New Perspectives in Clinical Microbiology

Series editor: W. Brumfitt

Volume 2

New Perspectives in Clinical Microbiology

Series editor: W. Brumfitt

Volume 1. New Perspectives in Clinical Microbiology, edited by W. Brumfitt.
Assistant-editor: J. M. T. Hamilton-Miller.
Kluwer Medical - London/Martinus Nijhoff - The Hague 1978. ISBN 90 247 2074 5

Volume 2. Aspects of Slow and Persistent Virus Infections, edited by D. A. J. Tyrrell.
Martinus Nijhoff - The Hague - Boston - London 1979. ISBN 90 247 2281 0

Volume 3. Combined Antimicrobial Therapy, edited by W. Brumfitt, L. Curcio and
L. Silvestri. Martinus Nijhoff - The Hague - Boston - London 1979. ISBN 90 247 2280 2

Aspects of Slow and Persistent Virus Infections

Proceedings of the European Workshop sponsored by the Commission of the European Communities on the advice of the Committee on Medical and Public Health Research, held in London (U.K.), April 5-6, 1979

Edited by

D. A. J. Tyrrell

Clinical Research Centre, Harrow

Martinus Nijhoff Publishers - The Hague/Boston/London 1979
for
The Commission of the European Communities

The distribution of this book is handled by the following team of publishers:

for the United States and Canada

Kluwer Boston, Inc.
160 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:

Aspects of Slow and Persistent Virus Infections.

(New Perspectives in Clinical Microbiology = V. 2) + Papers and Discussions at a Workshop . . . in London on the 5th and 6th of April 1979 as a Part of the Programme of the the Commission of the European Communities on Medical and Public Health Research.

Includes index.

1. Virus Diseases, Slow–Congresses. I. Tyrrell, David Arthur John. II. Commission of the European Communities.
RC114.6.A84 616.9'2 79-26296
ISBN-13:978-94-009-9341-9 e-ISBN-13:978-94-009-9339-6
DOI: 10.1007/978-94-009-9339-6

ISBN-13:978-94-009-9341-9

Publication arranged by
Commission of the European Communities,
Directorate-General for Scientific and Technical Information and Information Management
Luxembourg

EUR 6582 EN

For further information:
Martinus Nijhoff Publishers b.v.,
P.O. Box 566
2501 CN The Hague
The Netherlands

PREFACE

This book records the papers and discussions at a Workshop which took place in London on the 5th and 6th of April 1979, as part of the programme of the Commission of the European Communities on Medical and Public Health Research. However the views expressed are those of the individuals concerned and not of the EEC or any of its organs. The object was to discuss certain biological aspects of natural and experimental slow virus infections. Because the amount of knowledge and the focus of interest varied in respect of each infection the approach and emphasis varied also. In the case of scrapie, we discussed the nature of the agent and the mode of pathogenesis, in the case of SSPE, the search for unusual features of the virus, and recent detailed work on the immunology of the disease. As for Visna we reviewed the present understanding of the virus and its pathogenicity and also field epidemiology and methods for its control. There were also general papers, on interferon and oncornaviruses for example. We thank all those who made the meeting possible and enabled us to produce this book quickly, so that those who could not attend the meeting may nevertheless be able to read a great deal of what went on at it. In particular we would thank the Ciba Foundation who allowed us the use of their premises and Mrs. Jean Ashley who dealt with most of the arrangements. Last but not least we thank Dr. R.N.P. Sutton who as supporter and discussion editor rapidly produced a summary of the discussion which took place.

D.A.J. Tyrrell

TABLE OF CONTENTS

LIST OF CONTRIBUTORS

G. Agnarsdottir
The Royal Postgraduate Medical School
Departments of Immunology and Virology
Du Cane Road
LONDON, W 12
England

M. Bergeret
Institut National de la Santé et de la Recherche Médicale
u.-43, Hôpital Saint-Vincent-de-Paul
PARIS, 75014
France

G.F. de Boer
Centraal Diergeneeskundig Instituut
Afd. Virologie
Houtribweg 39
8221 RA LELYSTAD
The Netherlands

M.F. Bourgade
Institut National de la Santé et de la Recherche Médicale
u.-43, Hôpital Saint-Vincent-de-Paul
PARIS, 75014
France

C. Chany
Institut National de la Santé et de la Recherche Médicale
u.-43, Hôpital Saint-Vincent-de-Paul
PARIS, 75014
France

F. Chany-Fournier
Institut National de la Santé et de la Recherche Médicale
u.-43, Hôpital Saint-Vincent-de-Paul
PARIS, 75014
France

P.W. Ewan
The Royal Postgraduate Medical School
Departments of Immunology and Virology
Du Cane Road
LONDON, W 12
England

H. Fraser
ARC Animal Breeding Research Organisation
King's Buildings, West Mains Road
EDINBURGH, EH9 3JQ
Scotland

K.B. Fraser
Department of Microbiology and Immunology
The Queen's University of Belfast
Grosvenor Road
BELFAST, BT12 6BN
Northern Ireland

G. Georgsson
Institute for Experimental Pathology
University of Iceland
KELDUR, REYKJAVÍK
Iceland

N.T. Gorman
MRC Group on Mechanisms in Tumour Immunity
The Medical School
Hills Road
CAMBRIDGE
England

J. Habicht
MRC Group on Mechanisms in Tumour Immunity
The Medical School
Hills Road
CAMBRIDGE
England

D.J. Houwers
Centraal Diergeneeskundig Instituut
Afd. Virologie
Houtribweg 39
8221 RA LELYSTAD
The Netherlands

R.H. Kimberlin
Institute for Research on Animal Diseases
Compton, Near Newbury
BERKS, RG16 ONN
England

W. Kreth
Institut für Virologie und Immunologie
der Universitat Wurzburg
Versbacher Landstrasse 7
WURZBURG 8700
Germany

P. Lachmann
Addenbrookes Hospital
Hills Road
CAMBRIDGE, CB2 2QQ
England

E. Lund
Department of Veterinary Virology and Immunology
The Royal Veterinary and Agricultural
University of Copenhagen
13, Bulowsvej
1870 COPENHAGEN
Denmark

J.R. Martin
Institute for Experimental Pathology
University of Iceland
Keldur
REYKJAVIK
Iceland

V. ter Meulen
Institut für Virologie und Immunbiologie
der Universtität Wurzburg
Versbacher Landstrasse 7
WURZBURG 8700
Germany

C.A. Mims
Department of Microbiology
Guy's Hospital Medical School
London Bridge
LONDON, SE1 9RT
England

N. Nathanson
Institute for Experimental Pathology
University of Iceland
Keldur
REYKJAVIK
Iceland

H. Pabst
Institut für Virologie und Immunologie
der Universität Würzburg
Versbacher Landstrasse 7
WURZBURG 8700
Germany

P.A. Pálsson
Institute for Experimental Pathology
University of Iceland
Keldur
REYKJAVIK
Iceland

A. Pauloin
Institut National de la Santé et de la Recherche Médicale
u.-43, Hôpital Saint-Vincent-de-Paul
PARIS, 75014
France

G. Pétursson
Institute for Experimental Pathology
University of Iceland
Keldur
REYKJAVIK
Iceland

D. Sergiescu
Institut National de la Santé et de la Recherche Médicale
u.-43, Hôpital Saint-Vincent-de-Paul
PARIS, 74014
France

J.R. Stephenson
Institute of Virology and Immunobiology
University of Würzburg
Versbacherstrasse 7
8700 WURZBURG
Germany

L.Thiry
Head, Department of Virology
Institut Pasteur du Brabant
Rue du Remorqueur 28
1040 BRUSSELS
Belgium

H. Valdimarsson
Department of Immunology
St. Mary's Hospital Medical School
Praed Street
LONDON W2
England

R.A. Weiss
Imperial Cancer Research Fund Laboratories
P.O. Box 123
Lincoln's Inn Fields
LONDON WC2A 3PX
England

INTRODUCTION (C. A. MIMS)

There are two ways of looking at slow and persistent virus infections. The first is to consider their immense biological interest, whether or not they are of any practical importance. For instance, I could maintain that the most fascinating persistent virus of all is lactic dehydrogenase virus in mice. But this infection, in which only macrophages are involved and in which there are puzzling immunological phenomena, causes no pathological changes, no illness, and is of little or no importance for the mouse. The second approach to slow and persistent virus infections is a clinical one and reflects our eagerness to discover that viruses are behind this or that chronic disease of unknown aetiology. The neurologists, rheumatologists, and those who deal with cancer are interested from this point of view. The two approaches often overlap. SSPE, for instance, although it is a clinical problem, has a wider biological interest. We cannot understand oncornaviruses or visna virus without considering their relationship with the host genes and with the host species - in other words their general biology. Much of the scrapie work focuses unashamedly on scrapie as a fascinating problem in general biology but scrapie is also a practical problem in sheep.

