interleukin 1. In: Lymphokines and thymic hormones: Their potential utilization in cancer therapeutics, AL Goldstein and MA Chirigos (eds). New York: Raven Press, 1981, pp 15–20.
169. Lachman LB, George IV. FW, Metzgar RS: Human interleukins 1 and 2: Purification and characterization. In: Lymphokines and thymic hormones: Their potential utilization in cancer therapeutics, AL Goldstein and MA Chirigos (eds). New York: Raven Press, 1981, pp 21–32.
170. Gillis S, Mochizuki D, Conlon PJ, Hefeneidee SH: Biochemical and biological characterization of murine and human interleukien 2. Proc 13th Annual Int Ca Cong, Seattle, WA, p 236, 1982.
171. Gillis S: New reagents for interleukin 2 production and detection. In: Lymphokines and thymic hormones: Their potential utilization in cancer therapeutics, Goldstein AL and Chirigos MA (eds). New York: Raven Press, 1981, pp 57–68.
172. Das SK, Stanley ER, Guilbert LJ, Forman LW: Human colony-stimulating factor (CSF-1) radioimmunoassay, resolution of 3 sub-classes of human colony-stimulating factors. Blood 58:630–641, 1981.
173. Ihle JN, Keller J, Lee JC, Farrar WL, Hapel AJ: Purification and biological properties of interleukin 3. In: Lymphokines and thymic hormones: Their potential utilization in cancer therapeutics, Goldstein AL and Chirigos MA (eds). New York: Raven Press, 1981, 77–94.
174. Farrar JJ, Hilfiker ML: Antigen-nonspecific helper factors in the antibody response. Fed Proc, 41:263–268, 1982.
175. Howard M, Kessler S, Chused T, Paul WE: Long-term culture of normal mouse B lymphocytes. Proc. NAS(USA) 78:5788-5792, 1981.
176. Dumonde DC, Pulley MS, Hamblin AS, Singh AK, Southcott BM, O'Connel D, Paradinas FJ, Robinson MRG, Rigby CC, Den Hollander F, Scheims A, Verbeil H, Van Vhet E: Short term and long term administration of lymphoblastoid cell line lymphokine (LCL-LK) to patients with advanced cancer. In: Lymphokines and thymic hormones: Their potential utilization in cancer therapeutics, Goldstein AL, Chirigos MA (eds). New York: Raven Press, 1981, pp 301–318.
177. Papermaster BW, Gilliland CD, McEntire JE, Rodes NE, Dunn PA: *In vivo* biological studies with lymphoblastoid lymphokines. In: Lymphokines and thymic hormones: Their potential utilization in cancer therapeutics, Goldstein AL, Chirigos MA (eds). New York: Raven Press, 1981, pp 289–300.
178. Fidler I, Berendt M, Oldham R: The rationale for and design of a screening procedure for the assessment of biological response modifiers for cancer treatment. J Biol Resp Mod 1:15–26, 1982.
179. Stolfi R, Martin D: Therapeutic activity of maleic anhydride-vinyl ether copolymers against spontaneous antochtonous murine mammary tumors. Ca Trt Rep 62:1791–1796, 1978.
180. Morahan P, Barnes D, Munson A: Relationship of molecular weight to antiviral and antitumor activities and toxic effects of MVE polyicons. Ca Trt Rep 62:1797–1805, 1978.
181. Dean J, Padarathsingh M, Keys L: Response of murine leukemia to combined BCNU-MVE in *in vitro* growth inhibition assay. Ca Trt Rep 62:1807–1816, 1978.
182. Mohr S, Chirigos M, Fuhrman F, Pryor J: Pyran copolymer as an effective adjuvant to chemotherapy against a murine leukemia solid tumor. Ca Res 35:3750–3754, 1975.
183. Mohr S, Chirigos M, Smith G, Fuhrman F: Specific potentiation of L1210 vaccine by pyran copolymer. Ca Res 36:2035–2039, 1976.
184. Chirigos M, Mastrangelo M: Immunorestoration by Chemicals. In: Immunochemical approaches to cancer therapeutics, Mihich E (ed). Wiley-Interscience 1982, pp 193–230.

185. Barnes D, Morahan P, Loveless S, Munson A: The effects of maleic anhydride-divinyl ether (MVE) copolymers on hepatic microsomal mixed-function oxides and other biological activities. J Pharm Exp Ther 208:392–396, 1978.
186. Bartocci A, Papademetriou V, Chirigos M: Enhanced macrophage and natural killer cell tumor activity by various molecular weight maleic anhydride-divinyl ether. J Immunopharmacaol 2:149–154, 1979.
187. Djeu J, Heinbaugh J, Horden H, Herberman R: Augmentation of mouse natural killer cell antitumor activity by interferon and interferon inducers. J Imm 122:175–179, 1979.
188. Santonni A, Pucceti P, Riccardi C, Herberman R, Bonmasser E: Augmentation of natural killer cell activity by pyran copolymer in mice. Int J Ca 24:656–660, 1979.
189. Rosenblum M, Powell M, Hersh E: Phase I study of MVE-2 therapy in human cancer. Proc ASCO 1:37, 1982.
190. Rinehart J, Wilson H, Neidhart J: Phase I evaluation of MVE-II in man. Proc ASCO 1:37, 1982.
191. Forbes J, Luck A, Greco A: MVE-2: Phase I study and evaluation of biological response. Proc AACR 23:134, 1982.
192. Wiener R: Personal communication.
193. Bicker U: Aziridine dyes: Preclinal and clinical studies. In: Current progress in cancer research and therapy, Hersh E, Chirigos M, Mastrangelo M, (eds). New York: Raven Press, 1981, pp 523–538.
194. Bicker U: Immunomodulating effects of BM 12.531 in animals and tolerance in man. Ca Trt Rep 62:1987–1996, 1978.
195. Stylos WA, Chirigos MA, Papademetriou V, Lauer L: The immunomodulatory effect of BM 12531 (Azimexon) on normal and tumored mice: *In vitro* and *in vivo* studies. J Immunopharmacol 2:113–120, 1980.
196. Bicker V, Hebold G, Ziegler AE, Maus W: Animal experiments on the compensation of the immunosuppressive action of cyclophosphamide by BM 12.531. Exp Pathol 15:49–55, 1978.
197. Matter A: The effects of muramyldipeptide (MDP) in cell-mediated immunity: A comparison between *in vitro* and *in vivo* systems. Ca Imm Immunother 6:201–210, 1979.
198. Taniyama T, Horden H: Direct augmentation of cytolytic acivity of tumor derived macrophages and macrophage cell lines by muramyl dipeptide. Cell Imm 48:369–374, 1979.
199. Tenu J, Lederer G, Petit J: Stimulation of thymocyte mitogenic protein secretion and of cytostatic activity of mouse peritoneal macrophages by trehalose dimycolate and muramyl dipeptide. Eur J Imm 10:647–653, 1980.
200. Tanaka A, Nagoa S, Nagao R, Kotani S, Shiba T, Kusumoto S: Stimulation of the reticuloendothelial system of mice by muramyl dipeptide. Inf & Imm 24:302–307, 1979.
201. Nagao S, Tanaka A, Yamamoto Y, Koga T, Onoue K, Shiba T, Kusomoto K, Kotani S: Inhibition of macrophage migration by muramyl peptides. Inf & Imm 24:308–312, 1979.
202. Wahl S, Wahl L, MacCarthy J, Chedid L, Mergenhagen S: Macrophage activation by mycobacterial water soluable compounds and synthetic muramyl dipeptide. J Imm 122:2226–2231, 1979.
203. Fidler I, Soue S, Fogler W, Barnes Z: Eradication of spontaneous metastases and activation of alvelolar macrophages by intravenous injection of liposomes containing muramyl dipeptide. Proc NAS (USA) 78:1680-1684, 1981.
204. Fidler I, Soue S, Fogler W, Smith D, Braun D, Tarcsey L, Gisler R, and Schroit A: Efficacy of Liposomes containing a lipophilic muramyl dipeptide derivative for activating the tumoricical properties of alveolar macrophages *in vivo*. J Biol Resp Mod 1:43–55, 1982.
205. Najjar VA: Biochemistry and Physiology of Tuftsin – Thr-Lys-Pro-Arg. In: The reticuloen-

dothelial system, Sbarra S and Strauss R (eds). New York: Plenum Pub. Corp., 1980, pp 45–71.
206. Catane R, Schlonger S, Gottlieb P, Halpern J, Treves A, Fuks Z, Friedkin M: Toxicology and antitumor activity of tuftsin in mice. Proc AACR 22:371, 1981.
207. Nishioka K, Amoscato AA, Babcock GA: Tuftsin: A hormone like tetrapeptide with antimicrobial and antitumor activities. Life Sci 28:1081–1090, 1981.
208. Najjar VA, Chandhurri M, Konopincka D, Beck B, Layne P, Lineharr L: Tuftsin (Thr-Lys-Pro-Ang) a physiological activator of phagocytic cells: A possible role in cancer therapy. In: Augmenting agents in cancer therapy, Hersh E, Chirigos M, Mastrangelo M, (eds). New York: Raven Press, 1981, pp 459–478.
209. Florentin I, Bruley-Rosset M, Kiger N, Umbach J, Winternitz F, Mathe G: *In vivo* stimulation by tuftsin. Ca Imm Immunother 5:211–216, 1978.
210. Phillips J, Babcock G, Nishioka K: Tuftsin a naturally occurring immunopotentiating Factor I: In vitro enhancement of murine natural cell-mediated immunity. J Imm 126:915–921, 1981.
211. Chirhara G: The antitumor polysaccharide lentinan: An overview. In: Manipulation of host defense mechanisms, Aoki T, Urushizaki I, Tsubura E (eds) New York: Excerpta Medica, 1981, pp 1–16.
212. Aoki T, Miyakoski H, Horikawa Y, Yoshimaru Y: Staphage lysate and lentinan as immunomodulators and/or immunoptentiators in clinical and experimental systems. In: Progress in cancer research and therapy, Hersh E, Chirigos M, Mastrangelo M (eds). New York: Raven Press, 1981, pp 101–112.
213. Chihara G, Maeda YY, Hamuro J, Sasaki T, Fukuoka F: Inhibition of mouse sarcoma 180 by polysaccharides from Lentius edodes (Berk). Sing. Nature, 222:687–688, 1969.
214. Chihara G, Hamura J, Maeda YY, Arai Y, Fukuoka F: Fractionation and purification of the polysaccharides with marked antitumor activity, especially Lentinan, from Lentinus edodes (Berk.) Sing. (an edible mushroom). Ca Res 30:2776–2781, 1970.
215. Maeda YY, Hamuro J, Yamada YO, Ishimura K, Chichara G: The nature of immunopotentiation by the antitimour polysaccharide Lentinan and the signifiancance of biogenic amines in its action. In: Immunopotentiation, Wolstenholme GEW, Knight J (eds). Ciba Found. Symposium, new series 18. Amsterdam: Elsevier, Excerpta-Medica, North-Holland, 1973, pp 259–268.
216. Zakany J, Chirhara G, Fachet J: Effect of Lentinan on tumour growth in murine allogenic and syngeneic hosts. Int J Ca 25:371–376, 1980.
217. Zakany J, Chihara G, Fachet J: Effect of Lentinan on the production of migration inhibitory factor induced by syngeneic tumor in mice. Int J Ca 26:783–788, 1980.
218. Hamada C: Inhibitory effects of lentinan on the tumorigenesis of adeno-virus type 12 in mice. In: Manipulation of host defense mechanisms, Aoki T, Urushizaki I, Tsubura E (eds). New York: Excerpta Medica, 1981, pp 76–87.
219. Maeda YY, Chihara G: Lentinan, a new immuno-accelerator of cell-mediated responses. Nature 229:634, 1971.
220. Maeda YY, Hamuro J, Chihara G: The mechanism of antitumour polysaccharides: The effect of antilymphocyte serum on the antitumour activity of Lentinan. Int J Ca 8:41–46, 1971.
221. Maeda YY, Chihara G: The effects of neonatal thymectomy on the antitumour activity of Lentinan, carboxymethylpachymaran and zymosan, and their effects on various immune responses. Int J Ca 11:153–161, 1973.
222. Maeda YY, Chihara G: Periodical consideration of establishment of antitumour action in host and activation of peritoneal exudate cell by Lentinan. Gann Jap J Ca Res 64:351–357, 1973.

223. Dresser DW, Phillips JM: The orientation of the adjuvant activities of Salmonella typhosa lipopolysaccharide and Lentinan. Imm 27:895–902, 1974.
224. Dennart G, Tucker D: Antitumor polysaccharide Lentinan, a T cell adjuvant. JNCI 51:1727–1729, 1973.
225. Hamuro J, Rollinghoff M, Wagner H: Induction of cytotoxic peritoneal exudate cells by T cell immune adjuvants of the B(1,3)-glucan-type Lentinan and its analogues. Imm 39:551–559, 1980.
226. Hamuro J, Rollinghoff M, Wagner H: B(1,3)-glucan-mediated augmentation of alloreactive murine cytotoxic T-lymphocytes *in vivo*. Ca Res 38:3080–3085, 1978.
227. Hamuro J, Wagner H, Rollinghoff M: B(1,3)-glucans as a probe for T cell specific immune adjuvants: II. Enhanced *in vitro* generation of cytotoxic T lymphocytes. Cell Imm 38:328–335, 1978.
228. Sendo F, Siji K, Fuyama S, Wztabe S, Arai S: Augmentation of natural cell mediated cytotoxicity by administration of lentinan in mice. In: Manipulation of host defense mechanisms, Aoki T, Urushizaki I, Tsubura E (eds). New York: Excerpta Medica, 1981, pp 178–187.
229. Herberman R, Nunn-Hargrove M: Augmentation of natural killer (NK) cell activity by lentinan. In: Manipulation of host defense mechanisms, Aoki T, Urushizaki I, Tsubura E (eds). New York: Excerpta Medica, 1981, pp 188–189.
230. Akiyama Y, Kashima S, Hayami T, Izawa M, Mitsugi K, Hamuro K: Immunological characteristics of antitumor polysaccharides, lentinan and its analogs as immune adjuvants. In: Manipulation of host defense mechanisms, Aoki T, Urushizaki I, Tsubura E (eds). Excerpta Medica, 1981, pp 222–243.
231. Falk R, Makowka L, Nossal N, Falk J, Fields J, Asculai S: Modulation of the immune response and control of tumour spread by a new synthetic compound. Br J Surg 66:861–863, 1979.
232. Falk R, Makowka L, Nossal N, Falk J, Rotstein L, Fields J, Asculai S: Mechanism of increased antitumor immunity by a synthetic polymer. J Surg Res 28:485–491, 1980.
233. Makowka L, Falk R, Nossal N, Rotstein L, Falk J: Activation of the immune system to control experimental neoplastic growth by NED-137. In: Augmenting agents in cancer therapy, Hersh E, Chirigos M, Mastrangelo M (eds). New York: Raven Press, 1981, pp 295–311.
234. Okamoto H, Shoin S, Koshimura S, Shimizu R: Studies on the anti-cancer and Streptolysin S-forming abilities of hemolytic streptococci: A review. Jap J Microbial 11:323–331, 1967.
235. Sakurai Y, Tsukagoshi S, Satoh H, Akiba T, Suzuki S, Takagaki Y: Tumor-inhibitory effect of a streptococcal preparation (NSC-B116209). Ca Chemo Rep 56:9–17, 1972.
236. Hoshino A, Kato T, Amo H, Ohta K: Antitumor effects of OK-432 on resistant cell lines of Yoshida sarcoma and L-1210 leukemia. Chemotherapy 20:598–602, 1972.
237. Aoki T, Kvedar JP, Hollis VW, Jr, Bushar GS: Brief communication: Streptococcus pyogenes preparation OK-432: Immunoprophylactic and immunotherapeutic effects on the incidence of spontaneous leukemia in AKR mice. JNCI 56:687–690, 1976.
238. Matsubara S, Suzuki F, Ishida N: Induction of Immune interferon in mice treated with a bacterial immunopotentiator, OK-432. Ca Imm Immunother 6:41–45, 1979.
239. Osaki T, Hamada T, Kurimura T, Hiramo A, Hama S: Effect of streptococcal preparation (Picibanil) on interferon production in oral squamous cell carcinoma patients. Yonago Acta Medica 22:100–105, 1979.
240. Oshimi K, Kano S, Takaku F, Okumura K: Augmentation of natural killer cell activity by a streptococcal preparation, OK-432. JNCI 65:1265–1269, 1980.
241. Uchida A, Micksche M: In vitro augmentation of natural killing activity by OK-432. Int J Immunopharmacol 3:365–375, 1981.

242. Oshimi K, Wakasugi H, Seki H, Kano S: Streptococcal preparation OK-432 augments cytotoxic activity against an erythroleukemic cell line in humans. Ca Imm Immunother 99:187–191, 1980.
243. Wakasugi H, Oshimi K, Miyata M, Morioka Y: Augmentation of natural killer cell activity by a streptococcal preparation, OK-432, in patients with malignant tumors. J Clin Imm 1:154–162, 1981.
244. Talmadge J, Fidler I: Personal communication.

8. Therapy of Established Tumors by Adoptive Transfer of T Lymphocytes

PHILIP D. GREENBERG, MARTIN A. CHEEVER and ALEXANDER FEFER

1. INTRODUCTION

The demonstrations that tumor cells express surface antigens which can serve as targets for immunologic attack have long enticed laboratory and clinical investigators into attempts at manipulating the immune system to promote *in vivo* tumor destruction. Modulation of the immune system of a tumor-bearing host has been attempted by a variety of experimental approaches, from highly nonspecific by administering chemical immunoadjuvants which enhance general immunologic reactivity to highly specific by infusing purified monoclonal antibodies or cloned T cells which recognize and react to only a single antigenic determinant on tumor cells. Unfortunately, although tumor cells can be readily killed *in vitro* by many distinct immunologic effector mechanisms, attempts to amplify and utilize the same effector mechanisms *in vivo* for the therapy of established tumors has been difficult in animal models and generally unsuccessful in human tumor therapy. The difficulties encountered in treating established tumors have served to highlight the need for developing and studying animal models in which the individual parameters for successful immunotherapy can be isolated and examined, the immunologic mechanisms potentially operative *in vivo* for lysis of established tumor can be elucidated and amplified, and the factors which limit the efficacy of immunotherapy can be identified and eliminated. In this chapter, studies in animal models from our and other laboratories pertaining to one method of modulating the immune system of the host to facilitate tumor destruction will be reviewed – adoptive cellular immunotherapy with immune T cells.