It is a great pleasure to see that both the biological and the more clinical or practical aspects of these infections have been so neatly fitted into our programme. Its good also to see some immunology because immunology comes into everything, and you cannot understand any infectious process without looking at the immune response.

The rest of my short introductory talk consists of three points: First, there must be some more persistent viruses waiting to be discovered in man. I do not refer to C - type viruses, which for all I know have already been discovered in the form of nucleic acid sequences or virus - specific enzymes. But there are the papovaviruses, a fine set of persistent viruses, many of which are still what we used to call orphan viruses, looking for diseases. JC and BK viruses, excreted in the urine of transplant patients and pregnant women, infect most of us, and we need to learn more about

them. But there appear to be other human viruses in this group, because non - BK non - JC viruses have also been isolated. Even the common wart virus has now been unequivocally divided into at least four distinct types by restriction enzyme analysis. Dr. Kalder at the San Antonio Primate Centre now has seven antigenically distinct simian foamy viruses. Surely there are some human foamy viruses. If so, then it is possible they have no effects on their host, in which case their biological interest is great but their practical importance zero. Are there representatives of visna virus in man? Were the reports of visna antibodies in human serum false alarms?

The second point is that we may discover that some of the old viruses do unexpected things. If human picornaviruses are capable of persisting or remaining latent like Theiler's virus in mice, it will raise many possibilities. Chronic infection with Theiler's virus sometimes causes an immunologically mediated demyelinating disease in mice. There have been attempts to find poliovirus RNA sequences in amyotrophic lateral sclerosis, but so far these have been unsuccessful. Even C - type viruses can be neurotropic, and one of them causes a chronic neurological disease in the mouse, probably by a direct effect of the virus rather than via the immune response. Yellow fever virus may seem an odd one to mention at such a meeting, but I have noticed how difficult it is to explain to immunologists how neutralizing antibodies to yellow fever remain at high levels for 50 - 70 years after the primary infection, when the virus was presumably eliminated from the body. Could it be that in some corner of the lymphoreticular system viral antigens persist, or there is a very slow turn over of productive infection? Hepatitis B virus certainly persists, but little is known about its ability to infect or remain latent in parts of the body other than the liver.

My last point is about viruses and the immune system. This is an area of research which seems full of opportunities. If a virus is to establish a persistent infection it must come to terms with immune responses, either by-passing them, avoiding them, or inducing ineffective responses. It can be no accident that nearly all persistent viruses, and also scrapie, go first to lymphoid tissues. To <u>evade</u> host defences, what

more audacious but logical a strategy than to invade and in some way weaken these defences. There are various fascinating possibilities. We have suitable experimental techniques for dissecting out this interaction of viruses with lymphoreticular tissues, and by using the in vitro spleen cell system for instance it should be possible to discover a great deal that is relevant for persistence.

And now with great pleasure I will make way for those who have some hard data to present.

THE BIOLOGY OF SCRAPIE AGENT

H. KIMBERLIN

1. TRANSMISSIBILITY OF SCRAPIE

Scrapie is a fatal disease of the CNS that occurs naturally in sheep and goats (1). The clinical signs are variable but affected animals have either incoordinated movements, particularly in the hind limbs or show signs of intense pruritis. Commonly, both types of abnormality occur. The disease is diagnosed by clinical signs and the presence of vacuolated nerve cells in histological sections of brain. Interstitial spongy degeneration is often found in the same areas as neuronal vacuolation and occasionally there may be neuronal loss. Hypertrophy of astrocytes occurs as an additional but non-specific lesion. Demyelination is either very slight or absent and there are no inflammatory changes to indicate the presence of an infectious agent (Chapter 4 and reference 2).

However, there is no doubt that scrapie is caused by a transmissible agent. The injection of brain homogenates from affected sheep will transmit the disease to other sheep after long incubation periods which sometimes last for several years (1). The transmissible agent can be filtered (3,4) and experimentally passaged in sheep to extremely high dilutions of original inoculum (5) thus demonstrating the existence of a replicating, virus-like agent. Experimental forms of scrapie have been produced in many species (Table 1), notably mice and hamsters. It is important that several strains of mouse passaged agent have been injected into sheep and produced scrapie (7). As discussed later, scrapie is an infectious disease (section 3.3.1) and the causal agent shows the expected micro-biological properties of strain variation (section 3.4) and

mutation (section 3.7).

Table I. Known susceptible hosts for experimental scrapie

Group	Species
Ruminant	Sheep, Goat
Carnivore	Mink
Old World Monkey	Cynomolgous
New World Monkey	Squirrel, Capuchin, Spider
Rodent	Mouse, Rat, Gerbil, Vole
	Hamster (Syrian and Chinese)

Adapted from reference 6

2. PHYSICOCHEMICAL PROPERTIES OF SCRAPIE AGENT

Despite intensive study, there is little firm information on the nature of the scrapie agent (8). The only available assay is by titration in animal hosts, which even in the quickest model of scrapie (strain 263K in hamsters; 9) takes 150-200 days. Infectivity titres accurately reflect amounts of agent in inocula that are chemically similar but the proportionality between titre and agent changes when some chemical treatments are used, for example sodium dodecyl sulphate (SDS) (10). This happens because highly purified agent is not available and the non-scrapie components in a tissue extract may become chemically modified on treatment and, as a consequence, the efficiency of infection is altered. Hence much of the published data are difficult to interpret, particularly when infectivity titres differ by only 1 to 2 $\log_{10}$ LD_{50} units.

Most studies have been carried out with the 139A strain of mouse passaged agent or with other strains from the 'drowsy-goat' source. In retrospect this may have been a mistake because there are some indications that biologi-

cally different strains of agent have different physicochemical properties. For example, the inactivation of the 22C strain of agent was about 3 $\log_{10}$ LD_{50} units greater than that of the 22A agent when 10 percent saline homogenates of scrapie mouse brain were autoclaved at 110°C for 30 minutes (11). Because of these findings it may be premature to draw general conclusions about the nature of the scrapie agent.

Another limitation of past work is that most of it has been carried out with scrapie brains taken in the clinical stage of the disease. Table 2 shows the results of three preliminary experiments on the effects of SDS on titre in scrapie brains taken at different times during incubation. There is a clear pattern showing an apparent increased inactivation of scrapie (strain 139A) at earlier times than at later times. This pattern could be due to structural differences between early and late synthesised agent or, alternatively, to an alteration in brain tissue as lesions develop in the second half of the incubation period.

With these limitations in mind, the following is a brief summary of the main findings on the nature of the 139A (Chandler) strain of agent. In general the agent is highly stable when exposed to many physicochemical treatments, for example wet heat, alkylating agents, organic solvents, concentrated salt solutions and many detergents (8). This stability is probably related to the common finding that infectivity is functionally associated with cell membranes particularly in the microsome fraction. In one study of the SMB cell line (12), derived from a scrapie-affected brain and persistently infected with agent, the highest infectivity titres were found in the plasma membrane of the cell. Treatments which disaggregate membrane structures, e.g. 80% 2-chloroethanol, 90% phenol, 5% SDS (8,13), also appear to destroy most of the scrapie infectivity, again suggesting a link between agent and membranes. The agent has not been identified by the

Table 2. Effect of SDS on scrapie infectivity in brain homogenates prepared at different times in the incubation period

Days after i.c. infection with strain 139A	Infectivity titres ($-\log_{10}$ i.c. LD_{50} units/.03g)			
	Agent titre in brain	Loss of titre after treatment with SDS		
		Expt. 1	Expt. 2	Expt. 3
35	5.25		$\geqslant$ 2.50	
46	6.17	2.17		
49	6.21		2.14	
64	6.50		2.25	
68	7.27			3.25
76	7.00	1.17		
96	7.29			2.61
112	7.50		1.56	
117	7.33	1.00		
126	7.88		1.88	
138	7.88			2.41

Pooled mouse brains were homogenised in 0.32M sucrose at a concentration of 10% w/v and centrifuged at 1,000*g* for 10 min. to remove nuclei, myelin and unbroken cells. The supernatants were further centrifuged at 100,000*g* for 1 h. to sediment particulate material and most of the scrapie infectivity. The pellets were resuspended in saline at a concentration equivalent to 10% whole brain and aliquots were incubated with equal volumes of 1% SDS at pH 8.4. In experiments 1, 2 and 3, incubations were carried out at 20°C for 1 h., 37°C for 1 h. and 37°C for 2 h., respectively. Titrations of infectivity were performed on serial ten-fold dilutions injected intracerebrally (i.c.) into Compton white mice. Unpublished data of Kimberlin and Walker.

electron microscope and no one has purified scrapie infectivity by more than 50-fold. Because of these results, it has been suggested that scrapie agent may not exist as a nucleoprotein structure of regular morphology.

The minimal operational size of the infectious scrapie agent (assuming a spherical particle) has been estimated at approximately 30 nm by membrane filtration (14), greater than 5 x 10^7 molecular weight by gel filtration (14) and at least 40 S by sedimentation properties (15). The target size to ionising radiation is very small indeed, the calculated molecular weight being about 10^5 (16,17,18). This value is consistent with the very high doses of u.v. irradiation needed to inactivate the agent (19) and it is tempting to suggest the target to ionising radiation and the chromophore for u.v. absorption is a scrapie nucleic acid of about 10^5 molecular weight. However, if the chromophore is nucleic acid then the unusual u.v. inactivation spectrum for scrapie infectivity (18,20) indicates that it is associated with other macromolecules, possibly protein. This suggestion is supported by the enhanced inactivation of scrapie suspensions when exposed to ionising radiation in the presence of oxygen (21). In summary, the simplest interpretation of available data is that the agent is made up of a small scrapie specific nucleic acid, functionally associated with proteins (and perhaps other macromolecules) to give an infectious complex which *in vivo* is located in cell membranes.

Until recently there was no direct evidence for the existence of a scrapie specific nucleic acid. However, studies in which a hamster-passaged strain of agent was exposed to DNAase have produced the first direct evidence for a DNA component (22). This DNA has not yet been identified but at least one possible candidate has been described (23,24).

3. THE NATURAL DISEASE

3.1. *Contagious spread*

The results of three major studies, shown in Table 3, leave no doubt that horizontal or contagious spread of scrapie agent occurs naturally when previously unexposed sheep are maintained with infected flocks. Experiment II is impressive because the exposed sheep came from a flock in which no cases of scrapie had been seen in over 18,000 sheep during 13 years, and in experiment III, the numbers of sheep exposed to scrapie and eventually developing the disease were exceptionally large.

Although the mode of transmission of agent is not known for certain, several experimental routes of infection have been identified which are likely to be routes of natural infection. These include oral dosing (28), scarification (5) and via the conjunctiva (29). Studies in sheep and goats have failed to detect agent in secretions but agent is present in many tissues (1). Relatively large amounts are present in foetal membranes (28) making this tissue a probable source of infection. Other, but untested possibilities are cells sloughed off from mucous membranes or damaged skin.

There is some tentative evidence that sheep may become infected by grazing infected pastures (30) and the stability of scrapie agent to adverse conditions (section 3.2) makes this a plausible idea. Persistence of agent in the environment, on pastures or in buildings, is the likely explanation for the failure to eradicate Rida (Icelandic scrapie) from farms that were left free from sheep for at least 1 year before restocking from Rida free areas; the disease reappeared later but only on farms where it had previously occurred (31).

3.2. *Maternal transmission*

In general the probability of a lamb developing scrapie

Table 3. Contagious spread of scrapie

Experiment number	Date	Number of sheep or goats		Percent with scrapie	Reference
		Exposed	Developing scrapie		
I	1968	7 sheep	3	43	25
		17 goats	10	59	25
II	1974	75 sheep	21	28	26
III	1979	95 goats	50	53	27
		263 sheep	48	18	27

depends more on the eventual scrapie status of the ewe than of the ram (26,27,32). This bias indicates maternal transmission of the agent but the route by which this occurs is not firmly established. In part, maternal transmission can be explained by contagion during the months of intimate contact between ewe and lamb. However there is reasonable evidence that infection of the embryo occurs although this has not been shown directly. First, when ewes were experimentally infected with the SSBP/1 source of scrapie at the time of conception, some of the lambs developed scrapie at an exceptionally early age (4,33). This suggests pre-natal infection, particularly as there is independent evidence that lateral spread does not occur under these conditions (25). Secondly, there is the evidence mentioned above that foetal membranes from scrapie affected dams contain appreciable amounts of agent (28).

Several important questions concerning the spread of infection remain unanswered. For example, scrapie occurs most frequently in sheep between 2½ and 4½ years of age (1). A ewe could become infected before birth and could produce several lamb crops before she herself developed the

disease. But it is not known how efficiently an infected ewe can transmit infection to her offspring at different stages during the incubation period.

3.3. *Host genetic factors*

Although scrapie is caused by an infectious agent there is clear evidence that host genetic factors are important to the development of disease. As discussed elsewhere (1,34) the nature of the genetic control of natural scrapie is ill defined and difficult to study. The most fundamental problem is that there is no means of assessing the degree of exposure of individual sheep to scrapie agent and hence, one cannot tell whether an animal fails to develop the disease because of its genes or because it never has a chance to become infected.

This problem is removed by infecting sheep experimentally. Lines of Cheviot and of Herdwick sheep have been bred for increased (positive line) or decreased (negative line) incidence of scrapie in response to a standard dose of the SSBP/1 source of agent (35,36). Line crossing experiments have shown that the response to experimental infection is mainly controlled by a single gene with the dominant allele conferring susceptibility (1,35).

The difference between positive and negative lines was seen in terms of the incidence of scrapie and in the length of incubation which was very long in the relatively few cases that occurred in negative line animals. By analogy with the genetic control of scrapie in mice (Sections 3.4.2 and 3.9), it seems likely that the genotype of sheep influences incubation period (which possibly exceeds natural life-span in some negative line sheep) by controlling the dynamics of agent replication.

Positive and negative line sheep that were selected against SSBP/1 have been injected with other strains of agent and in general the responses were predictable (7).

However, at least one strain of agent is known (CH1641) which produces scrapie in both lines of Cheviot sheep with similar incidences but with shorter incubation periods in the negative line (7). This variation in response with agent strain is reminiscent of the diversity of interactions between strains of mouse passaged agent and the alleles of *Sinc* gene (Section 3.4.2) and it complicates the possible use of genetical methods of controlling the natural disease (34). The genetic control of natural scrapie may be even more complex than is indicated by these experiments because additional genetic factors may control stages of infection that are by-passed when sheep are infected experimentally. Hence, the genetic control of scrapie could vary with the predominant route of natural infection as well as with the strain of agent (1).