Adoptive immunotherapy as a means for treating tumors is based on the suppositions that tumor cells express surface antigens which can serve as targets for immunologic attack, that lymphocytes recognizing these antigens can be obtained in sufficient number, and that adoptive transfer of these

R.B. Herberman (ed), Basic and Clinical Tumor Immunology. ISBN-13: 978-1-4613-3875-8
© 1983. Martinus Nijhoff Publishers, Boston.

antigen-specific lymphocytes into a tumor-bearing host can mediate an antitumor effect without undue toxicity to the host. During the last decade, animal models have been developed in which adoptively transferred immune cells can reproducibly mediate an *in vivo* therapeutic effect against established tumors, and recent advances in murine immunology have provided the tools to rigorously analyze these models. Parallel technologic advances in human immunology have created optimism for the eventual clinical application of cellular immunotherapy to the treatment of human tumors. These advances have included: (a) methods to isolate and purify subpopulations of potential effector cells on the basis of phenotypic expression of antigens and receptors on the cell membrane [1, 2], (b) methods to generate large numbers of potential effector T cells by *in vitro* sensitization of lymphocytes to tumor cells and numerical expansion of reactive cells by reculture in medium containing T cell growth factors such as IL 2 [3–5], (c) methods to modulate *in vivo* immunoregulatory circuits which potentially limit the efficacy of adoptively transferred cells [6–9], (d) demonstrations that some human tumors express tumor-associated antigens (TAA) which can be immunogenic and serve as targets for tumor lysis [4, 10, 11], (e) the demonstration in animal models that normal differentiation antigens expressed on tumor cells rather than only tumor-specific antigens can serve as targets for specific immunotherapy without excessive host toxicity [12] and (f) the demonstration that a graft-versus-leukemia reaction may contribute to tumor eradication following human allogeneic marrow transplantation, confirming that under appropriate conditions therapy of human tumors can be facilitated by adoptive transfer of immune effector cells which recognize antigens expressed on the tumor cells [13, 14].

In this chapter the principles of and prerequisites for successful adoptive immunotherapy of established tumors as elucidated by studies in animal models will be examined. Tumor therapy models using adoptive transfer of syngeneic immune lymphocytes have been the most thoroughly investigated and will be discussed in detail, since these represent prototypes of what might be accomplishable with appropriately selected and amplified tumor-reactive autologous lymphocytes obtained from a tumor-bearing host. Tumor therapy with adoptively transferred allogeneic lymphocytes has been less well studied and poses unique problems, including toxicity to the host from graft-versus-host disease and a diminished antitumor effect due to rejection of the transferred cells by the host. However, since alloreactive cells by definition recognize a variety of antigens expressed on the host tumor cells and possess a clear therapeutic potential which might be translatable to the clinical setting, the experimental results and principles underlying adoptive therapy with allogeneic cells will also be reviewed.

2. ADOPTIVE IMMUNOTHERAPY WITH SYNGENEIC LYMPHOCYTES

Adoptive immunotherapy models in which the lymphocyte donor, tumor-bearing host, and the tumor are all syngeneic permit analysis of the underlying mechanisms and therapeutic efficacy without the complications presented by incompatibility between donor and host. Syngeneic models have generally utilized transplantable tumors with well-described growth patterns and immunogenicity, and donor lymphocytes from normal animals which have been rendered immune by *in vivo* and/or *in vitro* sensitization to immunogenic tumor-associated antigens. Clinical trials of analogous adoptive cellular therapy in humans have generally been unsuccessful and presumably premature, since it has been difficult to identify immunogenic determinants on human tumor cells which can induce and serve as targets for autologous immune effector cells [15]. However, the recent technologic advances in clinical immunology, which permit isolation of purified subpopulations of lymphocytes and identification and expansion of antigen-reactive cells, support the hope that it may be possible in the future to select from a tumor-bearing patient a small number of potential effector cells, which have been limited in quantity in the host because the tumor is weakly immunogenic and/or induces dominant immunosuppression, and expand these cells to large numbers such that reinfusion into the host exerts an antitumor effect.

2.1 *Adoptive transfer of syngeneic lymphocytes as the only form of therapy*

Lymphocytes have been shown to inhibit the *in vivo* growth of transplanted tumors when administered shortly before, with, or shortly after the inoculation of tumor. However, even under optimal conditions, when the tumor is immunogenic and sensitive to immune-mediated lysis and large numbers of immune lymphocytes can be obtained and administered, it is rarely possible to obtain a significant antitumor effect from adoptive transfer of cells as the sole form of therapy of an established tumor. These disappointing therapeutic results have reflected, at least in part, biologic consequences of a growing tumor, such as a large tumor burden and induction of suppressor cells and/or suppressor factors in the host [16–18]. Nevertheless, occasional impressive complete regressions of advanced tumors have been reported after treatment with syngeneic immune lymphocytes alone, and these studies have helped identify some of the problems which limit such a therapeutic approach.

Although the precise problems associated with a large tumor burden have not all been well-defined, it is evident that immune cells have great difficulty eradicating a large tumor load *in vivo*. In an early report of successful

immunotherapy, mice bearing an established small, 6 to 10 mm, highly immunogenic sarcoma were treated with syngeneic cells from multiply immunized donors [19]. Despite these conditions, a total dose of less than 5×10^8 lymphocytes (i.e. approximately the number contained in the spleens of 5 donor mice) was ineffective. Moreover, the efficacy of cell doses of 5×10^8 to 4×10^9 infused over several days was primarily reflected by only a slowing of tumor progression rather than tumor regression. Thus, even enormous numbers of immune lymphocytes had only minimal activity against a small established tumor. In studies examining immunotherapy of established transplanted intradermal tumors with local lymph node metastases, immune lymphocytes became decreasingly effective if administered either after a large initial tumor inoculum or if adoptive transfer was delayed until the host had a larger tumor burden [20]. Similar difficulties have also been noted in our laboratory in adoptive immunotherapy of FBL-3, a Friend virus-induced erythroleukemia of C57BL/6 origin [21]. Mice inoculated with 10^4 FBL-3 on day 0 were cured by infusion of 5×10^6 syngeneic immune cells on day 1, but, if treatment was delayed until day 3, even 10^8 immune cells exerted no effect. Since FBL-3 has a doubling time of approximately 24 hours, the predicted ratio of effector cells to tumor targets would be 150:1 on day 1 when therapy was effective and 1250:1 on day 3 when therapy was no longer effective. Thus, the obstacles presented by a large tumor burden reflect not only quantitative considerations, but qualitative problems induced by the growing tumor.

One major biologic consequence of an established growing tumor is the induction of suppressor T cells in the host. Recent studies, comparing adoptive immunotherapy in normal hosts and hosts which have been rendered T deficient by adult thymectomy followed by lethal irradiation and reconstitution with T-depleted bone marrow, have convincingly demonstrated that suppressor T cells present in the host interfere with the adoptive transfer of tumor immunity. Although immunotherapy was effective if cells were infused into normal recipients bearing a methylcholanthrene-induced fibrosarcoma early after tumor implantation, immune cells were no longer effective if administered 7 days after tumor inoculation. By contrast, adoptive transfer of immune cells 7 days after tumor inoculation into T deficient hosts resulted in complete rejection of large tumors [16, 22]. Moreover, the efficacy of adoptive immunotherapy in these T deficient recipients was abrogated by infusion of T cells obtained from normal recipients with a growing tumor. The presence of suppressor T cells in tumor-bearing hosts which inhibit adoptive immunotherapy was similarly demonstrated in studies of therapy of a disseminated syngeneic mastocytoma in normal and T deficient mice [23]. This interpretation that progressive tumors induce suppressor T cells which inhibit adoptive therapy is consistent with observa-

tions of successful immunotherapy of established tumors with lymphocytes alone in several other models, such as palpable Moloney sarcoma virus-induced tumors growing in immunologically suppressed or T-depleted adult mice [24, 25], and progressive fibrosarcomas growing in hosts which had been rendered relatively T deficient by irradiation or cyclophosphamide prior to tumor implantation [26, 27].

These few positive studies and the much larger number of negative studies attest to the difficulty encountered in attempting to achieve a therapeutic benefit from adoptive transfer of immune cells as the sole form of therapy of established antigenic tumors. The resistance of growing tumors to eradication by the immune system reflects central inhibition of effector cell expression and expansion by suppressor cells as described above, as well as other factors which might operate locally or centrally. For example, growing tumors have been shown to produce factors which locally interfere nonspecifically with immunologic effector functions - in essence, establishing a growing tumor nodule as a relatively immunologically-privileged site [28]. Thus, in addition to providing or promoting immune effector cells, successful immunotherapeutic approaches have generally required that the underlying host-tumor relationship be modified prior to adoptive transfer of lymphocytes.

2.2 *Adoptive immunotherapy following reduction of tumor load*

Since adoptive immunotherapy potentially provides unique antitumor specificity but is limited, at least in part, by the size of the tumor load and/or the associated immunologic suppression, immunotherapy might be best used to specifically destroy residual tumor cells remaining after cytoreductive surgery, radiation, or chemotherapy. Moreover, such modification of the host-tumor relationship prior to adoptive therapy might theoretically uncover potential benefits of immunotherapy which previously were undetectable due to consequences of the large tumor burden.

Effective adoptive immunotherapy of micrometastatic pulmonary lesions has been reported following surgical removal of a growing primary implant of Lewis lung carcinoma (3LL) cells [29]. This experimental setting was somewhat similar to clinical settings for which adjuvant therapy is considered, in that, following removal of the primary 3LL tumor growing in the foot pad, no residual disease was detectable but most mice eventually died from outgrowth of microscopic pulmonary metastases. Therefore, one day following surgery, mice received no additional therapy or infusion of either normal syngeneic lymphocytes, lymphocytes sensitized to syngeneic fibroblasts, or lymphocytes sensitized *in vitro* to syngeneic 3LL tumor cells. Mice treated with cells sensitized to 3LL had a significantly lower incidence of pulmonary metastases and better survival than the other treatment groups.

Of interest, although *in vitro* sensitization of 3LL cells rendered responder cells active in immunotherapy, syngeneic responder lymphocytes obtained from normal donors were more effective than responder cells obtained from tumor-bearing hosts. Potentially, this could result from expression in a host bearing a progressive tumor of specific and nonspecific immunologic suppression which can interfere with the generation and detection of antitumor effector cells. Thus, although surgical removal of a primary tumor may facilitate immunotherapy of micrometastatic disease, purification of specific lymphocyte effector populations, and, possibly selective expansion *in vitro*, may be important if cells from a tumor-bearing host are to be used for therapy of autochthonous tumor.

Radiation therapy as an adjunct to adoptive immunotherapy with specifically immune lymphocytes has not been extensively studied. Most studies utilizing radiation therapy in animal models have used supralethal doses of total body irradiation plus rescue by infusion of syngeneic marrow cells to repopulate the hematopoietic and immune systems. This approach has been effective against some murine hematologic malignancies, particularly if instituted shortly after tumor inoculation and if lymph node cells were infused with bone marrow cells [30, 31]. Unfortunately, no attempts were made in these studies to increase the potential therapeutic effectiveness of the syngeneic lymphoid cells by using lymphocytes immune to tumor, and thus it is difficult to assess whether the infused cells which reconstituted the host in these models provided any antitumor effect. Radiation therapy has not been pursued in animal models for therapy of established disease in part due to practical reasons - systemic or local radiotherapy is time consuming and difficult to deliver in a uniform fashion to rodents in comparison to other cytoreductive modalities. However, for application to treatment of human tumors, radiotherapy prior to adoptive cell transfer should theoretically be very useful - firstly, since many tumors are radiosensitive, it should reduce the tumor burden; and secondly, since suppressor T cells are radiosensitive, it might enhance the adoptive transfer of tumor immunity. For example, adoptive immunotherapy of an established fibrosarcoma in rats was more effective in hosts that were sublethally irradiated prior to tumor implantation than intact tumor-bearing hosts, presumably due to ablative radiation effects on the host suppressor cells [26].

Cytoreductive chemotherapy as an adjunct to immunotherapy has been the most extensively studied combined modality approach. Chemotherapeutic agents, which can destroy a large tumor load, have relative nonspecificity for target tissues, and thus are generally limited in use or dose by toxicity to normal host tissues. Since syngeneic lymphocytes immune to tumor-associated antigens have little if any toxicity for normal host tissues but are generally limited by the inability to eliminate a large tumor load,

therapy with both chemotherapy and immune cells offers the opportunity for maximizing the therapeutic potential of each modality while overcoming the individual limitations. Thus, animal models have been developed in which established syngeneic tumors can be eradicated by a combination of nonlethal noncurative chemotherapy plus lymphoid cells immune to tumor antigens. This approach has been successful in several strains of mice against a variety of advanced syngeneic tumors including Moloney leukemia [32], L1210 leukemia [33, 34], Friend leukemia [35], EL-4 lymphoma [36, 37], Rauscher leukemia [38], and Meth A sarcoma [27], as well as against L_2C leukemia in guinea pigs [39]. These adoptive chemoimmunotherapy models, as described in the next section, have been very useful for elucidating the requirements for effective immunotherapy and for analyzing the mechanisms by which immune cells facilitate *in vivo* eradication of tumors.

2.3 *Adoptive chemoimmunotherapy (ACIT)*

Several murine models have been developed in our laboratory for the treatment of established disseminated syngeneic leukemias and lymphomas by chemotherapy with cyclophosphamide followed by adoptively transferred syngeneic immune cells [21]. In these adoptive chemoimmunotherapy

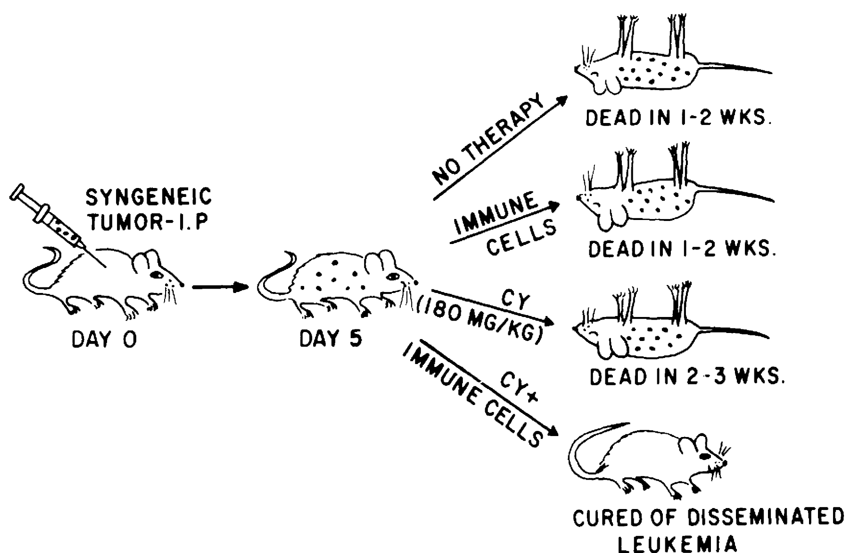

Figure 1. Adoptive chemoimmunotherapy (ACIT). Mice are inoculated with syngeneic tumor intraperitoneally. Five days later, after tumor has disseminated to blood and spleen, therapy with immune cells alone has no antitumor effect, chemotherapy with cyclophosphamide prolongs survival but cures no mice, and combination therapy with cyclophosphamide (CY) and syngeneic cells immune to the tumor can cure mice of disseminated disease.

(ACIT) models, mice are inoculated with syngeneic tumor intraperitoneally on day 0 (Figure 1). Five days later, tumor growth and dissemination can be readily demonstrated by bioassay of peripheral blood or spleen for tumor cells [40]. Mice receiving no therapy on day 5 develop progressive ascites, splenomegaly and lymphadenopathy and die within 1 to 2 weeks. Treatment on day 5 with immune cells alone, even in large numbers, has no apparent *in vivo* antitumor effect. Chemotherapy on day 5 with 180 mg/kg cyclophosphamide (CY) prolongs survival for 2 to 3 weeks, but cures no mice. By contrast, therapy on day 5 with CY followed in 6 hours by immune cells can eradicate tumor and cure mice.

The mechanisms of tumor elimination by ACIT, the nature and specificity of the effector cells, and the factors which can interfere with the efficacy of ACIT have been most extensively analyzed in the treatment of disseminated FBL-3, a Friend virus-induced leukemia of C57BL/6 origin. FBL-3 was selected for study for several reasons – it grows reproducibly and is fatal in syngeneic hosts; it induces specific T cell immunity following both *in vivo* and *in vitro* sensitization with tumor cells [37]; it is sensitive to the tumoridical activity of cyclophosphamide [40]; and it possesses immunogenic tumor-associated antigens which cross-react with antigens on other tumors induced by Friend, Moloney, or Rauscher (FMR) viruses [41].

2.3.1 *Kinetics of tumor elimination by ACIT*

Early studies of ACIT demonstrated that tumor eradication was dependent upon infusion of immune T cells which could proliferate in the host after adoptive transfer [21]. Since the requirement for donor T cell proliferation in the host might reflect an immunologic requirement for a prolonged *in vivo* antitumor response, tumor-bearing mice were examined at different time points before or after therapy for the presence of disseminated tumor by bioassay of peripheral blood or spleen cells [40]. Bioassay confirmed that leukemia was widely disseminated at the time of therapy on day 5, and that cyclophosphamide (CY) significantly reduced the tumor burden by a direct tumoricidal effect. Therapy with CY and immune cells resulted in a greater initial reduction of tumor cells than CY alone; however, lysis was incomplete and, even in mice which would ultimately be cured by the therapy with CY and immune cells, a 2 to 3 week period of subclinical tumor regrowth was observed before the tumor was completely eliminated. Rejection of the residual growing tumor cells was immunologically mediated, since mice immunosuppressed two weeks after ACIT by inoculation of antithymocyte serum exhibited lethal tumor recurrences. Thus, effective tumor therapy not only requires adoptive transfer of immune T cells, but these cells must be capable of persisting in the host and mediating a prolonged antitumor effect.

The prolonged time period required for complete tumor elimination by immune cells does not appear to be limited to the ACIT models studied in our laboratory, but rather may reflect a general principle of adoptive immunotherapy. Following adoptive therapy with immune cells which eventually resulted in complete elimination of an established rat sarcoma, an established murine fibrosarcoma, and a disseminated murine mastocytoma, measurable tumor growth continued for one to two weeks after cell transfer before the onset of tumor rejection became apparent [16, 23, 26]. Although the time required for tumor elimination in these models might partially reflect the quantity of immune cells administered, high cell doses were used in all the experiments described above. Moreover, it is likely that the observed kinetics reflect at least in part the necessity for infused cells to traffic to and establish operational effector mechanisms at sites of tumor growth. Recognition of this prolonged time period required for complete tumor eradication may be extremely important, since it may be possible intentionally or otherwise to either enhance or interfere with the efficacy of immune cells after cell transfer.