3.4. *Control*

Without an efficient diagnostic test to identify infected animals it would seem that eradication of endemic scrapie is impossible at the moment. Selective breeding may one day be a useful method of control in certain circumstances but meanwhile, some kind of slaughter policy is the only practicable way of coping with natural scrapie (1,34). The slaughter of both affected flocks and source flocks is an effective method of control. Less drastic measures depend on limiting the maternal and lateral spread of infection by selective culling of the female line of scrapie cases and by such measures as the removal of afterbirths at lambing.

3.5. *Related diseases*

Scrapie is the best understood of a group of four slow transmissible encephalopathies, the other three being kuru,

Creutzfeldt-Jakob disease (CJD) and transmissible mink encephalopathy (TME). The main link between these diseases is the broadly similar pattern of histological lesions found in brain (6,37). In addition, some combinations of scrapie strain and mouse genotype are associated with cerebral amyloid plaques which are a conspicuous feature of Alzheimer's disease and other dementias (38,39). Hence scrapie is an important model of some human CNS diseases. This does not necessarily imply that scrapie agent is the cause of human disease, but the possibility should be considered.

TME is a rare disease and epidemiological studies have pointed towards a source of agent outside the mink population (40,41). There is evidence that the source of TME agent is scrapie infected carcasses and that the natural route of infection is via bite wounds inflicted by littermates, particularly at feeding times (42). It has been postulated that the occurrence of CJD may also be related to the ingestion of scrapie contaminated food (43). However the epidemiological evidence conflicts with this view because the world-wide distribution of CJD does not correlate with the distribution of sheep or with the occurrence of scrapie (34). It may be nearer the truth to think of the CJ agent as a relatively common infection in the human population which only very rarely produces disease.

4. IDENTIFICATION OF AGENT STRAINS

4.1. *Lesion profiles*

The absence of serological or cell culture methods for identifying different strains of scrapie agent has led to the development of alternative techniques. One of these is based on a quantitative assessment of the severity of

vacuolar lesions in 9 grey matter areas and 3 white matter areas of mouse brain (2). The resulting lesion profile is influenced by many variables but if these are carefully controlled it can be used to identify different strains of agent (37). The technique is very sensitive because many agents produce characteristic but different profiles in mice of different *Sinc* genotypes (see section 3.4.2). In addition, there are qualitative differences between agent strains. For example, strains 79A and 22C are rarely if ever associated with cerebral amyloidosis whereas 87A and 87V produce many amyloid plaques and show some asymmetric vacuolation in certain areas of the brain (37, 44).

4.2. *Biological properties*

The incubation period of scrapie in mice is under the control of a gene, *Sinc* which has two alleles, s7 and p7. One group of agent strains behaves like ME7 and has a relatively short incubation period in mice with the *Sinc* genotype s7s7 and a much longer incubation period in mice homozygous for $Sinc^{p7}$. Another group, exemplified by 22A, shows the reverse properties (46). However many members of both the ME7 and 22A groups can be distinguished when the incubation periods in the heterozygotes are compared with those found in the parental, homozygous genotypes. For example, within the ME7 group of agents, ME7 itself shows no dominance, 22C shows partial dominance of the s7 allele, 79A, partial dominance of p7 and 139A, overdominance of p7 (47,48). This remarkable diversity of interactions between strains of agent and the alles of *Sinc* gene is not only of great theoretical importance (section 3.9) but also provides a way of identifying different strains which is independent of the lesion profile system described above. The combined use of these techniques, i.e. the comparison of incubation periods and lesion profiles in mice of the

three *Sinc* genotypes has led to the identification of about 15 different strains of scrapie agent (7). The fastest combinations of agent strain and mouse genotype produce clinical scrapie after about 100-150 days but other combinations have incubation periods that exceed the natural lifespan of laboratory mice (49).

5. ISOLATION OF AGENT STRAINS IN MICE

Isolations are usually made by injecting homogenates of sheep brain intracerebrally into mouse strains of the *Sinc* genotypes s7s7 and p7p7. Single strains of agent can be obtained by repeated intracerebral passage in mice or better still, by passage at limiting infectious dilutions of scrapie brain, i.e. cloning. It is known that several strains of agent may be involved in a single outbreak of scrapie. More important, two or more strains are frequently isolated from individual sheep (1).

However, primary isolations of scrapie agent are often difficult to make and there are some strains, for example CH1641, which cannot be transmitted to mice. The common finding is that incubation periods at first passage in mice are greatly extended and more variable than those found at second passage. This phenomenon is known as the species barrier effect and its existence suggests that some selective pressures may operate at primary passage which influence the strains of agent isolated. Some of the processes that take place on crossing the species barrier are discussed more fully later on (section 3.6) and strain selection is one of them.

In recent studies, scrapie agent has been isolated from several natural cases taken from the same outbreak. Table 4 shows that one group of sheep brains gave relatively short incubation periods at first passage, in the range of 470 to 479 days. Those from a second group produced scrapie in mice after 536 to 588 days, and in a single

Table 4. Mean incubation periods at first passage in mice of scrapie agent from natural cases

Individual sheep	Incubation periods in mice (days ± SEM)
C 58*	470 ± 13
M575	471 ± 9
F 47*	479 ± 11
G137	536 ± 26
H 77	561 ± 7
F 70*	585 ± 14
F 85*	588 ± 19
A 8*	671 ± 21
Pool	472 ± 11

The sheep came from the same flock of Herdwicks kept at Compton and were clinically and histologically positive for scrapie. Brain homogenates (5 or 10% w/v) were prepared in saline and 0.03 ml of each were injected intracerebrally into groups of 16 Compton white mice (*Sinc*^{S7}). *Denotes individual sheep brains used to make up the pool. Unpublished data of Kimberlin and Walker.

case, the mean incubation period was 671 days (Table 4). These preliminary results suggest that 2 or 3 different strains of agent were involved in the outbreak. However the injection of a representative pool of these sheep brains gave an incubation period of 472 days, indicating the selection of a quicker agent (Table 4).

The second passage was carried out using 5-8 individual mouse brains taken from the first passage of each sheep source of scrapie agent. The second passage from individual sheep brains was extremely variable with some incubation periods in the range of 139-163 days but

Table 5. Variation in incubation periods at second passage in mice of scrapie agent from natural cases

Individual sheep	No. of 1st passage mouse brains tested	Mean incubation periods at second passage in mice (days)
C58	5	147 150 189 358 395
F47	5	145 146 148 181 193
F70	6	163 227 239 243 249 391
F85	6	139 142 179 203 253 286
A 8	6	139 190 429 477
Pool of above	8	150 151 154 155 165 175 176 248

Brain homogenates (1% w/v) were prepared from individual mice with clinical scrapie at the first passage (Table 4) and 0.03 ml was injected intracerebrally into groups of 16 Compton white mice to initiate the second passage. The standard error of the mean incubation period was always less than 5 percent and usually less than 2 percent. Incubation periods of 176 days or less are underlined (see text). Unpublished data of Kimberlin and Walker.

others were two or three times longer (Table 5). In contrast, the second passage from the pool of sheep brains was far less variable with all but one of the incubation periods falling in the range of 150-176 days. These findings support the conclusion drawn from the data in

Table 4, namely that different strains of scrapie agent were involved in the outbreak and that isolation of agent from pooled sheep brains tended to select a quicker strain (in *Sinc*^{S7} mice).

The results in Table 5 also indicate that both 'short' and 'long' incubation strains of agent seem to be present in some sheep brains (e.g. C58 and A8) and that they can be isolated by passage in a single strain of mouse (Compton white; *Sinc*^{S7}). If this is the case then it is not immediately obvious why the long incubation strains of agent were isolated at all since the shorter ones should have had the selective advantage. Possible explanations could invoke differences between strains in their relative concentrations in brain and in the efficiency with which they infected mice at first and second passage. Alternatively there may have been blocking of one strain by another, or perhaps mutations occurred on passage to give variants with different incubation properties. Competitive interactions are known to occur when different strains of mouse passaged agent are injected into the same mice at different times (section 3.8) but there is no direct evidence at the moment that agents in the same inoculum can compete. However, there is good evidence that scrapie strains can mutate (section 3.7).