2.3.2 *Specificity of immune cells for tumor and major histocompatibility antigens*

Immunotherapy of tumors by ACIT has been accomplished with both *in vivo* and *in vitro* sensitized cells. The therapeutic effect of the lymphoid cells has been assumed to result from specific immunization of T cells, since cells immune to tumor-associated antigens were significantly more effective than nonimmune cells, cells immune to unrelated antigens, or tumor-immune cells depleted of T cells [21, 35]. Similar observations have been made in other immunotherapy models [20, 29, 42].

Rigorous proof of immunologic specificity, however, requires controls demonstrating that the cells effective in therapy of the immunizing tumor are ineffective against an antigenically distinct non-crossreactive tumor which can be similarly eradicated by appropriately directed immunotherapy. In ACIT in C57BL/6 mice, treatment of FBL-3, a Friend virus-induced tumor, was compared to EL-4(G-), a dimethylbenzanthracene carcinogen-induced lymphoma. These two tumors of C57BL/6 origin are antigenically distinct, since spleen cells from mice immunized *in vivo* with FBL-3 or EL-4(G-) and reexposed to the sensitizing tumor *in vitro* displayed cytotoxic reactivity specific for the immunizing tumor [37]. Moreover, immune spleen cells were effective only in ACIT of the sensitizing tumor. These studies not only demonstrated that the effector cells active in ACIT are immunologically specific T cells, but also the corollary that relatively unique tumor-associated antigens expressed on tumor cells can be targets for tumor-specific immunotherapy. Similar studies in immunotherapy of chem-

ically-induced and virus-induced sarcomas in BN rats have confirmed the requirement for immunologically specific T cells [42, 43].

Another aspect of T cell recognition of membrane antigens must be considered when assessing T cell specificity for tumor cells. According to the concept of H-2 restriction of T cell function, T cells are not sensitized to isolated tumor antigens but rather are sensitized to such tumor antigens in association with major histocompatibility complex (MHC) antigens expressed on the immunizing cell – the immune T cells subsequently recognize only tumor targets expressing both the tumor and MHC antigens present during sensitization [44]. Although lysis of tumors by cytotoxic T cells (CTL) has been shown to be H-2 restricted in 4-hour *in vitro* assays, prolongation of the *in vitro* assays for 12 to 20 hours has permitted detection of apparent tumor antigen specific nonrestricted lysis [45–48]. Since tumor eradication by ACIT requires a prolonged time period and should potentially permit protracted interactions between effector and target cells, these studies raised questions of whether H-2 restriction was important for tumor therapy. Therefore, to examine the role of H-2 restriction in adoptive immunotherapy and avoid problems imposed by allogeneic effects, models were developed for ACIT in (BALB/c × C57BL/6) F_1 hybrid mice of parental strain tumors induced by Moloney virus (LSTRA of BALB/c origin) and Friend virus (FBL-3 of C57BL/6 origin). These FMR virus-induced parental strain tumors are disparate at H-2 but express cross-reactive tumor-associated antigens [41, 49]. The results demonstrated that effector cells operative in ACIT recognize tumor antigens only in the context of an MHC product present on the immunizing cell [50]. These studies affirm not only that tumor eradication *in vivo* is H-2 restricted, but emphasize the importance of the MHC antigens expressed on the immunizing cell. Thus, if tumors expressing immunogenic tumor-associated antigens are to be used for generating cells for tumor therapy, the sensitizing tumor will have to either be MHC identical to the tumor target or the tumor antigen will need to be isolated and processed by a syngeneic macrophage for sensitization so that it is presented to T cells in the context of appropriate MHC determinants.

2.3.3 *Effector cells operative in ACIT*

Although immune destruction of tumor cells *in vitro* has been demonstrated to be mediated by macrophages, T cells, B cells and natural killer (NK) cells, the critical effector cell required for *in vivo* adoptive immunotherapy in all the established tumor models tested so far has been a T cell [16, 21, 25, 26]. In ACIT, the efficacy of immune cells is not diminished by passage over nylon wool to remove B cells and macrophages or by passage through a magnetic field after carbonyl iron ingestion to further deplete

phagocytic cells, but the therapeutic activity is ablated by removal of T cells [51]. These studies, while demonstrating a central role for immune T cells, do not necessarily imply that only T cells participate in tumor destruction *in vivo* following adoptive therapy, but rather that non-T effector cells are not limiting tumor elimination in the absence of enhanced T cell immunity. It should be noted that, in an ACIT model in which mice were inoculated on day 0 with several log fewer tumor cells than the standard therapy model, a small *in vivo* antitumor effect was detected following transfer of NK-like effector cells; however, even in this setting of minimal tumor burden, immune T cells remained dramatically more effective than NK cells [52]. This NK-like therapeutic activity was no longer detectable with a larger initial tumor inoculum. Thus, it is conceivable that marked augmentation of the reactivity of cell subpopulations other than T cells might uncover previously unidentified therapeutic potential. However, transfer of immune T cells results in readily demonstrable activity in tumor therapy and consequently T cells have been the most extensively studied effector population.

The mechanisms by which immune T cells effect tumor destruction *in vivo* have only recently been examined, and appear more complex than initially expected. Although it had simplistically been assumed that tumor eradication following ACIT reflected destruction of a large fraction of the tumor by drug followed by rapid killing of the residual tumor cells by infused cytotoxic donor cells, tumor eradication was demonstrated to require several weeks for completion [40], and the efficacy of immune cells in therapy did not correlate with either the immediately detectable cytolytic activity or the cytolytic potential revealed after reexposure to tumor antigens by *in vitro* sensitization [53]. Moreover, although the cytotoxicity of immune cells measured in a 4-hour *in vitro* assay was not diminished by γ-irradiation with 1000 R, such irradiation rendered the immune population unable to proliferate and ineffective in ACIT [54].

The above findings prompted an analysis of the role of T cell subpopulations, as defined by Lyt phenotype, in ACIT. Expression of Lyt antigens has been shown to be useful for separating functional T cell subsets [1, 55]. By negative selection using cytotoxic depletion with antibody and complement, T cells can be operationally separated into Lyt 1^+2^- cells containing the amplifier/inducer, helper, and delayed type hypersensitivity (DTH) effector cells, Lyt 1^-2^+ cells containing cytotoxic and suppressor cells, and an Lyt 1^+2^+ compartment containing precursors for the other subsets as well as a portion of cytotoxic and suppressor effector cells [1, 55]. In particular, cytotoxic T cells (CTL) to syngeneic tumors appear to reside in both the Lyt 1^-2^+ and Lyt 1^+2^+ compartments [56-60]. Analysis of the T cell

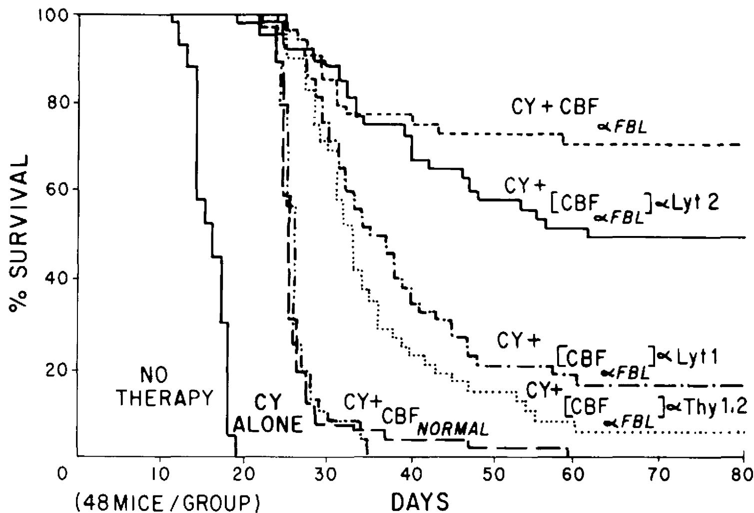

Figure 2. Efficacy of T cell subpopulations in adoptive chemoimmunotherapy (ACIT) of disseminated FBL-3 leukemia. (BALB/c × C57BL/6)F_1 mice, denoted CBF, were inoculated with 5×10^6 FBL-3 intraperitoneally on day 0 and left untreated (— No Therapy), treated on day 5 with 180 mg/kg of cyclophosphamide (— ·· — CY Alone), or treated with CY plus 5×10^6 CBF donor spleen cells obtained either form normal nonimmune CBF mice (— CBF_{NORMAL}) or CBF mice that had been primed and boosted *in vivo* with irradiated FBL-3. Donor immune spleen cells were used either unseparated (- - - $CBF_{\alpha FBL}$) or after depletion of subpopulations by *in vitro* incubation prior to adoptive transfer with complement and antibody to Thy 1.2 (··· [$CBF_{\alpha FBL}$]αThy 1.2), Lyt 1 (— · — [$CBF_{\alpha FBL}$]αLyt 1), or Lyt 2 (— $CBF_{\alpha FBL}$]αLyt 2).

subsets reactive to the FBL-3 tumor used in our models confirmed that an Lyt 1^+2^- amplifier/inducer T cell was necessary for the generation of CTL, while the actual cytolytic cells were of the Lyt 1^-2^+ and Lyt 1^+2^+ phenotypes [61].

A graphic depiction of the results of ACIT experiments, in which the role of T cell subpopulations in therapy was examined, is shown in Figure 2 [61]. (BALB/c × C57BL/6)F_1 hosts, denoted CBF, were inoculated with the parental strain tumor, FBL-3, and treated 5 days later with CY plus immune cells. Donor immune cells were generated by priming and boosting CBF donor mice *in vivo* with irradiated FBL-3 tumor cells, and the spleen cells were used in therapy either unfractionated or depleted of selected subpopulations by incubation with complement and monoclonal antibody to Thy 1.2, Lyt 1 or Lyt 2. Mice receiving no therapy died by day 19, and, although therapy on day 5 with CY alone or CY plus normal nonimmune spleen cells prolonged survival, all mice died of progressive tumor. Therapy with CY plus unfractionated immune spleen cells further prolonged survival and cured 71% of mice. Depletion of T cells from the immune population by *in vitro* incubation with αThy 1.2 and complement prior to adoptive

transfer abrogated most of the therapeutic effect. The small residual antitumor effect observed with T-depleted immune cells probably reflected incomplete removal of all T cells, since previous studies have demonstrated that neither B cells nor macrophages are responsible for the observed therapeutic activity [51]. Immune cells depleted of Lyt 1^+ cells with αLyt 1 and complement, so that only the Lyt 1^-2^+ T cell subset remained, were also ineffective in therapy, similar to the results with immune cells depleted of all T cell subsets. By contrast, immune cells depleted of Lyt 2^+ cells, which thereby contained only the Lyt 1^+2^- T cell subset and lacked CTL and CTL precursors, retained most of the therapeutic potential of the initial unfractionated population. Thus, although other T cell subsets may have a slight additive effect, the major therapeutic effect provided by adoptive transfer of immune spleen cells is mediated by a population of noncytolytic Lyt 1^+2^- T cells [61].

The above studies were performed with T cell subsets obtained by negative selection (i.e. cytotoxic depletion of other subsets), and the results were confirmed in additional studies with Lyt 1^+2^- T cells obtained by positive selection (i.e. labelled with fluoresceinated and rhodaminated antibodies to Lyt 1 and Lyt 2 respectively and selected on a cell sorter) [54]. The therapeutic efficacy observed with donor cells which lack cytotoxic potential implies that a host cell can contribute to tumor eradication. Thus, the effector mechanism operative *in vivo* against established tumor must reflect one or more of the immune functions of the Lyt 1^+2^- T cell subset and an interacting host cell – i.e. donor helper T cells and host B cells for antibody responses, donor initiator T cells and host macrophages for DTH responses, and/or donor amplifier T cells and host CTL precursors for CTL responses.

The precise function of Lyt 1^+2^- donor cells *in vivo* has not yet been elucidated. A helper function for enhancement of *in vivo* antibody responses seems least likely, since immunotherapy of established tumors by infusion of antibody has had very limited success [15], even when enormously high-titered monoclonal antibody has been utilized [12]. Unfortunately there is as yet insufficient experimental data to distinguish between the other two distinct functions of Lyt 1^+2^- T cells. Examination of regressing sarcomas has provided data consistent with both functions, since T cells and inflammatory cells potentially reflecting an ongoing DTH response as well as CTL and CTL precursors have been detected in the tumor mass [62, 63]. In support of an *in vivo* role for DTH, adoptive transfer of a tumor-specific DTH response with immune Lyt 1^+2^- T cells has been demonstrated [64], but the role of such T effector cells in the rejection of established tumors has not been defined. Perhaps the strongest evidence suggesting a central role for DTH in tumor eradication has been generated in a nontumor allograft rejec-

tion model, in which CTL had been generally presumed to be the critical effector cell – in adoptive transfer experiments utilizing totally T-deficient hosts, rejection of skin allografts required transfer of Lyt 1^+2^- T cells capable of inducing DTH, whereas transferred alloreactive CTL were unable to mediate graft rejection [65].

Alternatively, if Lyt 1^+2^- T cells function predominantly as amplifier cells in the host, then ultimate *in vivo* tumor rejection would be mediated by tumor-specific CTL. Amplifier T cells have been shown to augment generation of tumor-specific CTL *in vitro* and enhance neutralization of tumor *in vivo* [66]. Bioassay data, demonstrating a period of subclinical tumor growth after ACIT with unfractionated immune cells before complete tumor elimination occurs [40], are consistent with a critical role for an amplifier cell, since, unlike CTL, transfer of such nonlytic cells would be expected to result in a delay in tumor eradication while the necessary *in vivo* expansion of the pool of cytotoxic effector cells occurred. However, the hypothesis that transferred Lyt 1^+2^- T cells function to amplify a CTL response *in vivo* must contend with the observation that immune cells depleted of Lyt 1^+ cells, while lacking amplifier cells, still contained CTL and CTL precursors and yet had only a minimal effect in ACIT (Figure 2). This may have reflected largely a quantitative phenomenon, in that an inadequate number of CTL may have been present in the infusate to eradicate the tumor, and the CTL provided may have been incapable of sufficient expansion in the absence of amplifier cells. Additionally, Lyt 1^-2^+ CTL memory cells appear to have a short functional lifespan *in vivo* [59, 67, 68], and thus may be unable to mediate a prolonged *in vivo* antitumor effect. Therefore, continued generation of cytolytic effector cells *in vivo* might require the infusion of Lyt 1^+2^- amplifier cells, which appear to have a long lifespan *in vivo* [59, 67, 68], and the presence of CTL precursors in the host.

Conclusions as to the actual mechanisms by which adoptive transfer of immune cells results in eradication of a tumor must await further experimentation. Definitive studies will require treatment of selectively immunodeficient hosts (i.e. mice rendered totally T-deficient or deficient in selected subpopulations of lymphocytes, as described by Huber et al. [68] with adoptively transferred subpopulations of immune cells, so that the potential effector mechanisms operative *in vivo* are limited. The importance of understanding how tumor is eradicated in the ACIT model has been emphasized by recent results in other experimental immunotherapy models which have similarly demonstrated a requirement for transfer of noncytolytic T cells rather than CTL. In adoptive therapy of an established syngeneic virus-induced rat sarcoma, transfer of immune T cells depleted of CTL, but containing a purified T cell subset analogous to the inducer Lyt 1^+2^- subset in

mice, was even more effective on a cell-for-cell basis than unfractionated immune spleen cells [26, 42]. These results were confirmed in adoptive therapy of an antigenically distinct chemically induced rat sarcoma [43]. Thus, although the precise mechanism of action has not yet been resolved, the efficacy of immune cells in adoptive immunotherapy of both hematologic malignancies and solid tumors appears to be dependent upon the transfer of noncytolytic T cells functioning as helper, amplifier and/or DTH inducer cells.

2.3.4 *Influences of suppressor cells on the efficacy of ACIT*

Progressive growth of an antigenic tumor implies that the underlying host immune response is inadequate to mediate tumor eradication. Induction of suppressor cells which limit the generation and expression of effector cells may be responsible, at least in part, for this failure of the host to control tumor growth. The preferential generation of either effector or suppressor cells following exposure to tumors depends on factors such as the nature of the antigen, mode of antigen presentation, quantity of antigen, etc. [69]. Unfortunately, growing tumors, despite expression of immunogenic moieties on the cell surface, frequently induce specific and/or nonspecific immunologic suppression. This may be due to secretion of directly immunosuppressive soluble factors [70], or to induction of lymphoreticular cells which suppress the immune response. Macrophages, B cells, and T cells have been demonstrated to mediate nonspecific suppression of tumor immunity, whereas most studies of specific suppression have identified a T suppressor cell [69]. The importance of such specific and nonspecific suppressor cells on the generation of effector cells and control of tumor growth in the host has been well demonstrated. For example, inoculation into tumor-bearing hosts of antibodies to I-J determinants, which are MHC-linked alloantigens selectively expressed on suppressor T cells, has been shown to deplete suppressor cells *in vivo* and facilitate tumor rejection [6]; and nonspecific suppressor macrophages need to be depleted from spleens of rats bearing a progressing fibrosarcoma or mice bearing a growing plasmacytoma to permit generation of effector cells operative *in vivo* in immunotherapy or *in vitro* in tumor lysis [43, 71].

Eradication of established tumors by adoptive immunotherapy requires a prolonged *in vivo* antitumor response, and the transferred cells must proliferate in the host to be effective [16, 26, 40, 54]. As a reflection of these requirements, the efficacy of adoptive immunotherapy would be expected to be limited by the same suppressor mechanisms which interfere with the development of immunity in a tumor-bearing host. Indeed, suppressor cells induced by growing tumors have been shown to represent a major obstacle to immunotherapy. Adoptive transfer of immune cells into mice bearing

established fibrosarcomas or mastocytomas produced no antitumor effect; however, if the hosts were rendered T-deficient prior to establishment of the tumor to prevent the generation of tumor-induced T suppressor cells, adoptive immunotherapy resulted in tumor eradication [16, 23]. The importance of T suppressor cells in these models was further confirmed by infusing T cells from intact tumor-bearing hosts into the T-deficient hosts prior to the transfer of effector cells, which resulted in inhibition of the adoptive therapy. Similarly, immunotherapy of fibrosarcomas in two rat models was more effective if the hosts were irradiated prior to establishment of the tumor, presumably because this ablated radiation sensitive precursors of suppressor cells [42, 43].

The potential for suppressor cells to inhibit the efficacy of ACIT has also been demonstrated [27, 72]. Although cyclophosphamide was originally chosen for use in ACIT as a means of reducing the tumor burden prior to infusion of immune donor cells, the drug probably also depletes the host of suppressor cells which interfere with immunotherapy. The importance of the immunologic effect of cyclophosphamide was originally suggested in ACIT models in which treatment with lympholytic chemotherapeutic agents with only minimal tumoricidal activity uncovered potent therapeutic activity in transferred immune cells which had been totally ineffective when infused without prior chemotherapy [27, 38]. Cyclophosphamide has been shown to enhance T cell immunity *in vivo* by preferentially depleting suppressor cells and suppressor precursors more than other T cell subsets in the host [73-75]. Moreover, in ACIT of a fibrosarcoma, the cyclophosphamide has been specifically shown to eliminate tumor-induced suppressor cells in the host which otherwise prevent the transferred immune cells from expressing any antitumor activity [27].

In addition to host-derived suppression which may be largely ablated in ACIT by the cyclophosphamide, suppressor T cells, if present in the infused donor population, can limit the efficacy of coexistent effector cells. The extent to which suppressor cells contaminate a potential effector population is in large part dependent upon the source of donor cells and the method used for generating or purifying immune cells. For example, during *in vitro* sensitization in mixed lymphocyte tumor cultures, not only are effector cells for immunotherapy generated, but a population of nonspecific suppressor T cells is induced [72]. These culture-induced suppressor cells have been shown to inhibit allogeneic and tumor-specific immune responses *in vitro*, and, if inoculated with immune effector cells, diminish the therapeutic activity in ACIT [72].

Another source of suppression may be immunologic circuits involving interactions between host and donor cells. Such circuits can provide an autoregulatory function to limit the magnitude of an immune response and

the size of the pool of effector cells and thus may significantly modify the efficacy of adoptive immunotherapy. Although not yet studied in tumor systems, such a feedback suppression circuit in which effector cells induce suppressor cells has been shown to severely limit the adoptive transfer of immunity to sheep RBC [7-9]. The T cell subsets involved in this circuit can be distinguished by selective expression of Qal and Lyt antigens. Helper T cells are composed of Lyt 1^+2^- Qal^+ and Lyt 1^+2^- Qal^- cells. After activation by antigen, the immune Lyt 1^+2^- Qal^+ subpopulation can both mediate a helper function and induce a subpopulation of naive Lyt 1^+2^+ Qal^+ cells to provide feedback suppression of the helper T cell response by either amplifying or becoming Lyt 1^-2^+ suppressor cells [7-9]. Potentially, such immunoregulatory circuits could have great impact on the efficacy of adoptive immunotherapy - negative feedback limiting expansion of the number of effector cells might partially explain the lack of efficacy observed when treating large tumor burdens with only immune cells.

Thus, suppressor cells which interfere with tumor therapy can be of host origin or donor origin, or may result from interactions between host and donor cells. Although the nature of the suppressor cell and the mechanism of inhibition may vary depending on the tumor and type of immune response being examined, suppression of tumor immunity represents a major obstacle to immunotherapy. Development of methods to abrogate suppression should not only enhance the efficacy of immune cells under conditions in which immunotherapy has demonstrated activity, but permit detection of therapeutic potential in situations for which immunotherapy has had no apparent benefit.

2.3.5 *Contributions of host cells and donor cells to tumor immunity*

The outcome of adoptive immunotherapy reflects potentially complex interactions between host and donor cells and the tumor target. At the simplest level, the effector cells would all be provided by the donor and the host would not contribute to tumor lysis. However, host-derived suppressor cells can interfere with transferred donor immune cells [16, 23, 27], and theoretically other cells of host origin might positively influence the outcome of tumor therapy. The potential for a positive host contribution to tumor eradication by ACIT is suggested by both the demonstration that transferred Lyt 1^+2^- donor T cells are effective in therapy despite lacking cytolytic activity and thus must recruit or interact with a host-derived lytic cell [61], and the observation that a prolonged period of time after cell transfer is required for tumor eradication [40]. Thus, optimal tumor therapy may require not only deletion of host and donor-derived suppressor cells but proper orchestration of host and donor-derived effector cells.

Recent studies in our laboratory have begun to analyze the contributions of host and donor cells to tumor eradication [76]. To distinguish host T cells from donor T cells, mice congenic at the Thy locus were used. The two strains of mice utilized, C57BL/6, denoted B6, and congenic B6.PL(74NS), denoted B6/Thy 1.1, are genetically identical except for the Thy locus, at which the mice are respectively Thy 1.2 and Thy 1.1. B6 mice are low responders to Thy antigens and no immune response to disparities at the Thy allele between host and donor was detectable in our adoptive therapy studies [77].

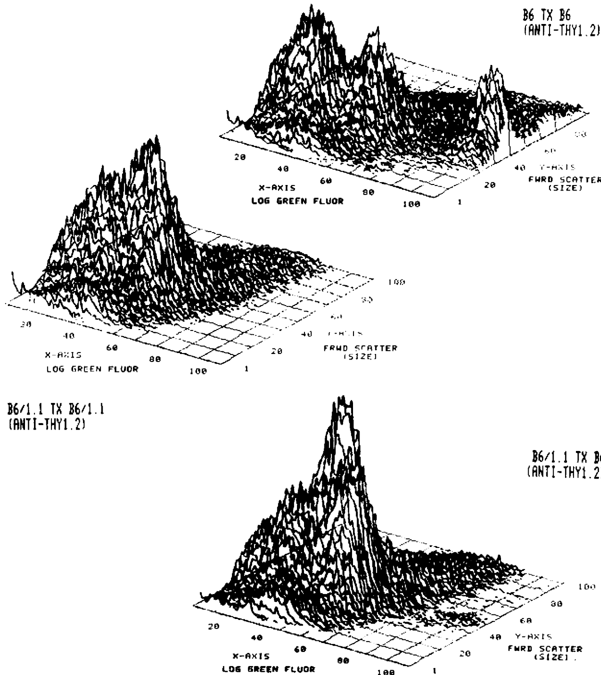

Figure 3. Persistence of donor cells in the host after ACIT. C57BL/6 mice, which are denoted B6 and express Thy 1.2 on their T cells, and congenic B6.PL(74NS) mice, which are denoted B6/1.1 and express Thy 1.1 on their T cells, were used as hosts and/or donors in these experiments. Host mice were inoculated with 5×10^6 FBL-3 on day 0 and treated on day 5 with cyclophosphamide plus 2×10^7 spleen cells from immune syngeneic or congenic donor mice which had been primed and boosted *in vivo* with irradiated FBL-3. Mice were sacrificed 120 days after curative tumor therapy, and the spleen cells were placed into suspension, labelled with fluoresceinated αThy 1.2, and analyzed on the fluorescence-activated cell sorter. The data is plotted with log fluorescence on the x-axis, size on the y-axis, and cell number on the z-axis. The upper left hand graph represents αThy 1.2 labelled spleen cells from B6 hosts that had been treated with immune B6 donor cells (i.e. both host and donor T cells are Thy 1.2); the upper right-hand graph represents αThy 1.2 labelled spleen cells from B6/1.1 hosts that had been treated with immune B6/1.1 donor cells (i.e. both host and donor T cells are Thy 1.1), and the lower graph represents αThy 1.2 labelled spleen cells from B6/1.1 hosts that had been treated with immune B6 donor cells (i.e. host T cells are Thy 1.1 and donor T cells are Thy 1.2).

To determine if donor cells persist, host mice bearing disseminated FBL-3 tumor were treated with cyclophosphamide plus a curative dose of immune cells from syngeneic or congenic donors. Cured mice were sacrificed 120 days after therapy, and suspensions of spleen cells were labelled with fluoresceinated anti-Thy 1.2 and examined on a fluorescence-activated cell sorter, as shown in Figure 3 [76]. Spleen cells obtained from B6 hosts 120 days after therapy with CY and cells from immune syngeneic B6 donors, so that both host and donor cells were Thy 1.2, contained a large population of fluoresceinated, Thy 1.2^+ medium-sized T cells. This distribution of spleen cells from cured mice appeared identical to the distribution of spleen cells obtained from normal B6 mice. Spleen cells from B6/Thy 1.1 hosts treated with cells from immune syngeneic B6/Thy 1.1 donors, so that both host and donor T cells were Thy 1.1, exhibited a similar size distribution, but did not label with fluoresceinated antibody to Thy 1.2 since no Thy 1.2^+ cells were present in the spleen. By contrast, spleens from B6/Thy 1.1 host mice cured with immune congenic B6 donor cells, so that host cells were Thy 1.1 and donor cells were Thy 1.2, contained a small population of cells reactive with fluoresceinated antibody to Thy 1.2. These persistent donor cells comprised 0.4% of the total spleen population. Thus, 120 days after curative ACIT, although the majority of splenic T cells were of host origin, a small fraction of residual donor T cells was present.

The immune reactivity of these congenic host and donor cells present 120 days after treatment of FBL-3 with ACIT was examined [76]. Spleens were removed, depleted of either host or donor T cells by incubation with the appropriate antibody and complement, sensitized for 5 days *in vitro* with syngeneic FBL-3 leukemia cells or allogeneic BALB/c cells, and the cytotoxic reactivity measured (Table 1). B6 hosts previously treated with B6 cells immune to FBL-3 generated a specific cytotoxic response to FBL-3, and a primary allogeneic response to BALB/c. Since both donor and host cells were Thy 1.2, depletion of Thy 1.2 cells abrogated both responses whereas treatment with αThy 1.1 had no effect. Spleen cells from B6 hosts treated with B6/Thy 1.1 donor cells immune to FBL-3 generated normal cytotoxic responses to tumor and alloantigens. Removal of host T cells with anti-Thy 1.2 ablated the allogeneic response but had little effect on the antitumor response, whereas removal of donor cells with anti-Thy 1.1 nearly ablated the antitumor response but had little effect on the allogeneic response. Thus, the small population of residual donor T cells, as demonstrated by quantitative analysis on the cell sorter 120 days after therapy, represented a minor fraction of the immunologically competent T cells but the majority of tumor-reactive T cells present in the host. Since this assay system only examines the function of amplifier and cytotoxic T cells, it is conceivable that there are functional subsets of tumor-immune T cells of

Table 1. Functional analysis of splenic T cells in host mice cured of disseminated leukemia by ACIT.

CTL generation test culture			Per cent Specific lysis [d]	
Source of responder cells [a]	T cell depletion [b]	Stimulator [c]	FBL	BALB/c
B6 hosts treated with $B6_{\alpha FBL}$ donor cells	–	FBL	35	0
		BALB/c	0	35
B6 hosts treated with $B6_{\alpha FBL}$ donor cells	αThy 1.2 +C	FBL	0	0
		BALB/c	0	0
B6 hosts treated with $B6_{\alpha FBL}$ donor cells	αThy 1.1 +C	FBL	39	0
		BALB/c	0	44
B6 hosts treated with B6/Thy $1.1_{\alpha FBL}$ donor cells	–	FBL	43	0
		BALB/c	1	48
B6 hosts treated with B6/Thy $1.1_{\alpha FBL}$ donor cells	αThy 1.2 +C	FBL	42	1
		BALB/c	1	9
B6 hosts treated with B6/Thy $1.1_{\alpha FBL}$ donor cells	αThy 1.1 +C	FBL	9	0
		BALB/c	0	35

[a] Responder spleen cells were obtained on day 125 from C57BL/6 hosts (B6), which have Thy 1.2^+ T cells, that had received 5×10^6 FBL-3 on day 0 and were cured of disseminated FBL-3 leukemia by treatment on day 5 with 180 mg/kg of cyclophosphamide and 2×10^7 spleen cells from B6 donors immune to FBL-3 ($B6_{\alpha FBL}$) or spleen cells from immune congenic donors (B6/Thy $1.1_{\alpha FBL}$) which have Thy 1.1^+ T cells. On day 125, these ACIT-treated hosts were healthy and free of tumor.

[b] Prior to culture the designated responder cells were incubated for 1 hour with either monoclonal αThy 1.2 plus complement or monoclonal αThy 1.1 plus complement to deplete T cells of the appropriate phenotype.

[c] 60×10^6 responder cells were cultured for 5 days with either 3×10^6 γ-irradiated syngeneic FBL-3 tumor stimulator cells or 15×10^6 γ-irradiated allogeneic BALB/c spleen cells.

[d] Effector cells obtained after 5 days of *in vitro* sensitization were tested in a 4-hour chromium release assay for cytotoxicity to labelled syngeneic FBL-3 tumor or allogeneic BALB/c Con A-induced blast cells at an effector-to-target ratio of 20:1.

host origin which are present but not being identified. However, the data suggest that the host generally does not provide effector T cells for participation in tumor lysis following ACIT. Further studies under conditions in which the absolute number or specific functional type of donor cells is limited will be necessary for defining if the host can potentially provide T cells which participate in tumor eradication.

3. GENERATION OF EFFECTOR CELLS FOR TUMOR THERAPY

Immunotherapy with adoptively transferred cells is predicated on the existence of methodologies for generating adequate numbers of therapeutically active effector cells. Most of the early studies of immunotherapy utilized immune syngeneic lymphocytes, obtained from normal donors which had been sensitized *in vivo* with tumor cells, as a prototype of what might be accomplished with appropriately selected and amplified autologous lymphocytes obtained from the tumor-bearing host. However, eventual application of immunotherapy to the clinical setting will require methods for generating effector cells by *in vitro* sensitization, since exposure of a normal syngeneic donor if available (i.e. identical twin) to tumor cells or extracts might pose unacceptable risks, and augmentation of the tumor immunity of the tumor-bearing host by *in vivo* sensitization may not be feasible due to the presence of factors such as tumor-induced suppressor cells [17] and blocking factors [18] which limit expansion of the effector population. *In vitro* sensitization should not only provide a means of bypassing the mechanisms which interfere with induction of effector cells *in vivo*, but should also permit analysis of all the end-products of sensitization, with subsequent selection for therapy of only the subpopulations of cells which have a beneficial effect *in vivo*. Therefore, much effort has been dedicated to developing methods and defining optimal conditions for *in vitro* sensitization of lymphocytes to antigens expressed on syngeneic tumor cells.

3.1 *Primary in vitro* sensitization

Primary *in vitro* sensitization provides a means for rendering immunologically naive lymphocytes specifically immune to tumor antigens. Such immunization of naive lymphocytes which have never been exposed to tumor antigens might reflect the situation with lymphocytes obtained for sensitization and subsequent use in therapy from a host bearing only a small local tumor or a tumor growing in an immunologically privileged site, as well as lymphocytes obtained from a normal syngeneic donor. Primary *in vitro* sensitization has been difficult to accomplish, in part due to immunologic considerations such as the more complex antigen presentation requirements for generating a primary rather than secondary response [78], and in part due to technical considerations such as problems establishing optimal *in vitro* conditions due to difficulties demonstrating sensitization since the response is of low magnitude and the assay systems are relatively insensitive. Nevertheless, primary *in vitro* sensitization of T cells to some tumor antigens has been achieved by a variety of techniques, including culture on antigen-fed macrophages [79, 80], on tumor monolayers [81], and in mixed lymphocyte-tumor suspensions [36, 82, 83]. Documentation of *in vitro* sensitization

has been accomplished largely by measurement of direct *in vitro* cytotoxicity [84] and prevention of tumor growh *in vivo* following adoptive transfer to nonimmune mice [85, 86].

The ability of lymphocytes primed *in vitro* to eradicate an established tumor has been examined in only a few murine models. Lymphocytes cultured with Lewis lung carcinoma cells and injected into mice after surgical removal of the primary 3LL tumor inhibited the development of pulmonary metastases [29], and lymphocytes cultured with the chemically-induced leukemia EL-4 eradicated established tumor when inoculated as an adjunct to chemotherapy [36]. In the previously described ACIT models, primary *in vitro* sensitization was shown to be a potential means for generating specifically immune effector T cells for therapy of two non-crossreactive tumors [87].

The few published positive studies demonstrating the generation of effector cells for tumor therapy by primary *in vitro* sensitization can no doubt be matched by many more unpublished as well as published negative studied [88]. Analysis of the cell populations generated by primary *in vitro* sensitization has permitted identification of some of the major problems, such as the small magnitude of the immune response with induction of only a limited number of effector cells, and the generation of suppressor cells during *in vitro* culture which interfere with the *in vivo* efficacy of the concurrently generated effector cells [72, 86]. However, the studies do suggest that, with appropriate techniques for selection and expansion of effector cells, primary *in vitro* sensitization represents a potential means of generating the requisite effector cells for tumor therapy.

3.2 *Secondary in vitro sensitization*

Progressive growth of immunogenic tumors despite evidence of concomitant tumor immunity has suggested not only that modulation of host immune reactivity *in vivo* might facilitate tumor rejection, but also that the tumor-bearing host might be a source of primed lymphocytes which, if appropriately amplified and selected, could be reinfused as a form of tumor therapy. Secondary *in vitro* sensitization of primed lymphocytes can generate strong, easily detected immune responses and has been shown to increase the number of antitumor effector cells [89]. Thus, removal of lymphocytes from a host bearing an immunogenic tumor, followed by *in vitro* sensitization and infusion back into the host, should be useful in situations in which the absolute number of effector cells is a limiting factor for tumor elimination.

Secondary *in vitro* sensitization of cells primed *in vivo* has been accomplished with many tumors using a variety of *in vitro* culture techniques [84]. Although in most studies sensitization was documented by enhancement of

in vitro cytotoxicity or increased activity in tumor neutralization assays [90], secondary *in vitro* sensitization has been shown to render primed cells more effective in therapy of established tumors in several models including a rat fibrosarcoma [42] and a murine mastocytoma [91]. Moreover, the therapeutic efficacy of cells from secondary mixed lymphocyte-tumor culture has been extensively studied in ACIT of disseminated FBL-3 leukemia. These studies have shown that *in vivo* primed cells can be rendered more cytotoxic *in vitro* and more effective in ACIT by secondary *in vitro* sensitization [92], that enhancement of the therapeutic efficacy of *in vivo* primed cells by secondary *in vitro* sensitization is dependent upon the method of priming and cannot be predicted by results of *in vitro* cytotoxicity or *in vivo* tumor neutralization assays [92], and that the generation of cells expressing *in vitro* cytolytic reactivity and cells expressing *in vivo* therapeutic efficacy exhibit disparate time courses during *in vitro* sensitization, implying different cell requirements and/or regulation for these two effector functions [53]. The precise effector cell operative in ACIT being induced and/or expanded during *in vitro* culture has not yet been identified. Although secondary *in vitro* sensitization increases CTL number and reactivity [89], this does not necessarily imply that the therapeutic efficacy of cells generated by secondary *in vitro* sensitization is due to transfer of CTL. Analysis of the cells generated by *in vivo* sensitization has revealed that the major effector cell for ACIT is a noncytolytic Lyt $1^{+}2^{-}$ T cell [61]. Moreover, in immunotherapy of an established rat sarcoma with cells generated by secondary *in vitro* sensitization, the effector cell operative *in vivo* was derived from the pool of noncytolytic T cells rather than CTL [26]. Thus, further studies will be needed to determine if CTL, either in very large number or by repeated infusion, can facilitate eradication of established tumors.