6. THE SPECIES BARRIER

The most detailed studies of the species barrier effect have been carried out with hamsters and mice and three underlying processes have been identified (50).

Although there is no specific immune response to scrapie infection (51), the intimate association between scrapie agent and tissue components makes it likely that there is an immune response to the antigenically foreign inoculum which could lead to a reduced efficiency of infection. Two studies have been made showing that this is

probably true. First, pretreatment of mice with normal hamster brain decreased the proportion of scrapie cases and increased incubation times following infection with hamster passaged scrapie agent (52). In the second study, mouse passaged ME7 agent was transmitted to sheep and then reisolated in mice. On reisolation, the incubation period of ME7 was extended at first passage in mice relative to the second but the increase was exactly in accord with the lower estimated titre in mice of the sheep passaged agent (53,54).

However a reduced efficiency of infection is an inadequate explanation of the species barrier effect when the incubation period at first pass falls outside the normal dose-response range of the agent in the new host. It should also be emphasised that sometimes the species barrier effect is minimal as when hamster passaged 431K is transmitted to mice ($Sinc^{S7}$) (55).

The second process associated with interspecific passage of scrapie has already been mentioned, namely strain selection. In one study it was found that the early passages of a source of scrapie agent in hamsters were associated with the loss of strain 431K which had a longer incubation period than strain 263K (55).

In the third process a prolonged 'zero-phase' occurs at first passage which is either absent or much reduced on second passage in the new host (56). The term 'zero-phase' is used to describe the period of time before agent replication can be detected, and in mouse passaged scrapie its duration is controlled by *Sinc* gene (section 3.9). In one study of the transmission of hamster scrapie to mice it was found that the dynamics of agent replication in brain at first and second passage were almost identical so that the difference in incubation periods was entirely accounted for by a prolonged zero-phase at first passage (56). This is summarised by the following equation:-

Incubation period 1st pass	minus	Zero-phase 1st pass	=	Incubation period 2nd pass
(325 days)	minus	(175 days)	=	(149 days)

The reason for such a prolonged zero-phase at first passage is not known. In part it may be due to a decreased efficiency of infection but other factors must also be involved, for example, re-routing of hamster associated agent along different and less efficient pathways.

7. BIOLOGICAL STABILITY OF AGENTS

There is now strong evidence that some strains of scrapie agent can undergo mutation on passage in mice (53) and Table 6 gives a list of the stability classes found so far. With class II agents, there is a gradual change in properties which is indicative of accumulated point mutations over several passages. Agent strains in class III are different in that the change in properties is unpredictable and discontinuous. Class III agents include 87A and other strains that are associated with a high incidence of amyloid plaques in brain and with asymmetrical vacuolation. When these agents change, the new or modified strains appear to be the same in each case. This regularity suggests either that mutations or deletions occur at the same site or that the host selects strongly in favour of one strain if many are produced. Probably both factors determine the uniformity of the resulting strain.

8. COMPETITION BETWEEN AGENTS

There is considerable evidence for competitive interactions between some strains of agent (47). The basic experiment is to inject mice with an agent strain (blocking agent)

Table 6. Biological stability of different classes of scrapie agent

Class	Example	Characteristics
I	ME7	Completely stable on passage in either *Sinc*^{s7} or *Sinc*^{p7} mice.
II	22A	Stable on passage in the *Sinc* genotype in which they were isolated but properties change on passage in mice of the other homozygous genotype.
III	87A	Unstable even on serial passage in the mouse genotype in which they were isolated.

Information taken from reference 53.

that is operationally slow in the genotype used, and after an interval, to inject an agent that is quicker (killing agent). Competition or blocking is seen as an increased incubation of the second agent which can be identified by its lesion profile. The original experiments (57,58) were carried out with agents from the ME7 and 22A groups, and by changing the genotype of mouse the 'blocking' and 'killing' roles of the agents could be reversed. However, blocking has now been demonstrated between agents within the ME7 group (48). Blocking can be achieved using either the intracerebral or the intraperitoneal route and it is independent of the mouse genotype in which the agents were passaged (47,48).

The simplest and most convincing explanation of blocking is that the first agent occupies a proportion of a finite and relatively small number of agent replication

sites so that fewer are available when the second agent is injected. As a consequence the effective dose of the second agent is reduced and incubation is prolonged (48). In some cases blocking may be total (58). It is important that the extent of blocking can be precisely controlled by varying the respective doses of the two agents. For example, blocking is greater if the dose of the first agent is increased and that of the second agent reduced. Blocking is also greater if the interval between injections is increased because this allows more time for the slower, blocking agent to replicate and occupy sites that would otherwise be used by the killing agent (47,48).

There is now good evidence that blocking only occurs with an agent that under the conditions of the experiment can replicate. For example, a dose of agent that is too low to produce infection will not block (48). No blocking has been demonstrated with the related transmissible mink encephalopathy agent which is not transmissible to any of the 14 strains of mice tested (48). Neither does blocking occur with strain 22A that has been inactivated by chemical or physical treatment (59).

9. REPLICATION SITE HYPOTHESIS FOR SCRAPIE

The pathogenesis of scrapie is discussed in detail in the next Chapter but it is necessary at this stage to emphasise some of the main features.

With the short incubation models of scrapie, agent replication occupies most of the incubation period (48,50, 51). Following peripheral routes of infection, agent replicates rapidly in spleen to reach plateau concentrations of infectivity within a few weeks. Agent also replicates in other lymphoid organs but spleen is known to play an important role because its removal lengthens incubation period (60-63). At a later stage, agent enters the CNS and a prolonged period of replication follows to give

concentrations of agent in the CNS that are much higher than those found elsewhere. It is significant that histological lesions of scrapie only develop in the CNS (2) and that the sharp onset of clinical disease appears to be triggered by a certain critical concentration of agent in brain or in, as yet unidentified, areas of brain (64). It is also significant that agent replication occurs without the apparent stimulation of a specific immune response to infection and without the involvement of interferon (see 34,51). However the absence of a continuing host response is consistent with the remarkable predictability of incubation period which is a general feature of the pathogenesis of scrapie.

The same sequence of events occurs in the long incubation models of scrapie except that there is a prolonged zero-phase before agent replication begins and the duration of the zero-phase in mice is precisely controlled by *Sinc* gene (48,49,65,66). It is clear, therefore that agent replication is the central process in the pathogenesis of mouse scrapie and that the slowness and predictability of disease development is due to an overiding genetic control exerted by the host. Since genetic factors influence the development of scrapie in sheep (section 3.3.3) it is reasonable to suggest that genes equivalent to *Sinc* exert a similar control on the natural disease.

The interaction between strains of scrapie agent and the alleles of *Sinc* gene described earlier (section 3.4.2) is of great theoretical importance as well as being of practical value in strain-typing. In particular, the occurrence of overdominance indicates that the two alleles of *Sinc* do not act independently and Dickinson has suggested that each allele contributes a monomer to a multimeric structure which is involved in agent replication (46-48). These suggestions have been developed into the Scrapie Replication Site Hypothesis which states that the slow development of clinical scrapie in mice is due to a

restriction in the number of agent replication sites which are multimeric structures specified by the alleles of *Sinc* gene.

There is a considerable amount of experimental data to support this hypothesis, and so far, none to refute it. The most important evidence comes from the many studies described earlier (section 3.8) showing competition between strains of agent. The concept of a limited number of agent replication sites is further supported by the occurrence of a plateau concentration of agent in spleen (45,49,61,67). The important role of the spleen in pathogenesis probably arises because this organ contains a high proportion of the peripheral replication sites in mice. Splenectomy removes these sites which do not appear to be replaced (63) so that agent has to replicate in a smaller number of sites and incubation period is prolonged. The hypothesis predicts that when the dose of injected agent is less than the number of replication sites in spleen, splenectomy will have no effect and the estimated infectivity titres in splenectomised and intact mice will be the same. Both these predictions have been fulfilled (48).