Analysis of the end products of sensitization and the efficiency of effector cell generation has identified many of the problems associated with the use of secondary *in vitro* sensitization as a means of generating cells for tumor therapy. Firstly, primed cells obtained from tumor-bearing hosts may contain suppressor cells which limit the generation or activity of effector cells. Such suppression may be due to macrophages [43] or T cells [17]. Secondly, suppressor T cells may be generated during the course of secondary *in vitro* sensitization which interfere with the therapeutic activity of effector T cells [72]. These problems should be successfully resolved with definition of optimal culture conditions and identification and positive selection of the subset of cells responsible for *in vivo* therapy. However, the major limitation elucidated by these studies, which has required the development of new culture techniques as described below, is the inefficient expansion of absolute effector cell number accomplished by secondary *in vitro* sensitization.

3.3 *Expansion of effector cell number by culture with Interleukin 2 (IL 2)*

Tumor eradication by adoptive immunotherapy requires the infusion of large numbers of effector cells. With the standard culture conditions used for *in vitro* sensitization, only minimal increases in the total number of effector cells occurs, in part due to progressive cell loss during culture [89]. Therefore, a variety of organic and inorganic compounds have been added to the culture medium in an effort to identify reagents which promote cell survival and selective expansion *in vitro* of antigen-reactive cells. Several cytokines (i.e. secreted cell products) and in particular one lymphokine, Interleukin 2 (IL 2), have been recently identified and purified, and have exhibited great potential for enhancing the efficacy of sensitization and augmenting the expansion of effector cells during *in vitro* sensitization.

The T cell growth factor IL 2 is a soluble protein produced by Lyt $1^{+}2^{-}$ amplifier T lymphocytes following interaction with mitogens or antigens and macrophages [93, 94]. IL 2 binds selectively to activated T cells and addition of this lymphokine to cultures containing antigen-activated lymphocytes induces continued proliferation of stimulated T cells [95, 96]. Since the cells proliferating in response to IL 2 become dependent upon exogenous IL 2 for survival, large numbers of specifically activated helper and cytotoxic T cells have been generated and maintained *in vitro* in cultures continually supplemented with IL 2 [97–99]. Addition of IL 2 to mixed lymphocyte tumor cultures has been shown to enhance sensitization to tumor antigens - thus rendering weak responses more easily detectable [43, 91], and tumor specific cytotoxic T lymphocytes cultured for prolonged periods in IL 2 have been shown to inhibit the growth of syngeneic tumor when inoculated concurrently in a Winn assay [100].

The *in vivo* efficacy and specificity of tumor-specific T cells expanded by culture in IL 2 have been examined in ACIT [101]. Primed lymphocytes were activated by culture with tumor cells for 7 days, and induced to proliferate further by addition of IL 2. At day 19, such cells displayed greater than seven-fold numerical expansion and retained specificity for the tumor antigen as determined by *in vitro* cytotoxicity. Moreover, such cells exhibited specificity and dose-dependent efficacy in ACIT of two non-crossreactive tumors [101]. The phenotype of the effector cell generated by long-term culture in IL 2 which is operative in ACIT of disseminated leukemia and the mechanism of action *in vivo* have not yet been determined, but these effector cells did demonstrate altered kinetics of tumor elimination and relative resistance to γ-irradiation when compared in ACIT to noncultured immune cells [102].

Therapy with IL 2 dependent cultured T cells is potentially limited by problems such as short survival and abnormal trafficking patterns *in vivo* [103, 104]. Infusion of exogenous IL 2 into the host may represent a

means of promoting survival and proliferation of these IL 2 dependent cells *in vivo,* and thus may augment therapeutic efficacy. In ACIT of disseminated leukemia, the efficacy of therapy with long-term cultured cells can be significantly enhanced by inoculation of IL 2 into the treated hosts [105]. The mechanism of action of IL 2 in this setting remains to be elucidated, but the demonstration that exogenous IL 2 can have activity *in vivo* may prove to be of general importance in immunotherapy. Pharmacologic doses of this lymphokine may potentially supplement or substitute for transfer of noncytolytic Lyt $1^{+}2^{-}$ T cells, which are active in tumor therapy possibly due to the production of IL 2 *in vivo* by amplifier cells [61, 93]. Although further studies will be necessary to identify all the possible roles of IL 2 and long-term cultured cells in immunotherapy, these studies have suggested that methods for augmenting even weak immune responses to syngeneic tumors may become readily available, and that generation of effector cells in adequate number for therapy need not remain a major obstacle to the eventual clinical application of these therapeutic approaches to the treatment of human tumors.

4. ADOPTIVE IMMUNOTHERAPY WITH ALLOGENEIC LYMPHOCYTES

Allogeneic lymphocytes can readily lyse tumor cells *in vitro* and can prevent allogeneic tumors from growing *in vivo* through recognition of histocompatibility antigens. Attempts to utilize this antitumor activity of alloreactive cells for the treatment of established tumors by adoptive transfer of allogeneic lymphocytes have been impeded by two major obstacles – excessive host toxicity due to reactivity of donor cells with normal host tissues and limited therapeutic efficacy due to rejection of donor cells by the host. However, clinical studies have already suggested that, despite these obstacles, allogeneic adoptive therapy has sufficient therapeutic potential to warrant further study in preclinical animal models. Following human allogeneic bone marrow transplantation for the treatment of acute leukemia, the risk and incidence of leukemic relapse is significantly diminished in patients with graft-versus-host disease (GVHD) as compared to patients with no GVHD [13, 14]. The therapeutic benefit observed in patients exhibiting GVHD is presumably the result of a graft-versus-tumor (GVT) effect mediated by the transplanted allogeneic cells.

Allogeneic adoptive therapy has some practical and theoretical advantages, particularly in settings in which tumor cells may not be highly immunogenic or in which the alloreactive effector cells could be delivered in relative proximity to the tumor target. Sensitization to alloantigens expressed on tumor cells would be easier to accomplish than to tumor-specific

antigens, since many alloantigens are highly immunogenic and tumor cells which may be direclty suppressive or poor stimulators need not necessarily be used as the sensitizing cell. Perhaps more importantly, alloreactive cells would be capable of recognizing a variety of antigenic determinants on tumor cells, depending upon the nature and extent of the histocompatibility disparity between donor and host, and thus would not require that all tumor targets express the unique tumor-specific antigens.

The extent of histocompatibility disparity between host and donor cells should in part determine the *in vivo* activity of infused allogeneic cells. Cells obtained from allogeneic donors matched at the MHC would be expected to persist longer and induce more limited acute toxicity in the host than MHC incompatible donor cells, since minor antigens are less immunogenic than MHC antigens to the host immune system and have a more limited distribution on host target cells. The potential for using normal differentiation antigens or minor histocompatibility antigens present on tumor cells as targets for immunologic attack has been well demonstrated in animal models. For example, treatment of mice bearing a T cell lymphoma with monoclonal antibody to the Thy 1.1 antigen resulted in a significant reduction of tumor mass [12], and treatment of rats bearing a T cell lymphoma with lymphocytes obtained from allogeneic MHC-identical donors immune to determinants on normal host lymphocytes resulted in a significant prolongation of survival [106].

The efficacy of allogeneic cells in the therapy of established leukemias, lymphomas and sarcomas has been demonstrated in several experimental animal models [32, 36, 39, 106–111]. In most of these models the hosts were treated with high dose chemotherapy and/or radiotherapy not only to reduce the tumor burden, as required for syngeneic adoptive therapy, but also to either ablate or severely suppress the host immune system so as to prevent rapid injection of the infused allogeneic cells. Although the effector cell requirements and immunologic mechanisms responsible for tumor elimination still require elucidation, several fundamental principles have emerged from these studies. MHC-compatible cells were generally more effective and less toxic than MHC-incompatible cells. Sensitization to normal host histocompatibility and/or differentiation antigens prior to adoptive transfer rendered unprimed allogeneic cells more effective in tumor therapy [106, 112]. Eradication of the tumor after cell transfer required a minimum of several days, and persistence of donor cells in the host during this time period was mandatory [107, 108, 111]. Thus, allogeneic donor cells can recognize host tumor cells *in vivo*, and donor cells which persist after cell transfer can mediate a therapeutic antitumor effect.

The efficacy of the GVT effect observed in these therapy models was not correlated with the severity of the induced GVHD [112, 113]. However,

hosts generally needed to be severely immunosuppressed in order to permit donor cells to mediate an antitumor effect, and early intervention to prevent the development of fatal GVHD was generally required. Rescue from GVHD was accomplished by *in vivo* depletion of allogeneic donor cells using techniques such as infusion of alloantiserum, chemotherapy, radiotherapy, or infusion of syngeneic lymphocytes to reject the allogeneic cells. Unfortunately, since rescue was generally ineffective once GVHD was manifest in the host, methods to eliminate donor cells were usually initiated shortly after cell transfer and before any antitumor effect or toxicity was detectable. This apparent necessity for early eradication of all donor lymphocytes prior to knowing whether the desired antitumor effect has been completed has emphasized the importance of defining the kinetics, mechanisms, and effector cells responsible for both tumor elimination and GVHD following transfer of allogeneic cells. At the present time it is unclear whether tumor lysis and GVHD are mediated by the same effector mechanism. The effector cell required for tumor elimination by syngeneic adoptive therapy is a noncytolytic Lyt 1^+2^- donor T cell [61], whereas the effector cell required for expression of GVHD following allogeneic marrow transplantation across minor histocompatibility differences is a potentially cytolytic Lyt $1^{\pm}2^+$ donor T cell [114]. Thus, it is conceivable that allogeneic donor cells can be manipulated to promote the antitumor activity and diminish the toxicity to the host.

The generation of immune cells for allogeneic adoptive therapy is less problematic than for syngeneic tumor therapy. For therapy across MHC barriers, strong primary *in vitro* responses are easily generated. For therapy with allogeneic MHC identical cells, effector cells recognizing minor histocompatibility antigens can be generated by primary *in vivo* and secondary *in vitro* sensitization of donor cells to host normal lymphocyte stimulator cells [115]. However, since this latter method would require *in vivo* exposure of the donor to host cells potentially contaminated by tumor cells or genomic material, the generation of T effector cells to minor histocompatibility antigens by primary *in vitro* sensitization may be preferable. This requires using *in vitro* techniques to abrogate the influence of suppressor cells and augment the activity of helper cells [116, 117]. One additional approach for generating alloreactive effector cells for tumor therapy needs to be explored. Using techniques analogous to those described in Section 3.3 for expansion of syngenic effector cells by culture with IL 2, it should be possible to amplify and clone IL 2 dependent populations of alloreactive cells following *in vitro* sensitization to minor histocompatibility antigens. Such cloned populations could be screened against a panel of host target cells, and only those clones which recognize determinants expressed on the tumor cell but with limited tissue distribution would be selected for use in

therapy. This selection process should permit enhancement of the antitumor reactivity and reduction of the toxicity to the host. Moreover, IL 2 dependent alloreactive cloned cells survive only briefly *in vivo* without exogenous IL 2 and should be unable to induce a persistent GVH response. Since such cells may therefore have minimal toxicity to the host, it may be possible to prolong the *in vivo* survival and facilitate the antitumor activity of cloned alloreactive cells by inoculation of pharmacologic doses of IL 2 into the host until such time as toxicity becomes limiting [105].

These studies strongly suggest that allogeneic adoptive tumor therapy has potential beyond that already observed with allogeneic bone marrow transplantation. Although transfer of allogeneic cells may be toxic to the host, such cells exhibit unequivocal therapeutic activity. The obvious challenge is to identify means of augmenting the GVT effect and decreasing the GVHD so that allogeneic therapy can become a clinically useful modality for the treatment of established tumors.

5. CONCLUDING REMARKS

In this chapter the experimental and theoretical basis for the use of adoptively transferred immune T cells for the therapy of established tumors as elucidated by animal models has been reviewed. In general, adoptive transfer of immune T cells as the sole form of therapy has been ineffective, in large part due to physical and immunologic consequences of a large tumor burden. However, following cytoreductive therapy of the host with surgery, radiation, or chemotherapy, transferred immune cells can be shown to mediate a potent and potentially curative antitumor effect. Complete tumor eradication requires transfer of specifically immune, MHC-restricted T cells that mediate a prolonged antitumor effect *in vivo*. Surprisingly, the requisite donor effector cell for syngeneic immunotherapy appears to be a noncytolytic T cell which must induce other cells of host and/or donor origin to lyse the tumor cells. Donor immune cells must proliferate and persist in the host after cell transfer and are subject to suppression and immunoregulation after adoptive transfer.

Translation to human tumor therapy of the insights derived from studying the mechanisms of tumor eradication by adoptive immunotherapy in animal models is largely dependent on the suppositions that human tumor cells express surface antigens which can serve as targets for immunologic attack, and that lymphocytes recognizing these antigens can be generated in sufficient quantity for adoptive transfer. Using modern *in vitro* technology, it is now possible to activate antigen-reactive cells by *in vitro* sensitization, and, with the addition of IL 2, a T cell growth factor, even weak immune

responses can be augmented so that large numbers of clonally-expanded antigen-specific T cells can be generated. Such long-term cultured IL 2 dependent T cells have been shown to function in adoptive therapy of established tumors in animal models. Adaptation of this technology should permit both detection of autologous lymphocytes from a cancer patient which recognize tumor-associated antigens on the autochthonous tumor, and expansion of such tumor-specific T cells, if present, to sufficiently large numbers for reinfusion in adoptive therapy. The generation of effector cells for human tumor therapy should be further enhanced by improved methods of distinguishing lymphocyte subpopulations, since sensitization should be improved by deletion of suppressor cells and positive selection for the desired effector cells and necessary accessory and/or helper cells.

Unfortunately, it is possible that many human tumors will neither be immunogenic nor express unique tumor-associated antigens, and thus would not be amenable to therapeutic approaches requiring tumor-specific effector cells. However, minor histocompatibility and differentiation antigens on tumor cells can serve as alternative targets for immunologic attack by allogeneic cells. Transferred alloreactive cells have already been demonstrated to mediate an antitumor effect in both animal models and human allogeneic bone marrow transplantation, but such cells recognize normal host cells and can induce significant toxicity. Although further studies into methods to promote the antitumor activity and diminish the antihost activity of transferred alloreactive cells will be necessary, allogeneic adoptive therapy holds great promise as a modality for treating established tumors.

During the past decade the rationale for the use of adoptively transferred lymphocytes for tumor therapy has been demonstrated, animal models to examine the biologic principles have been established, and many of the prerequisites for and obstacles to therapeutic efficacy have been identified. Although it is unclear precisely how these approaches will be translated to the clinical setting, it seems likely that methods which harness the immune system to promote tumor eradication will become an important part of the therapeutic armamentarium for the treatment of human malignant disease.

ACKNOWLEDGEMENT

The investigations reported herein were supported in part by Grants CA 10777, CA 30558, CA 303384 and CB 84247 from the National Cancer Institute, DHHS, and IM 304 from the American Cancer Society.

REFERENCES

1. McKenzie IFC, Potter T: Murine lymphocyte surface antigens. Adv Immunol 27:179, 1979.
2. Reinherz EL, Schlossman SF: The differentiation and function of human T lymphocytes. Cell 19:821, 1980.
3. Gillis S, Smith KA: Long-term culture of tumor-specific cytotoxic T cells. Nature 268:154, 1977.
4. Zarling JM, Bach FH: Continuous culture of T cells cytotoxic for autologous human leukemia cells. Nature 280:685, 1979.
5. Gillis S, Baker PE, Ruscetti FW, Smith KA: Long-term culture of human antigen-specific cytotoxic T cell lines. J Exp Med 148:1093, 1978.
6. Perry LL, Benacerraf B, McCluskey RT, Greene MI: Enhanced syngeneic tumor destruction by *in vivo* inhibition of suppressor T cells using anti-IJ alloantiserum. Am J Path 92:491, 1978.
7. Earley DD, Hugenberger L, McVay-Boudreau L, Shen FW, Gershon RK, Cantor H: Immunoregulatory circuits among T-cell sets: I. T-helper cells induce other T-cell sets to exert feedback inhibition. J Exp Med 147:1106, 1978.
8. Cantor H, McVay-Boudreau L, Hugenberger J, Naidorf K, Shen FW, Gershon RK: Immunoregulatory circuits among T-cell sets: II. Physiologic role of feedback inhibition *in vivo:* Absence in NZB mice. J Exp Med 147:1116, 1978.
9. Cantor HJ, Hugenberger J, McVay-Boudreau L, Eardley DD, Kemp J, Shen FW, Gershon RK: Immunoregulatory circuits among T-cell sets: Identification of a subpopulation of T-helper cells that induces feedback inhibition. J Exp Med 148:871, 1978.
10. Lee SK, Oliver RTD: Autologous leukemia-specific T-cell-mediated lymphocytotoxicity in patients with acute myelogenous leukemia. J Exp Med 147:912, 1978.
11. McGee J O'D, Woods J, Ashall F, Bramwell ME, Harris H: A new marker for human cancer cells: 2. Immunohistochemical detection of the Ca antigen in human tissues with the CA1 antibody. Lancet 2:7, 1982.
12. Badger CC, Bernstein ID: Therapy of murine leukemia with monoclonal antibody against a normal differentiation antigen. J Exp Med, in press.
13. Weiden PL, Flournoy N, Thomas ED, Prentice R, Fefer A, Buckner CD, Storb R: Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. New Engl J Med 300:1068, 1979.
14. Weiden PL, Sullivan KM, Flournoy N, Storb R, Thomas ED, the Seattle Marrow Transplant Team: Antileukemic effect of chronic graft-versus-host disease: Contribution to improved survival after allogeneic marrow transplantation. N Engl J Med 304:1529, 1981.
15. Rosenberg SA, Terry WD: Passive immunotherapy of cancer in animals and man. Adv Cancer Res 25:323; 347; 366, 1977.
16. Berendt MJ, North RJ: T-cell-mediated suppression of antitumor immunity: An explanation for progressive growth of an immunogenic tumor. J Exp Med 151:59, 1980.
17. Fujimoto S, Greene MI, Sehon AH: Regulation of the immune response to tumor antigens: I. Immunosuppressor cells in tumor-bearing hosts. J Immunol 116:800, 1976.
18. Hellström KE, Hellström I: Lymphocyte-mediated cytotoxicity and blocking serum activity to tumor antigens. Adv Immunol 18:209, 1974.
19. Borberg H, Oettgen HF, Choudry K, Beathie EJ: Inhibition of established transplants of chemically induced sarcomas in syngeneic mice by lymphocytes from immunized donors. Internatl J Cancer 10:539, 1972.
20. Smith HG, Harmel RP, Hanna MG, Zwilling BS, Zbar BS, Rapp HJ: Regression of esta-