It is obvious from this discussion that our detailed understanding of scrapie pathogenesis is still very incomplete. However, studies of the biology of scrapie agent have produced a well tested hypothesis which can explain why scrapie is a slow disease and they have identified some of the key questions to be answered by future research. One of these is, what is the normal function of *Sinc* gene?

REFERENCES

1. Dickinson, AG: Scrapie in sheep and goats. In: Slow virus diseases of animals and man, Kimberlin RH (ed), Amsterdam, North Holland, 1976, p 209-241.
2. Fraser, H: The pathology of natural and experimental scrapie. In: Slow virus diseases of animals and man, Kimberlin, RH (ed), Amsterdam, North Holland, 1976, p 267-305.
3. Wilson, DR, RD Anderson, W Smith: Studies in scrapie. J Comp Pathol 60: 267-282, 1950.
4. Gordon, WS: Review of work on scrapie at Compton, England, 1952-1964. In: Report of scrapie seminar, ARS 91-53, Washington, DC, US Department of Agriculture, 1966, p 8-40.
5. Stamp, JT, JG Brotherston, I Zlotnik, JMK Mackay, W Smith: Further studies on scrapie. J Comp Pathol 69: 268-280, 1959.
6. Marsh, RF: The subacute spongiform encephalopathies. In: Slow virus diseases of animals and man, Kimberlin, RH (ed), Amsterdam, North Holland, 1976, p 359-380.
7. Dickinson, AG, H Fraser: An assessment of the genetics of scrapie in sheep and mice. In: Slow transmissible diseases of the nervous system, Hadlow, WJ, SB Prusiner (eds), New York, Academic Press, in press.
8. Millson, GC, GD Hunter, RH Kimberlin: The physicochemical nature of the scrapie agent. In: Slow virus diseases of animals and man, Kimberlin, RH (ed), Amsterdam, North Holland, 1976, p 243-266.
9. Kimberlin, RH, CA Walker: Characteristics of a short incubation model of scrapie in the golden hamster. J Gen Virol 34: 295-304, 1977.
10. Kimberlin, RH: Biochemical approaches to scrapie research. Trends in Biochemical Sciences 2: 220-223, 1977.
11. Dickinson, AG, DM Taylor: Resistance of scrapie agent to decontamination. N Engl J Med 299: 1413-1414, 1978.
12. Clarke, MC, GC Millson: The membrane location of scrapie infectivity. J Gen Virol 31: 441-445, 1976.
13. Millson, GC, EJ Manning: The effect of selected detergents on scrapie infectivity. In: Slow transmissible diseases of the nervous system, Hadlow, WJ, SB Prusiner (eds), New York, Academic Press, in press.

14. Kimberlin, RH, GC Millson, GD Hunter: An experimental examination of the scrapie agent in cell membrane mixtures. III. Studies of the operational size. J Comp Pathol 81: 383-391, 1971.
15. Prusiner, SB, WJ Hadlow, CM Eklund, RE Race, SP Cochran: Sedimentation characteristics of the scrapie agent from murine spleen and brain. Biochemistry 17: 4987-4992, 1978.
16. Alper, T, DA Haig: Protection by anoxia of the scrapie agent and some DNA and RNA viruses irradiated as dry preparations. J Gen Virol 3: 157-166, 1968.
17. Field, EJ, F Farmer, EA Caspary, G Joyce: Susceptibility of scrapie agent to ionizing radiation. Nature (London) 222: 90-91, 1969.
18. Latarjet, R: Inactivation of the agents of scrapie, Creutzfeldt-Jakob disease and kuru by radiations. In: Slow transmissible diseases of the nervous system, Hadlow, WJ, SB Prusiner (eds), New York, Academic Press, in press.
19. Alper, T, DA Haig, MC Clarke: The exceptionally small size of the scrapie agent. Biochem Biophys Res Commun 22: 278-284, 1966.
20. Latarjet, R, B Muel, DA Haig, MC Clarke, T Alper: Inactivation of the scrapie agent by near monochromatic ultraviolet light. Nature (London) 227: 1341-1343, 1970.
21. Alper, T, DA Haig, MC Clarke: The scrapie agent: evidence against its dependence for replication on intrinsic nucleic acid. J Gen Virol 41: 503-516, 1978.
22. Marsh, RF, TG Malone, JS Semancik, W Lancaster, RP Hanson: Evidence for an essential DNA component in the scrapie agent. Nature (London) 275: 146-147, 1978.
23. Corp, CR, RA Somerville: Nucleic acids associated with detergent-treated synaptosomal plasma membranes from normal and scrapie-infected mouse brain. Biochemical Soc Trans 4: 1110-1112, 1976.
24. Hunter, GD: The enigma of the scrapie agent: biochemical approaches and the involvement of membranes and nucleic acids. In: Slow transmissible diseases of the nervous system, Hadlow WJ, SB Prusiner (eds), New York, Academic Press, in press.
25. Brotherston, JG, CC Renwick, JT Stamp, I Zlotnik, IH Pattison: Spread of scrapie by contact to goats and sheep. J Comp Pathol 78: 9-17, 1968.
26. Dickinson, AG, JT Stamp, CC Renwick: Maternal and lateral transmission of scrapie in sheep. J Comp Pathol 84: 19-25, 1974.
27. Hourrigan, JL, AL Klingsporn, WW Clarke, M de Camp: Epidemiology of scrapie in the United States. In: Slow transmissible diseases of the nervous system, Hadlow, WJ, SB Prusiner (eds), New York, Academic Press, in press.

28. Pattison, IH, MN Hoare, JN Jebbett, WA Watson: Further observations on the production of scrapie in sheep by oral dosing with foetal membranes from scrapie-affected sheep. Br Vet J 130: lxv-lxvii, 1974.
29. Haralambiev, H, I Ivanova, A Vesselinova, K Mermerski: An attempt to induce scrapie in local sheep in Bulgaria. Zentralbl Veterinaermed B 20: 701-709, 1973.
30. Greig, JR: Scrapie in sheep. J Comp Pathol 60: 263-266, 1950.
31. Pálsson, PA: Rida: its epidemiology in Iceland. In: Slow transmissible diseases of the nervous system, Hadlow, WJ, SB Prusiner (eds), New York, Academic Press, in press.
32. Dickinson, AG, GB Young, JT Stamp, CC Renwick: An analysis of natural scrapie in Suffolk sheep. Heredity (Lond) 20: 485-503, 1965.
33. Dickinson, AG, GB Young, CC Renwick: Scrapie: experiments involving maternal transmission in sheep. In: Report of scrapie seminar, ARS 91-53, Washington DC, US Department of Agriculture, 1966, p 244-248.
34. Kimberlin, RH; Scrapie as a model slow virus disease: problems, progress and diagnosis. In: Comparative diagnosis of viral diseases. Vol III. Animal and related viruses, Kurstak, E, C Kurstak (eds), New York, Academic Press, in press.
35. Dickinson, AG, JT Stamp, CC Renwick, JC Rennie: Some factors controlling the incidence of scrapie in Cheviot sheep injected with a Cheviot-passaged scrapie agent. J Comp Pathol 78: 313-321, 1968.
36. Nussbaum, RE, WM Henderson, IH Pattison, NV Elcock, DC Davies: The establishment of sheep flocks of predictable susceptibility to experimental scrapie. Res Vet Sci 18: 49-58, 1975.
37. Fraser, H: Neuropathology of scrapie: the precision of the lesions and their diversity. In: Slow transmissible diseases of the nervous system, Hadlow, WJ, SB Prusiner (eds), New York, Academic Press, in press.
38. Bruce, ME, AG Dickinson, H Fraser: Cerebral amyloidosis in scrapie in the mouse: effect of agent strain and mouse genotype. Neuropathol Appl Neurobiol 2: 471-478, 1976.
39. Wisniewski, HM, ME Bruce, H Fraser: Infectious etiology of neuritic (senile) plaques in mice. Science 190: 1108-1110, 1975.
40. Hartsough, GR, D Burger: Encephalopathy of mink. I. Epizootiological and clinical observations. J Infect Dis 115: 387-392, 1965.
41. Burger D, GR Hartsough: Encephalopathy of mink. II. Experimental and natural transmission. J Infect Dis 115: 393-399, 1965.

42. Marsh, RF, RP Hanson: On the origin of transmissible mink encephalopathy. In: Slow transmissible diseases of the nervous system, Hadlow, WJ, SB Prusiner (eds), New York, Academic Press, in press.
43. Gajdusek, DC: Unconventional viruses and the origin and disappearance of kuru. Science 197: 943-960, 1977.
44. Fraser, H: Scrapie: a transmissible degenerative CNS disease. In: Progress in neurological research, Behan, PO, F Clifford Rose (eds), London, Pitman, in press.
45. Dickinson, AG, VMH Meikle, H Fraser: Identification of a gene which controls the incubation period of some strains of scrapie agent in mice. J Comp Pathol 78: 293-299, 1968.
46. Dickinson, AG, VMH Meikle: Host-genotype and agent effects in scrapie incubation: change in allelic interaction with different strains of agent. Mol Gen Genet 112: 73-79, 1971.
47. Dickinson, AG, H Fraser: Scrapie: pathogenesis in inbred mice: an assessment of host control and response involving many strains of agent. In: Slow virus infections of the central nervous system, ter Meulen, V, M Katz (eds), New York, Springer-Verlag, 1977, p 3-14.
48. Dickinson, AG, GW Outram: The scrapie replication-site hypothesis and its implications for pathogenesis. In: Slow transmissible diseases of the nervous system. Hadlow, WJ, SB Prusiner (eds), New York, Academic Press, in press.
49. Dickinson, AG, H Fraser, GW Outram: Scrapie incubation time can exceed natural lifespan. Nature (London) 256: 732-733, 1975.
50. Kimberlin, RH: Early events in the pathogenesis of scrapie in mice: biological and biochemical studies. In: Slow transmissible diseases of the nervous system. Hadlow, WJ, SB Prusiner (eds), New York, Academic Press, in press.
51. Outram, GW: The pathogenesis of scrapie in mice. In: Slow virus diseases of animals and man, Kimberlin RH (ed), Amsterdam, North Holland, 1976, p 325-357.
52. Kimberlin, RH, CA Walker, GC Millson: Interspecies transmission of scrapie-like diseases. Lancet ii: 1309-1310, 1975.
53. Bruce, ME, AG Dickinson: Biological stability of different classes of scrapie agent. In: Slow transmissible diseases of the nervous system, Hadlow, WJ, SB Prusiner (eds), New York, Academic Press, in press.
54. Dickinson, AG: personal communication.
55. Kimberlin, RH, CA Walker: Evidence that the transmission of one source of scrapie agent to hamsters involves separation of agent strains from a mixture. J Gen Virol 39: 487-496, 1978.

56. Kimberlin, RH, CA Walker: Pathogenesis of scrapie: agent multiplication in brain at the first and second passage of hamster scrapie in mice. J Gen Virol 42: 107-117, 1979.
57. Dickinson, AG, H Fraser, VMH Meikle, GW Outram: Competition between different scrapie agents in mice. Nature (New Biol) 237: 244-245, 1972.
58. Dickinson, AG, H Fraser, I McConnell, GW Outram, DI Sales, DM Taylor: Extraneural competition between different scrapie agents leading to loss of infectivity. Nature (London) 253: 556, 1975.
59. Kimberlin, RH, AG Dickinson: unpublished.
60. Fraser, H, AG Dickinson: Pathogenesis of scrapie in the mouse: the role of the spleen. Nature (London) 226: 462-463, 1970.
61. Clarke, MC, DA Haig: Multiplication of scrapie agent in mouse spleen. Res Vet Sci 12: 195-197, 1971.
62. Dickinson, AG, H Fraser: Scrapie: effect of Dh gene on incubation period of extraneurally injected agent. Heredity 29: 91-93, 1972.
63. Fraser, H, AG Dickinson: Studies of the lymphoreticular system in the pathogenesis of scrapie: the role of spleen and thymus. J Comp Pathol 88: 563-573, 1978.
64. Kimberlin, RH, CA Walker: Pathogenesis of mouse scrapie: effects of route of inoculation on infectivity titres and dose-response curves. J Comp Pathol 88: 39-47, 1978.
65. Dickinson, AG, H Fraser: Genetical control of the concentration of ME7 scrapie agent in mouse spleen. J Comp Pathol 79: 363-366, 1969.
66. Dickinson, AG, VMH Meikle, H Fraser: Genetical control of the concentration of ME7 scrapie agent in the brain of mice. J Comp Pathol 79: 15-22, 1969.
67. Hunter, GD, RH Kimberlin, GC Millson: Absence of eclipse phase in scrapie mice. Nature (New Biol) 235: 31-32, 1972.

THE PATHOGENESIS AND PATHOLOGY OF SCRAPIE

H. FRASER

1. INTRODUCTION

Although scrapie represents a major source of loss to the sheep industry, it has become increasingly apparent that its study can provide insight into an important group of enigmatic human neurological diseases. In addition however, it has a peculiar fascination for the biological scientist as it represents a uniquely perplexing subject as far as most current views on infectious processes are concerned.

The disease results from a progressive degeneration of the neuroparenchyma, which it has not been found possible to reverse. At present there is almost complete ignorance concerning the molecular basis of this deterioration, despite the large amount of relevant data which is now available. Scrapie is a naturally occurring disease in sheep and goats, but it is studies in laboratory rodents which are providing the means of unravelling its basic biology. The development of a procedure using inbred mice, for the isolation and discrimination of the many different strains of scrapie agent represents the single most significant advance in recent years. These studies have also identified a wide range in the manifestation of the disease, whose basis has become clearly established as a result of the experimental work with the inbred mice. This has led to a recognition that several human diseases have a variety of features in common with one or other of the various "types" of scrapie, and it cannot be anticipated where such similarities will end, because there is no reason to think that the full range of scrapie has yet been found. It is easy to recognise the analogy with Creutzfeldt-Jakob (C-J) disease and kuru, both of which have been assigned,

with scrapie and transmissible mink encephalopathy (TME) to a group of infectious degenerative encephalopathies. In addition, in the case of Alzheimer's disease, scrapie clearly provides a highly appropriate model for a variety of reasons (1) even if Alzheimer's disease does not turn out to have an infectious aetiology.

Although scrapie is unquestionably an infectious disease, both experimentally and in the field situation, there remain a number of issues where knowledge of it departs from expectations for conventional infectious processes. Firstly one of the most serious limitations in its identification and study is that it can only be recognised by clinical and pathological criteria, and all experimental work is completely dependent upon whole animal systems, usually in mice in which clinical disease occurs only after long incubation periods sometimes approaching or even exceeding median life span. In hamsters a "rapid" incubation period model has been developed, which has advantages for certain types of investigation (2). There is still no in vitro diagnostic recognition test for scrapie in infected or affected animals. Secondly it has not yet been possible to achieve biochemical purity of the agents of scrapie, and all transmissions and pathogenesis studies are therefore dependent upon more or less crude homogenates, usually of brain or spleen, but containing a wide range of tissue components and degeneration products. Thirdly it is not always possible even to transmit the natural disease to mice from all cases, the failure rate being around 20% (3), although this may to some extent reflect the limits imposed by the life span of the species concerned (4). An analogous situation has been found experimentally in which a scrapie agent passaged in hamsters, originally from a mouse isolate, has lost its infectivity for mice (5). Fourthly it has not been possible to recognise any scrapie agents ultrastructurally, and fifthly those agents that have been studied extensively do not express

antigenicity, although many of the more recently identified agents, such as those responsible for the induction of cerebral amyloid, have not been studied in this respect. Finally it should not be forgotten that it has not yet been possible to show that Koch's third postulate has been formally met with any of the agents isolated, by reproducing all the features of the natural disease in the species of origin.