blished intradermal tumors and lymph node metastases in guinea pigs after systemic transfer of immune lympoid cells. J Natl Cancer Inst 58:1315, 1977.
21. Fefer A, Einstein AB, Cheever MA, Berenson JR: Models for syngeneic adoptive chemoimmunotherapy of murine leukemias. Ann NY Acad Sci 276:573, 1976.
22. Berendt MJ, North RJ, Kirstein DP: The immunological basis of endotoxic-induced tumor regression: Requirement for T cell mediated immunity. J Exp Med 148:1550, 1978.
23. Dye ES, North RJ: T cell mediated immunosuppression as an obstacle to adoptive immunotherapy of the P815 mastocytoma and its metastases. J Exp Med 154:1033, 1981.
24. Fefer A: Immunotherapy of primary Moloney sarcoma virus-induced tumors. Internatl J Cancer 5:327, 1970.
25. Colavo D, Zanovello P, Leuchars E, Davies AJS, Chieco-Bianchi L, Biasi G: Moloney murine sarcoma virus oncogenesis in T-lymphocyte-deprived mice: Biologic and immunologic studies. J Natl Cancer Inst 64:97, 1980.
26. Fernandez-Cruz E, Woda BA, Feldman JD: Elimination of syngeneic sarcomas in rats by a subset of T lymphocytes. J Exp Med 152:823, 1980.
27. North RJ: Cyclophosphamide-facilitated adoptive immunotherapy of an established tumor depends on elimination of tumor-induced suppressor T cells. J Exp Med 55:1063, 1982.
28. Spitalny GL, North RJ: Subversion of host defense mechanisms by malignant tumors: An established tumor as a privileged site for bacterial growth. J Exp Med 145:1264, 1977.
29. Treves AJ, Cohen IR, Feldman M: Immunotherapy of lethal metastases by lymphocytes sensitized against tumor cells *in vitro*. J. Natl Cancer Inst 54:777, 1975.
30. Barnes DWH, Loutit JR, Neal FE: Treatment of murine leukemia with x-rays and homologous bone marrow. Br Med J 2:626, 1959.
31. DeVries MJ, Vos P: Treatment of mouse lymphosarcoma by total body x-irradiation and by injection of bone marrow and lymph node cells. J. Natl Cancer Inst 21:1117, 1958.
32. Fefer A: Adoptive chemoimmunotheraphy of a Moloney lymphoma. Internatl J Cancer 8:364, 1971.
33. Mihich E: Combined effects of chemotherapy and immunity against leukemia L1210 in DBA/2 mice. Cancer Res 29:848, 1969.
34. Vadlamudi S, Padarathsingh M, Bonmasser E, Goldin A: Effect of combination treatment with cyclophosphamide and syngeneic or allogeneic spleen and bone marrow cells in leukemic (L1210) mice. Internatl J Cancer 7:160, 1971.
35. Fass L, Fefer A: Factors related to therapeutic efficacy in adoptive chemoimmunotherapy of a Friend virus-induced lymphoma. Cancer Res 32:2427, 1972.
36. Kedar E, Raanan Z, Schwartzbach M: *In vitro* induction of cell-mediated immunity to murine leukemia cells: VI. Adoptive immunotherapy in combination with chemotherapy of leukemia in mice, using lymphocytes sensitized *in vitro* to leukemia cells. Cancer Immunol Immunother 4:161, 1978.
37. Cheever MA, Greenberg PD, Fefer A: Specificity of adoptive chemoimmunotherapy of established syngeneic tumors. J Immunol 125:711, 1980.
38. Einstein AB, Cheever MA, Fefer A: The use of dimethylmyleran in adoptive chemoimmunotherapy of two murine leukemias. J Natl Cancer Inst 56:609, 1976.
39. Klein DL, Brown W, Cote P, Garner W, Pearson JW: Chemoadoptive immunotherapy against a guinea-pig leukemia. Cancer Immunol Immunother 6:17, 1979.
40. Greenberg PD, Cheever MA, Fefer A: Detection of early and delayed anti-tumor effects following curative adoptive chemoimmunotherapy of established leukemias. Cancer Res 40:4428, 1980.
41. McCoy JL, Fefer A, Glynn JP: Comparative studies on the induction of transplantation resistance in BALB/c and C57BL/6 mice in three murine leukemia systems. Cancer Res 27:1743, 1967.

42. Fernandez-Cruz E, Halliburton B, Feldman JD: *In vivo* elimination by specific effector cells of an established syngeneic rat Moloney virus-induced sarcoma. J Immunol 123:1772, 1979.
43. Fernandez-Cruz E, Gilman SC, Feldman JD: Immunotherapy of a chemically-induced sarcoma in rats: Characterization of the effector T cell subset and nature of suppression. J Immunol 128:1112, 1982.
44. Zinkernagel RM, Doherty PC: MCH-restricted cytotoxic T cells: Studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction-specificity, function, and responsiveness. Adv Immunol 27:51, 1979.
45. Plata F, Jongeneel V, Cerottini J-C, Brunner KT: Antigenic specificity of the cytolytic T lymphocyte (CTL) response to murine sarcoma virus-induced tumors: I. Preferential reactivity of *in vitro* generated secondary CTL with syngeneic tumor cells. Eur J Immunol 6:823, 1976.
46. Gomard E, Duprez V, Henin Y, Levy JP: H-2 region product as determinant in immune cytolysis of syngeneic tumor cells by anti-MSV lymphocytes. Nature 260:707, 1976.
47. Holden HT, Herberman RB: Cytotoxicity against tumour-associated antigens not H-2 restricted. Nature 268:250, 1977.
48. Stutman O, Shen F-W: H-2 restriction and non-restriction of T-cell-mediated cytotoxicity against mouse mammary tumour targets. Nature 276:181, 1978.
49. Gomard E, Levy JP, Plata F, Henin Y, Duprez V, Bismuth A, Reme T: Studies on the nature of the cell surface antigen reacting with cytolytic T lymphocytes in murine oncornavirus-induced tumors. Eur J Immunol 8:228, 1978.
50. Greenberg PD, Cheever MA, Fefer A: H-2 restriction of adoptive immunotherapy of advanced tumors. J Immunol 126:2100, 1981.
51. Berenson JR, Einstein AB, Fefer A: Syngeneic adoptive immunotherapy and chemoimmunotherapy of a Friend leukemia: Requirement for T cells. J Immunol 115:234, 1975.
52. Cheever MA, Greenberg PD, Fefer A: Therapy of leukemia by non-immune syngeneic spleen cells. J Immunol 124:2137, 1980.
53. Cheever MA, Greenberg PD, Fefer A: Chemoimmunotherapy of a Friend leukemia with cells secondarily sensitized *in vitro:* Effect of culture duration on therapeutic efficacy. J Natl Cancer Inst 67:169, 1981.
54. Greenberg PD, Cheever MA, Fefer A: Prerequisites for successful adoptive immunotherapy: Nature of effector cells and role of H-2 restriction. In: The potential role of T cells in cancer therapy, Fefer A, Goldstein AL (eds). New York: Raven Press, 1982, pp 31–51.
55. Cantor H, Boyse E: Regulation of the immune response by T-cell subclasses. Contemp Top Immunobiol 7:47, 1977.
56. Shiku H, Takahashi T, Bean MA, Old LJ, Oettgen HF: Ly phenotype of cytotoxic T cells for syngeneic tumor. J Exp Med 144:1116, 1976.
57. Green WR, Nowinski RC, Henney CS: The generation and specificity of cytotoxic T cells raised against syngeneic tumor cells bearing AKR/Gross murine leukemia virus antigens. J Exp Med 150:51, 1979.
58. Nakayama E, Shiku H, Stockert E, Oettgen HF, Old LJ: Cytotoxic T cells: Lyt phenotype and blocking of killing activity by Lyt antisera. Proc Natl Acad Sci USA 76:1977, 1979.
59. Leclerc J-C, Cantor H: T cell-mediated immunity to oncornavirus-induced tumors: I. Ly phenotype of precursor and effector cytolytic T lymphocytes. J Immunol 124:846, 1980.
60. Stutman O, Shen F-W, Boyse EA: Ly phenotype of T cells cytotoxic for syngeneic mouse mammary tumors: Evidence for T cell interactions. Proc Natl Acad Sci USA 74:5667, 1977.
61. Greenberg PD, Cheever MA, Fefer A: Eradication of disseminated murine leukemia by

chemoimmunotherapy with cyclophosphamide and adoptively transferred immune syngeneic Lyt-$1^{+}2^{-}$ lymphocytes. J Exp Med 154:952, 1981.

62. Chapdelaine JM, Plata F, Lilly F: Tumors induced by murine sarcoma virus contain precursor cells capable of generating tumor-specific cytolytic T lymphocytes. J Exp Med 149:1531, 1979.
63. MacDonald HR, Cerottini J-C, Ryser, J.-E, Maryanski JL, Taswell C, Widmer MB, Brunner KT: Quantitation and cloning of cytolytic T lymphocytes and their precursors. Immunol Rev 51:93, 1980.
64. Perry LL, Dorf ME, Bach BA, Benacerraf B, Greene MI: Mechanisms of regulation of cell-mediated immunity: Anti-I-A alloantisera interfere with induction and expression of T-cell-mediated immunity to cell-bound antigen *in vivo. Clin Immunol Immunopathol* 15:279, 1980.
65. Loveland BE, Hogarth PM, Ceredig R, McKenzie IFC: Cells mediating graft rejection in the mouse: I. Lyt-1 cells mediate skin graft rejection. J Exp Med 153:1044, 1981.
66. Fujiwara H, Hamaoka T, Shearer GM, Yamamoto H, Terry WD: The augmentation of *in vitro* and *in vivo* tumor-specific T cell-mediated immunity by amplifier T lymphocytes. J Immunol 124:863, 1980.
67. Leclerc J-C, Cantor H: T cell-mediated immunity to oncornavirus-induced tumors: II. Ability of different T cell sets to prevent tumor growth *in vivo.* J Immunol 124:851, 1980.
68. Huber B, Cantor H, Shen F-W, Boyse EA: Independent differentiative pathways of Lyl and Ly23 subclasses of T cells: Experimental production of mice deprived of selected T-cell subclasses. J Exp Med 144:1128, 1976.
69. Noar D: Suppressor cells: Permitters and promotors of malignancy? Adv in Cancer Res 29:45, 1979.
70. Plescia OJ, Smith AH, Grinwich K: Subversion of immune system by tumor cells and role of prostaglandins. Proc Natl Acad Sci USA 72:1848, 1975.
71. Mokyr MB, Braun DB, Dray S: Augmentation of the antitumor cytotoxicity in MOPC-315 tumor bearer spleen cells by depletion of glass-adherent cells prior to *in vitro* activation. Cancer Res 39:785, 1979.
72. Greenberg PD, Cheever MA, Fefer A: Suppression of the *in vitro* secondary response to syngeneic tumor and of *in vivo* tumor therapy with immune cells by culture-induced suppressor cells. J. Immunol 123:515, 1979.
73. Turk JL, Parker D, Poulter LW: Functional aspects of the selective depletion of lymphoid tissue by cyclophosphamide. Immunology 23:493, 1972.
74. Fass L, Fefer A: The application of an *in vitro* cytotoxicity test to studies of the effects of drugs on the cellular immune response in mice: I. Primary response. J Immunol 109:749, 1972.
75. Glaser M: Regulation of specific cell-mediated cytotoxic response against SV40-induced tumor-associated antigens by depletion of suppressor T cells with cyclophosphamide in mice. J Exp Med 149:774, 1979.
76. Greenberg PD, Cheever MA, Fefer A: Role of host and donor cells in tumor elimination following adoptive chemoimmunotherapy (ACIT). Proc Am Assoc Cancer Res 23:1014, 1982 (abstract).
77. Zaleski M, Klein J: Genetic control of the immune response to Thy-1 antigens. Immunol Rev 38:120, 1978.
78. Hayry P, Anderson LC: Generation of T memory cells in one-way mixed lymphocyte culture: IV. Primary and secondary responses to soluble and insoluble membrane preparations and to ultraviolet light inactivated stimulator cells. Scand J Immunol 5:391, 1976.

79. Hellström I, Hellström KE: Specific sensitization of lymphocytes to tumor antigens by co-cultivation with peritoneal cells exposed to such antigens. Int. J Cancer 17:748, 1976.
80. Treves AJ, Schechter B, Cohen IR, Feldman M: Sensitization of T lymphocytes *in vitro* by syngeneic macrophages fed with tumor antigens. J Immunol 116:1059, 1976.
81. Kall MA, Hellström I: Specific stimulatory and cytotoxic effects of lymphocytes sensitized *in vitro* to either alloantigens or tumor antigens. J. Immunol 114:1083, 1975.
82. Plata F, Cerottini JC, Brunner KT: Primary and secondary *in vitro* generation of cytolytic T lymphocytes in the murine sarcoma virus system. Eur J Immunol 5:227, 1975.
83. Ting CC, Bonard GD: Cell-mediated immunity to Friend virus-induced leukemia: IV. *In vitro* generation of primary and secondary cell-mediated cytotoxic responses. J Immunol 116:1419, 1976.
84. Burton RB, Chism SE, Warner NL: *In vitro* induction and expression of T-cell immunity to tumor associated antigens. Contemp Top Immunobiol 8:69, 1978.
85. Röllinghoff M, Wagner H: Brief communication: *In vitro* protection against murine plasma cell tumor growth by *in vitro* activated syngeneic lymphocytes. J Natl Cancer Inst 51:1317, 1973.
86. Small M, Trainin N: Separation of populations of sensitized lymphoid cells into fractions inhibiting and fractions enhancing syngeneic tumor growth *in vivo.* J Immunol 117:292, 1976.
87. Cheever MA, Greenberg PD, Fefer A: Adoptive therapy of established syngeneic leukemia by cells primarily sensitized *in vitro.* Cancer Res 41:2658, 1981.
88. Burton RC, Warner NL: *In vitro* induction of tumor-specific immunity: IV. Specific adoptive immunotherapy with cytotoxic T cells induced *in vitro* to plasmacytoma antigens. Cancer Immunol Immunother 2:91, 1977.
89. Plata F, Jongeneel CV: Characterization of effector lymphocytes associated with immunity to murine sarcoma virus (MSV)-induced tumors: II. Repeated stimulation and proliferation *in vitro* of specific cytolytic T lymphocytes. J Immunol 119:623, 1977.
90. Bernstein I: Passive transfer of a systemic tumor immunity with cells generated *in vitro* by a secondary immune response to a syngeneic rat Gross-virus-induced lymphoma. J Immunol 118:122, 1977.
91. Mills GB, Carlson G, Paetkau V: Generation of Cytotoxic lymphocytes to syngeneic tumors by using co-stimulator (Interleukin 2): *In vivo* activity. J Immunol 125:1904, 1980.
92. Cheever MA, Greenberg PD, Fefer A: Tumor neutralization, immunotherapy, and chemoimmunotherapy of a Friend leukemia with cells secondarily sensitized *in vitro*: II. Comparison of cells cultured with and without tumor to noncultured immune cells. J Immunol 121:2220, 1978.
93. Wagner H, Röllinghoff M: T-T cell interaction during *in vitro* cytotoxic allograft responses: I. Soluble products from activated Ly 1^+ T cells trigger autonomously antigen-primed Ly 23^+ T cells to cell proliferation and cytolytic activity. J Exp Med 148:1523, 1978.
94. Shaw J, Caplan B, Paetkau V, Pilarski LM, Delovitch TL, McKenzie IFC: Cellular origins of co-stimulator (IL 2) and its activity in cytotoxic T lymphocyte responses. J Immunol 124:2231, 1980.
95. Bonnard GD, Yasaka K, Jacobson D: Ligand-activated T cell growth factor-induced proliferation: Absorption of T cell growth factor by activated T cells. J Immunol 123:2704, 1979.
96. Smith KA, Gillis S, Baker PE, McKenzie D, Ruscetti FW: T cell growth factor mediated T cell proliferation. Ann NY Acad Sci 332:423, 1979.
97. Gillis S, Mochizuki DY, Conlon PJ, Hefeneider SH, Ramthun CA, Gillis AE, Frank MB,

Henney CS, Watson JD: Molecular characterization of Interleukin 2. Immunol Rev 63:167, 1982.
98. Watson J: Continuous proliferation of murine antigen-specific helper T lymphocytes in culture. J Exp Med 150:1510, 1979.
99. Schreier MH, Tees R: Clonal induction of helper T cells: Conversion of specific signals into nonspecific signals. Int Archs Allergy Appl Immun 61:227, 1980.
100. Smith KA, Gillis S, Baker P: The inhibition of *in vivo* tumor growth by cytotoxic T cell lines. Proc of Am Assoc Ca Res 20:93, 1979. Abstract.
101. Cheever MA, Greenberg PD, Fefer A: Specific adoptive therapy of established leukemia with syngeneic lymphocytes sequentially immunized *in vivo* and *in vitro* and non-specifically expanded by culture with Interleukin 2. J Immunol 126:1318, 1981.
102. Cheever MA, Greenberg PD, Gillis S, Fefer A: Specific adoptive therapy of murine leukemia with cells secondarily sensitized *in vitro* and expanded by culture with Interleukin 2. In: The potential role of T cells in cancer therapy, Fefer A, Goldstein AL (eds). New York: Raven Press, 1982, p 127.
103. Lotze MT, Line BR, Mathisen DJ, Rosenberg SA: The *in vivo* distribution of autologous human and murine lymphoid cells grown in T cell growth factor (TCGF): Implications for the adoptive immunotherapy of tumors. J Immunol 125:1487, 1980.
104. Dailey MO, Fathman CG, Butcher EC, Pillemer E, Weismann I: Abnormal migration of T lymphocyte clones. J Immunol 128:2134, 1982.
105. Cheever MA, Greenberg PD, Fefer A, Gillis S: Augmentation of the anti-tumor therapeutic efficacy of long-term cultured T lymphocytes by *in vivo* administration of purified Interleukin 2. J Exp Med 155:968, 1982.
106. Wescott O, Dorsch S, Roser B: Adoptive immunotherapy of leukemia in the rat, without graft-vs-host complications. J Immunol 123:1478, 1979.
107. Boranic M, Tonkovic I: Time pattern of the antileukemic effect of the graft-versus-host reaction in mice. Cancer Res 31:1140, 1970.
108. Kende M, Keys LD, Gaston M, Goldin A: Immunochemotherapy of transplantable Moloney leukemia with cyclophosphamide and allogeneic spleen lymphocytes and reversal of graft-versus-host disease with alloantiserum. *Cancer Res* 35:346, 1975.
109. Putnam DL, Kind PD, Goldin A, Kende M: Adoptive immunochemotherapy of a transplantable AKR leukemia (K36). Internatl J Cancer 21:230, 1978.
110. Truitt RL, Bortin MM: Adoptive immunotherapy of malignant disease: Model systems and their relevance to human disease. In: Organ transplantation: Present status, future goals, Slavin S (ed). Amsterdam: Elsevier/North Holland. In press.
111. Yamada Y, Kawamura T, Gotohda E, Akiyama J, Hosokawa M, Kodama T, Kobayashi H: Effect of normal allogeneic lymphoid cell transfer in combination with chemotherapy on a transplantable tumor in rats. Cancer Res 40:954, 1980.
112. Bortin MM, Truitt RL: Graft-versus-leukaemia reactivity induced by alloimmunisation without augmentation of graft-versus-host reactivity. Nature 281:490, 1979.
113. Bortin MM, Rimm AA, Saltzstein EC, Rodey GE: Graft versus leukemia: III. Apparent independent antihost and antileukemic activity of transplanted immunocompetent cells. Transplantation 16:182, 1973.
114. Korngold R, Sprent J: Features of T cells causing H-2-restricted lethal graft-vs-host disease across minor histocompatibility barriers. J Exp Med 155:872, 1982.
115. Bevan MJ: Cytotoxic T-cell response to histocompatibility antigens: The role of H-2. Cold Spring Harbor Symp Quant Biol 41:519, 1976.
116. MacPhail S, Yron I, Stutman O: Primary *in vitro* cytotoxic T cell response to non-major histocompatibility complex alloantigens in normal mice. J Exp Med 156:610, 1982.
117. MacPhail S, Stutman O: Suppressor T cells activated in a primary *in vitro* response to non-major histocompatibility alloantigens. J Exp Med 156:1398, 1982.

Subject Index

A blood group antigen, 204
n-Acetylmuramyl-L-alanyl-D-isoglutamine (MDP), 280, 286
  action of, 282-283
  macrophages in metastases treatment and, 93, 94, 95
Acquired immunodeficiency syndrome (AIDS), 23, 60
ADCC assay, 229, 233-234, 236
  interferon and, 265, 266
  monoclonal antibodies and, 276
Adenocarcinomas
  biological heterogeneity of, 85
  lymphocyte response tests in, 223, 225
Adenomas, in nude mice, with immunological surveillance, 26, 28, 30
Adenovirus type 12 induced tumors, and lentinan, 284
Adherent cells, 240-241
Adoptive chemoimmunotherapy (ACIT), 307-310
  effector cells with, 310-315
  kinetics of tumor elimination by, 308-309
  specificity of immune cells in, 309-310
  suppressor cells in, 315-317
Adoptive immunotherapy, 310-329
  allogenic lymphocytes with, 325-328
  chemoimmunotherapy (ACIT), 307-310
  effector cell generation in, 321-325
  following reduction of tumor load, 305-307
  host cells and donor cells in, 317-320
  interleukin 2 (IL 2) and, 324-325
  radiation therapy and, 306
  syngeneic lymphocytes with, 303-320
Adriamycin, and antibody conjugation, 277
Agammaglobulinemia, and natural killer (NK) activity, 60
AKR tumor system
  azimexon with, 281
  interferon with, 262
  monoclonal antibodies in, 273
  picibanil and, 285
Alpha-1 thymosin, 272
Antibody formation, 221-222
Antilymphocyte serum (ALS), 8, 10
Antithymocyte serum (ATS), 8, 84
Anti-tumor immunity, 107-123
  active specific immunotherapy and, 118-120
  blocking factors in, 115, 119
  cellular immune response to tumors in, 132-138
  conditions before therapeutic use of, 107-108
  down-regulating, 119
  effector cells in tumor recognition in, 110-112
  generation of, 109-115
  immune recognition of malignant cells in, 108-109
  interferon and, 262
  modulation of, 115-118
  suppressor T cells and, 115-116
Antitumour T cells, 129-150
  culture of cytotoxic and proliferative lymphoid cells in, 138-143
  large granular lymphocytes (LGL) in, 138-139, 140
  specificity of cells in, 148

suppression of immunity in, 148-149
tumor heterogeneity in, 149
Asialo-G_{M1} antigen, 203
Astrocytoma antigens, and monoclonal antibodies, 180
Ataxia telangiectasia, 22, 60
Atheromas, 89
Autoimmune thyroditis, 24
Autologous mixed lymphocyte reaction (AMLR), 136
Azathioprine, 10
Azimexon, 280, 281-282, 286

Bacillus Calmette-Guerin (BCG), 258-259
active ingredients for adjuvant effect of, 259-260
followed by cytotoxic chemotherapy, 260-261
immunotherapy with, 118, 121
non-Hodgkin lymphoma (NHL) and, 24
target cell interaction with macrophages stimulated with, 88
tumor development and, 11-12, 123
tumor rejection with, 111-112
Basal cell carcinoma, and immunological surveillance (IS), 21
B cells
immunodilator testing and, 280
immunoglobulin levels and, 221, 222
immunosuppression and tumor growth and, 10
interleukin-3 and, 279
lymphokines and, 278
MDP and, 282
tumor cell marker and, 275
B cell tumors
interferon and, 264
monoclonal antibodies to, 196
BCG, *see* Bacillus Calmette-Guerin (BCG)
BCNU, with MVE-2 therapy, 280
Beige mice, and natural killer (NK) activity, 52-56
Biological response modifiers (BRM), 257-287
Bladder cancer
delayed type hypersensitivity (DTH) in, 218, 220
DNCB tests in, 226
PHA response in, 226
suppressor cells in, 242-243, 244
T cells in, 230
Blast antigens in leukemia, 193-194
Blastogenesis, 222
Bone marrow function, and immunodeficiency, 60
Boron-10, 277
Brain cancer
Ca antigen in, 200
monoclonal antibodies in, 174-180
Breast cancer
biological heterogeneity of, 85
carcinoembryonic antigen (CEA) in, 189
host immunity in, 84
immune response in, 132-133
immunogenicity of, 108-109
immunotherapy with, 122-123, 130
interferon and, 264, 266, 268-269
lung macrophages in, 90
lymphocyte test responses in, 224, 227, 228, 229
monoclonal antibodies to, 187-189
natural killer (NK) activity in, 45, 62, 235-236
picibanil and, 285
suppressor cells in, 14-15, 239, 244
thymosin and, 272
T lymphocytes in, 229, 231
Bronchial carcinoma
E-rosette forming cells in, 229, 230
immunoglobulins in, 221
Bronchogenic cancer
immunoglobulin levels in, 221
lymphocyte response test in, 226
Brucella endotoxin, 221
Burkitts lymphoma
delayed type hypersensitivity (DTH) in, 219
EBV particles associated with, 198
Busulphan, 130
1-butanol, 121

Ca antigen, and monoclonal antibodies, 199-200
Candida antigens, 218
Carcinoembryonic antigen (CEA), 189, 199
Carcinogenesis
anti-thymocyte (ATS) and antilymphocyte (ALS) serum in, 8, 10
immunopotentiation and, 11-13
mononuclear phagocytes and, 88-92

tumor development and, 7-10
Cardiac transplantation, 20
Chang target cells, 234
Chediak-Higashi syndrome, 60
Chromium, and T cells in tumor development, 134
Cis-platinum compounds, 62
Colon cancer
adherent suppressor cells in, 242
biological heterogeneity of, 85
carcinoembryonic antigen (CEA) in, 189
cytotoxic activity with disease progression in, 135
DNCB response in, 219
lymphocyte response in, 223, 224
monoclonal antibodies in, 123, 275
Colorectal cancer
ADCC assay in, 234
monoclonal antibodies to, 123, 179, 180-182
suppressor cells in, 243
Combination therapy in cancer, 149
Common acute lymphatic leukemia antigen (CALLA), 276
Concanavalin A (Con A), 223
recurrence of disease and, 223-224
suppressor cells and, 239-240, 243, 244
treatment results and, 225
Corynebacterium parvum
immunotherapy with, 118
target cell interaction with macrophages with, 88
tumor cell vaccination with, 261
tumor development and, 11
Cryptomnesia, in immunosuppression, 10, 39
CSF, 279
Cyclophosphamide (CY)
adoptive immunotherapy with, 305, 307, 308, 312, 316, 319
azimexon with, 282
combination therapy with immunosuppression and, 149
immunotherapy with, 119-120
suppressor cell action and, 13
thymosin with, 271
tumor development and, 38, 50
tumor immunity generation and, 117, 119
vaccinations combined with, 260-261
Cystadenocarcinoma, ovarian, and monoclonal antibodies, 185-186
Cytokines, 277-279
Cytotoxic T lymphocytes (CTL), 233
adoptive immunotherapy with, 310, 311-312, 313

Daunomycin, 277
Delayed type hypersensitivity (DTH), 217-220
adoptive chemoimmunotherapy (ACIT) and, 311, 313, 315
categories of, 218
natural killer (NK) cytotoxicity and, 236
recurrence of disease and, 219-220
thymosin and, 271, 272
Dermatophytin 'O, 220
Diethylstilbestrol (DES)-induced tumors
natural killer (NK) activity in, 48-49, 62
in nude mice, 33-35, 36-37
Dimethylbenzanthracene (DMBA), and natural killer (NK) activity, 50-51, 54-55
2.4 dinitrochlorobenzene (DNCB) testing, 218-219, 226
recurrence of disease and, 219-220, 224
T cell quantitation correlation with, 229
treatment results with, 225
Down's syndrome, 60

Ear duct carcinoma, 109
Effector cells
adoptive chemoimmunotherapy (ACIT) and, 310-315
adoptive immunotherapy and generation of, 321-325
function of, 112-115
immunotherapy with, 131-132
interferon and, 263
Interleukin 2 (IL 2) and, 324-325
T cell delayed type hypersensitivity (TDTH), 112, 113-114
tumor recognition with, 110-112
Ehrlich carcinoma, 285, 286
EL-4 lymphoma, 309, 322
Epstein-Barr virus (EBV), 142
Burkitt's lymphoma with, 198
immunodeficiency in malignancy and, 22-23
natural killer (NK) defect and, 59
E-rosette forming cells (E-RFC), 228-229
disease stage and, 230, 231

thymosin and, 271, 272
Erythroleukemia, 304
Erythropoiesis, and macrophages, 89
Ethylnitrosourea (ENU)-induced tumors, 35

Familial melanoma, 61
Fanconi's anemia, 60
Fetal antigens, 204
Fibrosarcoma
adoptive immunotherapy with, 305, 306, 309, 316, 323
azimexon with, 281
immunodepression and, 8, 10
immunosuppression in, 149
macrophages in, 91
nude mice, in immunological surveillance (IS), 25
pathogenesis of metastases in, 84-85
sneaking through phenomenon in, 20
suppressor cells and, 13
tuftsin and, 283
tumoricidal macrophages in, 87
N-2-fluorenylacetamide FAA-induced carcinoma, 109
Forssman antigen, 204
Fraction 5 thymosin, 271-272
Friend virus leukemia
adoptive immunotherapy with, 304, 307, 308
combination therapy with, 149
immunodepression with, 10
interferon with, 262

Gastric cancer, *see* Stomach cancer
Gastrointestinal cancer antigen (GICA), 204
Gastrointestinal malignancies, 276
Gluten enteropathy, 24
Graft-versus-host disease (GVHD), 130, 242, 325, 326-327
Granulocytic leukemia, 197-198
Growth factors, T cell (TCGF), 114, 132, 278

Head and neck cancers
adherent suppressor cells in, 242, 243
DNCB response in, 219
lymphocyte response test in, 225, 227, 228
thymosin and, 271
Helix pomatia haemocyanin, 221, 222
Helper T cells, 231-232
immune response and, 133
suppressor T cells and, 15
Hemangioendotheliomas, 29
Hemopoietic system, and immunodeficiency, 60
Hepatocarcinoma
BCG therapy in, 259
monoclonal antibodies and, 275
Hepatocellular carcinoma, 109
Hepatoma
anti-tumor immune response with, 118
effector cells and, 111-112
immunotherapy for, 121
natural killer (NK) cells and, 45
tumor-specific immunity induced in, 116, 117, 118
Hodgkin's disease
ADCC assay in, 234
delayed type hypersensitivity (DTH) in, 218-219
immunoglobulin levels in, 222
interferon and, 267
lymphocyte test responses in, 223, 224, 229
suppressor cells in, 239, 240, 241
T cell quantitation in, 229, 231
Host resistance
antitumor immunity and, 107-123
biological heterogeneity of malignant neoplasms and, 85-86
macrophages and, 83-96
pathogenesis of metastases and, 83-85
Human T-lymphoma virus (HTLV), 198-199
Hybridoma technique, with monoclonal antibodies, 159, 273
Hypogammaglobulinemia, 60, 238

Immune system
interferon and, 262-263
prognostic significance of information about, 216
techniques in studies of, 216-217
Immunocompetence, 215-246
delayed type hypersensitivity (DTH) in, 217-220
immunoglobulin quantitation in, 221-222
lymphocyte cytotoxic functions in, 232-237
lymphocyte proliferative function in, 222-228
suppressor cells in, 237-244

T cell quantitation in, 228-232
Immunodeficiency
malignancy and, 20-24
in non-Hodgkin lymphoma (NHL), 22-24
Immunodepression, and tumor development, 7-10
Immunodilators, 280-286
Immunoglobulins
quantitation of level of, 221-222
T suppressor cells and, 238, 239
Immunoglobulin A (IgA), 221, 222, 239
Immunoglobulin G (IgG), 221, 222
antibody therapy with, 274, 275, 276, 277
killer (K) cell receptors for, 233
natural killer (NK) cell receptors for, 234
T cell receptors for, 231
T suppressor cells and, 239
Immunoglobulin M (IgM), 274
natural killer (NK) cell receptors for, 234
T cell receptors for, 231
T suppressor cells and, 239
Immunological surveillance (IS) hypothesis, 1-64, 215-216
carcinogen dose and antigenicity in, 17-18
clinical evidence in, 20-24
clonality assessment in, 16
cryptomnesia in, 39
definitions in, 3-4
escapes from surveillance in, 5-6
experimental studies on, 7-20
immunodepression and tumor development in, 7-10
immunopotentiation and tumor development in, 11-13
lymphoma development and, 3, 30-32
natural cell-mediated cytotoxicity (N-CMC) in, 38-62
natural killer (NK) cells in, 43
selection of tumor variants in, 15-16
sneaking through phenomenon in, 19-20, 41
spontaneous tumors and lymphomas in nude mice in, 25-29
strain differences and, 18-19
studies on conventional, 7-24
suppressor cells and, 13-15
T cells and, 1-2
tumor development in nude mice in, 24-38
tumor-host interactions in, 4-5
tumor immunity appearance and, 4
tumor necrosis factor in, 6
viable yellow gene effect in nude mice in, 28-29
Immunopotentiation, and tumor development, 11-13
Immunosuppression
combination therapy with, 149
prostaglandin in, 149
Immunotherapy
active specific and nonspecific, 118-120, 258-261
adoptive, *see* Adoptive immunotherapy
conditions in, 107-108
cultured T lymphocytes in, 144-149
followed by cytotoxic chemotherapy, 260-261
introduction of cultured cells in, 144-145
metastatic tumors with, 120-123
parameters necessary for success in, 131-132
passive cellular, 130-132
regional, 122-123
specificity of T cells in, 148
Interferon, 261-271, 286
animal tumor models with, 262-263
antiproliferative effect of, 262-263
beta and gamma, 269
Biological Response Modifiers Program (BRMP) research in, 265-267
Con A induced suppressor cell and, 240
functions of, 261-262
human trials with, 263-264
immune, and T cell subsets, 115
immunotherapy with, 123
inducers of, 269-271
natural killer (NK) activity and, 44, 60, 61, 62, 235
recombinant, 268-269
suppressor cells and, 241, 242
tumor cytotoxic activity with, 137
Interleukin 1 (IL 1), 278-279
Interleukin 2 (IL 2), 324-325
Interleukin-3 (IL 3), 279
IS, *see* Immunological surveillance (IS) hypothesis

Jumping antigens, 203-204

Kaposi sarcoma, 23

Keyhole limpet haemocyanin, 218
Kidney transplantation, and natural killer (NK) activity, 60
Killer (K) cells, 233-234

Lentinan, 280, 284-285, 286
Leprosy, 60
Leukemia
  adoptive immunotherapy with, 307, 308, 324-325, 326
  allogeneic cells in treatment of, 326
  azimexon with, 281
  B-cell, 196-197
  BCG vaccination in, 258
  beige mice natural killer (NK) activity and, 55-56
  blast antigens in, 193-194
  candidate non-T, non-B leukemia specific antigen in, 197
  candidate T-leukemia-specific antigen in, 195-196
  chronic antigenic stimulation in, 30-31
  common acute lymphoblastic leukemia antigen (CALLA) in, 196-197
  granulocytic and monocytic, 197-198
  immunoglobulin levels in, 221
  IgM monoclonal antibody (CALL2) in, 195
  interferon and, 270
  low natural killer (NK) activity and, 56-58
  monoclonal antibodies in, 193-199, 276
  natural killer (NK) cells and, 51-52, 60, 62, 235, 237
  non-immunogenic, 109
  PHA response, in, 223
  recall antigens in, 220
  spontaneous, with natural killer (NK) cells, 45-47
  suppressor cells in, 238, 243-244
  T-cell, 194-196, 231
  thymus-leukemia (TL) antigens in, 194-195
  viral antigens related to, 198-199
Levamisole, 11, 12
Lewis lung carcinoma
  adoptive immunotherapy with, 305-306, 322
  azimexon with, 281
  interferon and, 262, 263
  lentinan and, 284
  macrophages and, 88
Lipid metabolism, and macrophages, 89
Lip tumors, 21
L1210 vaccine, 280
Lung cancer
  adoptive immunotherapy with, 305-306, 322
  azimexon with, 281
  biologic heterogeneity of, 85-86
  cyclophosphamide (CY) and, 50
  cytotoxic activity with disease progression in, 135
  delayed type hypersensitivity (DTH) in, 218
  immunoglobulin levels in, 221
  interferon and, 262, 263
  lentinan and, 284
  lymphocyte test responses in, 223, 224, 225-226, 228, 229
  monoclonal antibodies in, 182-184
  natural killer (NK) activity in, 49-50, 52, 54, 236
  in nude mice, in immunological surveillance (IS), 26, 28, 32-33
  suppressor cells in, 241, 243-243, 244
  thymosin and, 271, 272, 273
  T lymphocytes in, 229, 230
  see also Pulmonary cancer
Lymph node lymphocytes (LNL), 236, 244
Lymph node metastasis, 283
Lymphocytes
  activating factor, 278
  adoptive immunotherapy with, 303-320
  assessment of, 223
  cytotoxic functions, 232-237
  expression of data on, 226-227
  interferon and, 265
  large granular (LGL), 138-139, 140
  lymph node (LNL), 244
  MDP and, 282
  mixed culture (MLC), 224-225
  natural killer (NK) activity and, 236
  proliferative functions of, 222-228
  prostaglandins and, 242
  reactive, within tumor mass, 140-141
  recurrence of disease and, 223-224
  stage or outcome of disease and, 224, 225-226, 228
  stimulation of, in cancer, 135-136, 227
  traffic studies of human, 145-147
  treatment results and, 225

tumor infiltrating (TIL), 236, 244
Lymphocytic leukemia
BCG vaccination in, 258
immunoglobulin levels in, 221
interferon in, 270
monoclonal antibodies in, 276
natural killer (NK) cells in, 235
PHA response in, 223
suppressor cells in, 238, 243-244
T lymphocyte counts in, 231
Lymphohemopoiesis, and natural cell-mediated cytotoxicity (N-CMC), 63
Lymphoid cells
antitumor T cells and, 138-143
monoclonal antibodies to, 185
Lymphoid tumors
immunodeficiency and, 21-22
lymphokines and, 278
Lymphokines, 277-279
immune response with, 132
immunotherapy with, 122
macrophage activation with, 93
Lymphoma
adoptive immunotherapy for, 326
allogeneic cells in treatment of, 326
BCG therapy in, 259
beige mice natural killer (NK) activity and, 53-54, 55-56
biological heterogeneity of, 85
Ca antigen in, 200
chronic antigenic stimulation in, 30-31
immunodeficiency in, 21. 22-24
immunoglobulin levels in, 222
immunological surveillance (IS) in, 3
induction of, in nude mice, 30-32
interferon and, 264, 266, 267, 268, 269
lentinan and, 284
low natural killer (NK) activity and, 56-58
monoclonal antibodies in, 193-199, 273, 274, 275
natural killer (NK) activity and, 42, 45-47, 60
in nude mice, in immunological surveillance (IS), 24, 25-29
sneaking through phenomenon in, 41
tuftsin in, 283
viral antigens related to, 198-199
Lymphoproliferative disorders
lymphocyte transformation studies in, 227
natural killer (NK) activity in, 59, 60
Lymphoreticular tumors, 29, 30
Lymphosarcoma
immunoglobulin levels in, 221
PHA response in, 223

Macrophage activating factor (MAF), 279
metastases in, 92, 93, 95
Macrophages, 83-96
immune response with, 133
immunodilator testing and, 280
lentinan and, 284
target cell interaction with, 86-88
tumoricidal processes in, 86-87
Mammary cancer, *see* Breast cancer
Mastocytoma, 119, 309, 323
MDP, *see* Muramyl dipeptide (MDP)
Megakaryoblastic leukemia, 198
Melanocytes
differentiation in, and melanoma subsets, 173-174
high molecular weight antigen complex in, 161-164
malignant melanoma studies with, 160
Melanoma
antigen 3.1 in, 170
antigens Mel-5 and Mel-7 in, 170
BCG therapy in, 259
biologic heterogeneity of, 85
Ca antigen in, 200
delayed type hypersensitivity (DTH) in, 218, 219, 220
E-rosette forming cells in, 229
ganglioside (G_{D3}) antigen in, 170-172
gp74 and gp49 in, 170
gp94 on, 168-169
gp94/75/70/30 complex in, 169
gp95 in, 166-168
gp116/95/29/26 complex in, 166
gp130 in, 165-166
gp210 and gp155 in, 165
high molecular weight antigen complex in, 161-165
HLA-DR (Ia-like) antigens in, 173
immunoglobulin levels in, 221
immunotherapy in, 130
interferon and, 266, 271
lymphocyte response tests in, 224-225, 227
malignant transformation in differentiation stages in, 160
melanocyte differentiation and subsets in,

173-174
mixed lymphocyte culture (MLC) in, 224-225
monoclonal antibodies in, 123, 160-174, 185, 275
natural killer (NK) activity in, 61, 236, 237
origin of various types of, 161
suppressor cells in, 240, 241, 242, 244
T cells in, 230, 232
thymosin and, 272
tuftsin and, 283
Metastases
DNCB skin test in, 219-220
immunoglobulin levels in, 221
immunotherapy of, 120-123
mononuclear phagocytes and treatment of, 92-95
natural cell-mediated cytotoxicity (N-CMC) and, 63
natural killer (NK) activity in, 42, 235
pathogenesis of, 83-84
T cell counts in, 232
tumor-host interactions in, 4-5
Meth A fibrosarcoma
adoptive immunotherapy with, 307
immunosuppression in, 149
sneaking through phenomenon with, 20
Methylcholanthrene (MCA)
carcinogen dose and antigenicity in, 17, 18
clonality assessment with, 16
host immunity and, 84
immunotherapy for, 121
macrophages and, 91
natural killer (NK) activity and, 48, 54, 57-58
tumor development and, 11
tumor specific antigens and, 108
MHC antigens, 133
Milk fat globule antigens, 188-189
Mitomycin C-inactivated-tumor cells, 112
Mixed lymphocyte culture (MLC), 224-225, 236
MLTC
lymphocyte activation in tumors with, 135-136, 140
tumor proliferative and cytotoxic precursors and, 141-143
Moloney leukemia virus (M-MuLV)
adoptive immunotherapy in, 307, 308
natural killer (NK) cells in, 55
Moloney sarcoma virus
adoptive immunotherapy with, 305, 310
effector cells in anti-tumor responses in, 112
natural killer (NK) activity in, 55
Monoclonal antibodies (moAbs), 159-204, 273-277
advantages and disadvantages of, 160
astrocytome antigens and, 180
B-cell leukemias and lymphomas in, 196
brain cancer and, 174-180
breast cancer and, 187-189
Ca antigen with, 199-200
carcinoembryonic antigen (CEA) with, 199
classification of, 200-204
colorectal cancer with, 179, 180-182
common antigens with, 204
conjugation of. 277
differential antigens in, 202-203
fetal antigens and, 204
granulocytic and monocytic leukemias in, 197-198
human T-lymphoma virus (HTLV) in, 198-199
hybridoma technique with, 159, 273
immunomodulating agents with, 123
jumping and random antigens in, 203-204
leukemias and lymphomas and, 193-199
lung cancer and, 182-184
malignant melanoma with, 160-174
megakaryoblastic leukemia with, 198
milk fat globule antigens and, 188-189
non-T cell, non-B cell acute lymphocytic leukemias with, 196-197
osteogenic sarcoma with, 192-193
ovarian cancer and, 185-186
pancreatic cancer and, 192
prostate cancer and, 192
renal cancer and, 189-191
specificity of, 159
T cell function and, 274-275
T-cell leukemias and lymphomas and, 194-195
tumor-specific antigens in, 200-201
Monocytic leukemia, 197-198
Mononuclear phagocytes
carcinogenesis and progression of cancer and, 88-92
metastases treatment and, 92-95

target cell interaction with, 86-88
MSV-induced sarcoma, 149, 282
Multiple sclerosis, 59
Mumps skin test antigen, 218, 220
Muramyl dipeptide (MDP), 260, 280, 282-283, 286
immunotherapy with, 122
macrophages in metastases and, 93, 94, 95
Murine tumors
immunotherapy for, 120, 121
non-immunogenic, 109
in nude mice, in immunological surveillance (IS), 31-32
suppressor cells and, 14, 116, 119
tumor cell-*C. parvum* vaccination in, 261
MVE-2, 280-281, 286
Mycosis fungoides, 198
Myelocytic leukemia
immunoglobulin levels in, 221
natural killer (NK) activity in, 62
Myeloid leukemia, 235
Myeloma
immunoglobulin levels in, 221
interferon with, 263, 268, 271
natural killer (NK) activity in, 60, 61
PHA response in, 223
suppressor cells in, 237, 241

Nasopharynx cancer, 221
Natural cell-mediated cytotoxicity (N-CMC),
anti-tumor surveillance mediated by, 38-62
cis-platinum compounds and, 62
escapes from surveillance and, 5, 6
general properties of, 40-44
in high natural killer (NK) activity mouse strains, 44-45
immunological surveillance (IS) and, 1, 2, 4
natural cytotoxic (NC) branch of, 20
natural killer (NK) cells and, 40, 43
nude mice tumor development with, 24, 37
sneaking through phenomenon with, 41
spontaneous tumors in rats and mice with natural killer (NK) activity and, 45-48
tumor development in animals deficient in, 44-58
Natural cytotoxic (NC) cells, 40, 41, 42
Natural killer (NK) cells, 233, 234-237
adjuvant therapies and, 62
adoptive chemoimmunotherapy (ACIT) and, 310, 311
age-related decline of spleen activity of, 43-44
beige mice tumor development with, 52-56
carcinogens and depression of, 49-52
clinical evidence of, 58-62
cytotoxic activity in tumors and, 135
dimethylbenzanthracene (DMBA) and, 50-51
high activity mouse strains of, 44-45
immunodilator testing and, 280
immunological surveillance (IS) and, 1
immunopotentiators and, 12
immunotherapy agents and, 123
interferon and, 44, 261, 265, 266, 267, 268, 269, 270
lack of standardized technology with, 236
lentinan and, 284-285
leukomogenic split-dose radiation and, 51
low levels of, with lymphoma-leukemia development, 56-58
lymphoma in beige mice with, 53-54
metastasis of lymphomas and solid tumors and, 42
multiple sclerosis and, 59
natural cell-mediated cytotoxicity (N-CMC) and, 41
normal level definitions in, 58-59
specificity of cytotoxicity of, 235
spontaneous tumors in rats and mice and, 45-48
suppressor cells and, 13, 244
target preference of, 42
T cell delayed type hypersensitivity (TDTH) effector cells with, 114
tuftsin and, 283
tumor heterogeneity and, 149
urethan lung carcinoma and, 49-50, 54
N-CMC, *see* Natural cell-mediated cytotoxicity (N-CMC)
Neck cancer, *see* Head and neck cancers
NED-137, 280, 285, 286
Nevi, 161, 164, 165
Nitrogen mustard, 130
NK, *see* Natural killer (NK) cells
Nude mice
diethylstilbestrol (DES)-induced testicular tumors in, 36-37
lymphoma induction in, 30-32
monoclonal antibodies in, 276

skin tumors in, 33-36
spontaneous tumors and lymphomas in, 25-29
tumor development in, 24-38
viable yellow gene effect in, 28-29

Oropharyngeal cancer, 221
Osteomyelofibrosis, 60
Osteosarcoma
immunotherapy with, 123
interferon and, 263-264
monoclonal antibodies to, 192-193
Ovarian carcinoma
BCG therapy in, 259
biologic heterogeneity of, 85
immunoglobulin levels in, 221
immunotherapy in, 130
interferon with, 263, 264
monoclonal antibodies to, 185-186

Pancreatic cancer, 192
Papain extract, 116
Papilloma, 33-34, 35
P blood group antigen, 204
Penile cancer, 130
Phorbol acetate-induced skin tumors, 34-35
Phytohemmagglutinin (PHA), 222, 223
cytotoxic and proliferative lymphoid cells with, 138, 140
lymphocyte activation in tumors with, 135
natural killer (NK) activity and, 236
recurrence of disease and, 223-224, 226
stage of disease correlation to, 223
stimulation index in, 227
suppressor cells and, 240, 241-242, 243, 244
T cell quantitation correlation with, 229
treatment results and, 225
Picibanil, 280, 285-286
Plasma cell myeloma, 223
Plasmaphoresis in cancer therapy, 119
Pokeweed mitogen (PWM), 223-224
Polycythemia vera, 60
Polyriboinosinic:polyribocytidylic acid (Poly I:C), 269-270
PPD, *see* Tuberculin (PPD) test
Prednisolone, 130
Preleukemia, 60
Prostaglandins
lymphocyte response and, 242
MDP and, 282
stimulator tumor cells and, 135
suppression of immunity and, 149
suppressor cells in Hodgkin's disease and, 241
Prostatic cancer
Ca antigen in, 200
immunoglobulin levels in, 221
monoclonal antibodies in, 192
natural killer (NK) activity in, 62
Pulmonary tumors
interferon and, 263
lung macrophages in, 90
MDP and, 283
in nude mice, in immunological surveillance (IS), 26, 28, 30
pathogenesis of metastases in, 84-85
tumor rejection antigens in, 122
see also Lung cancer
Pyran copolymer, 11, 90

Radiation therapy, with adoptive immunotherapy, 306
Random antigens, 203-204
Rauscher viruses
adoptive immunotherapy with, 307, 308
interferon with, 262
Recall antigens, 218, 220
Renal cell carcinoma
interferon and, 267, 270, 271
monoclonal antibodies to, 189-191
thymosin and, 272
tumor rejection antigens in, 122
Renal transplantation, 20
Retrovirus particles, 198, 199
Rhabdomyosarcomas, 91
Rheumatoid arthritis, 24
Rosette forming cells (RFC), 228

Salmonella extract, 221
Sarcoma
adoptive immunotherapy with, 304, 305, 307, 309, 326
allogeneic cells in therapy of, 326
Ca antigen in, 200
delayed type hypersensitivity (DTH) in, 218, 219
effector cells in, 111-112
immunogenicity of, 108, 109
immunoglobulin levels in, 221

immunotherapy with, 119, 121
lentinan and, 284
lymphocyte response test in, 225
monoclonal antibodies to, 192-193
picibanil and, 285, 286
suppressor cells in, 13-14
tumor-specific immunity induced in, 116, 118
tumor specific antigens in, 108
Schistosomiasis, 24
Severe combined immune deficiency (SCID), 20, 22-23
Severe immunodeficiency syndrome, 20
Sheep red blood cell (SRBC) reaction, 228-229, 285
Sjogren's syndrome, 24
Skin tumors
immunodepression and, 7-10
immunoglobulin levels in, 221
immunological surveillance (IS) and, 21
in nude mice, 33-35
Small-cell carcinoma of lung
biologic heterogeneity of, 85-86
monoclonal antibodies to, 183
thymosin and, 271
Spontaneous tumors
immunogenicity of, 108-109
immunological surveillance (IS) and, 25-29
natural killer (NK) activity and, 45-48, 52-56
Squamous cell tumors
DNCB response in, 219
E-rosette forming cells in, 229, 230
immunoglobulins in, 221
immunological surveillance (IS) in, 21
lymphocyte response tests in, 225
monoclonal antibodies to, 183
thymosin and, 271
Stage-specific embryonic antigen (SSEA-1), 182, 203
Stomach cancer
biologic heterogeneity of, 85
DNCB response in, 219
PHA response in, 223
suppressor cells in, 240, 243
T cells in, 231
Streptokinase/streptodornase, 219
Suppressor cells, 231-232, 237-244
adherent cells in, 240-241
adoptive chemoimmunotherapy (ACIT) with, 315-317
adoptive immunotherapy and, 303, 304
cell types in, 237-238
Con A tests and, 239-240
definitions and meanings in, 242-243
immunological surveillance (IS) and, 5
immunotherapy with, 119
sneaking through phenomenon with, 20
spontaneous T cells and, 238
tumor development and, 13-15
tumor immunity and, 115-116, 118

T-cell leukemia and lymphoma, 278
C-type retrovirus particles with, 198
monoclonal antibodies to, 194-196, 274
T cells
adoptive chemoimmunotherapy (ACIT) with, 308
adoptive transfer of, *see* Adoptive immunotherapy
cellular immunity with cultured, 144-149
cytotoxic (CTL), 233
delayed type hypersensitivity (TDTH) effector cells with, 112, 113-114
differentiation antigens with, 274, 277, 279
effector cells in tumor rejection and, 110, 112
E-rosette forming cells (E-RFC) reaction in, 228-229
growth factor (TCGF), 114, 132, 278
helper and suppressor types in, 231-232
histocompatibility between target and effectors in lysis with, 134-135
human tumors and role of, 133-134
immune interferon and subsets of, 115
immunodilator testing and, 280
immunological surveillance (IS) hypothesis and, 1-2
interpretation problems with, 232
killer (K) cells with, 233
lentinan with, 284
lymphokines and, 278
MDP and, 282
monoclonal antibodies and, 274-275
natural cell-mediated cytotoxicity (N-CMC) and, 41
quantitation of, 228-232
sheep red blood cells (SRBC) reaction with, 228-229
sneaking through phenomenon with, 20

specificity of, in immunotherapy, 148, 261
stage of disease and, 229-231
subsets of, 231
suppression of immunity and, 148-149
suppressor activity with, 13-14, 15, 237-244
thymosin and, 271-273
traffic studies of cultured, 145-147
tumor cytotoxic activity with, 136-137
tumor development and immunodepression of, 7
tumor heterogeneity and, 149
tumor specific immunity with, 261
Testicular cancer, 36-37, 48-49, 200
Thiotepa, 130
Thy-1 antigen, 203
Thymocytic mitogenic protein (TMP), 282
Thymosin, 271-273, 286
Thymus, and leukemogenesis, 52
Thymus-leukemia (TL) antigen, 194-195
Thyroid cancer, 226
Thyroiditis, autoimmune, 24
Toxoplasma gondii, 90
Transitional cell carcinoma of bladder, 218
Transplantation
immunological surveillance (IS) hypothesis in, 20, 21
natural killer (NK) activity in, 60
Transplantation antigens, 4
Trisomy-21, 60
Tuberculin (PPD) test, 218, 220, 224
natural killer (NK) cytotoxicity and, 236
recurrence of disease and, 224
tumor growth and, 123
Tuberculosis, 89
Tuftsin, 280, 283, 286
Tumor antigens
classification of, 200-204
differentiation antigens in, 202-203
jumping and random antigens in, 203-204
tumor-specific, 200-201
Tumor-associated antigens (TAA), 302
Tumor cell immunogenicity, 91
Tumor infiltrating lymphocytes (TIL), 236, 244
Tumor necrosis factor, 6

Ultraviolet (UV)-induced tumors
immunotherapy with, 120
mononuclear phagocytes and, 90
suppressor cells in, 13-14
T cell activity in, 133-134
tumoricidal macrophages in, 87
tumor-specific antigens with, 200
Urethan-induced cancer
leukomenesis with, 51-52
natural killer (NK) activity in, 49-50, 52, 54
Urologic cancer, 229, 240
Uterine cancer
immunoglobulin levels in, 221
monoclonal antibodies to, 186
mononuclear phagocytes and, 89

Vitamin A, 11

Wiskott-Aldrich syndrome, 22, 60

X-linked agammaglobulinemia, 60
X-linked lymphoproliferative disorders, 59

YAC
age-related decline of natural killer (NK) activity and, 43
spontaneous tumors in mice with natural killer (NK) activity and, 45-48