A major difference in the behaviour of scrapie, with wide ranging epidemiological significance, is that, whereas it is both contagious in sheep and transmitted vertically from mother to offspring, neither of these phenomena have been shown to occur experimentally in mice.

2. PATHOGENESIS

Although scrapie is a neurological disease, resulting from changes induced in the central nervous system (CNS) many months after initial infection, this is preceded by replication of agent in several peripheral organs, and the only way of altering the progress of the infectious process is by interfering with it either during the prelude of the infection, when the early peripheral events are commencing, or during the peripheral replication but prior to CNS involvement. Once the CNS infection has become established it has not been possible to alter its progress in any way. If the initial infection is directly into the CNS, as, for example, with the intracerebral route of injection, replication proceeds there independently of replication elsewhere, and under these circumstances it has not been possible to impede or alter the progress of the disease at any stage.

The rate of agent replication in mice and the timing of events such as the onset of the clinical disease are all controlled by a single host gene called Sinc (scrapie incubation period). The action of Sinc distinguishes agents in a way which has now become

an important and established procedure in their classification and identification. Two alleles, *Sinc*^{s7} and *Sinc*^{p7}, have been identified, and the dozen or so agents which are currently known can be divided broadly into an "ME7 group", which have shorter incubation periods in *Sinc*^{s7} homozygotes, and a "22A group" with shorter incubation periods in the *Sinc*^{p7} homozygotes (6). A similar gene with two alleles *Sip* and *sip* has been identified in Cheviot sheep, based on their susceptibility to a particular experimental challenge agent (3, 7). On the basis of the differences in *Sinc* action, the *replication site hypothesis* has been proposed, which suggests that the gene products of *Sinc* associate as multimers and these are restricted in number (the multimers will include heteromers in the heterozygote). This hypothesis successfully explains many of the peculiar features of the pathogenesis of scrapie (6,8).

The absolute timing of the events controlled by *Sinc* is directly influenced by the number of infectious units of infectivity introduced at the initial infection, and so the five major variables which interact to produce the final phenotypic expression of incubation period are: 1. host genotype; 2. strain of agent; 2. route of inoculation; 4. dose of agent; 5. immunological status (including developmental maturity). It is possible to choose a combination of these variables such that a "very short" incubation period disease can be produced, "very short" being something in the region of 20 weeks in mice (the details will depend upon the precise criteria for measuring incubation period), and this is achieved with *intracerebral* (i.c.) injection of a *high dose*, using a *quick* agent combination (such as ME7, 79A, or 22C agents in *Sinc*^{s7} mice) at any age. Under such circumstances a sequence occurs in which, after a brief interval of a day or so, agent increases in titre in the lymphoreticular organs such as spleen (LRS) and reaches a plateau by around 3-4 weeks. Replication in the CNS commences at about 10 weeks and the titre continues to rise there until

clinical and eventually terminal disease supervenes (Figure 1). Under these conditions the programming for replication in the CNS is evidently initiated at the time of infection, and CNS replication proceeds independently of the earlier peripheral replication. This is concluded, among other reasons (9), from the failure of splenectomy, either before or after i.c. injections to impede the disease onset (10). On the other hand, if an intraperi--toneal (i.p.) route had been used an incubation period of around 35 weeks would have resulted and the occurrence and replication of agent in the CNS would then be dependent upon prior replication in the LRS (Figure 1). This can be shown by the profound delay in clinical onset which follows reduced peripheral agent replication. This can be achieved by splenectomy either prior to intraperitoneal injection, which removes replication sites, or following it, which removes replicating agent as well (10).

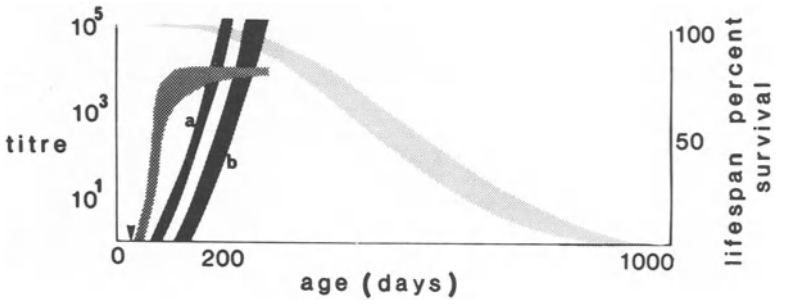


FIGURE 1: Diagram indicating range of titre changes in spleen and brain during pathogenesis of a "short" incubation period model of scrapie, following i.c. or i.p. injection of weanling mice. The bands indicate ranges over which the dynamics can be controlled with different agents and hosts. Survival is reduced by the necessary expediency of sacrificing a proportion of animals prior to natural death, and this band is therefore not a "lifespan curve". The entire sequence of events is completed well before median life span. ▼ - injection time; ▒ - spleen titre; ▓ - brain titre; (a - i.c. injection, b - i.p. injection; ▒ - survival curve.

In contrast, much more prolonged incubation periods follow the use of slow agent combinations (such as 22A in Sinc^{s7} mice). Here, by the simple expedient of reducing the dose of initial infection to around 100 LD_{50}'s, there is a prolonged interval, in excess of a year, during which the infectivity is not detected, but after which it rises rapidly in spleen to give a plateau just as in the quicker combinations (Figure 2). These are the types of circumstances in which the full sequence of events involving CNS replication and eventual neurological deterioration do not occur because of the limits imposed by life span (4).

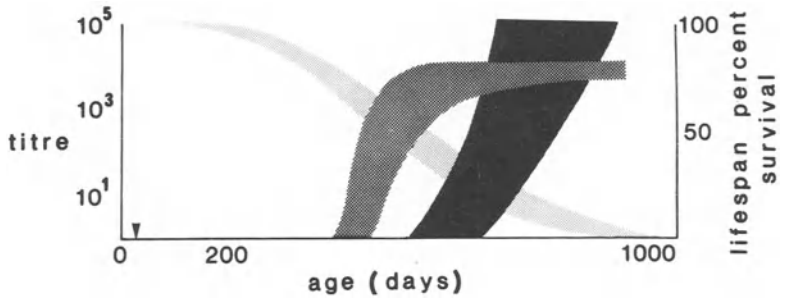


FIGURE 2: Diagram indicating range of titre changes in spleen and brain during pathogenesis of a "long" incubation period model of scrapie, following i.p. injection of weanling mice. With low levels of infection the sequence of events cannot be completed within the life span.
▼ - injection time; ▒ - spleen titre;
▓ - brain titre; ▒ - survival curve.

The interval during which there is no detectable infectivity is provisionally designated as the "zero-phase" (6), and has also been shown in mice following transmission from another species (5,11). It is inappropriate at present to describe this as an "eclipse-phase" because of a possibility that replication may still be occurring in a different organ system has not been fully excluded. Also a failure to detect infectivity may simply reflect the insensitivity of the in vivo assay. The sequence of events in pathogenesis, such as the duration of zero-phase, the rate of replication and the progress of the clinical disease are programmed by host-controlled steps which are initiated at the time of initial infection and are not a consequence of a later randomly-timed triggering of latent infection.

It has been impossible so far to establish the identity in the LRS of the systems engaged in the peripheral pathogenesis of scrapie, although recent work has cast some light on the biological properties of the cells involved. Newborn mice (up to 4 days old) are markedly less susceptible to i.p. infection than are older mice (12). However by the use of certain immunosuppressive procedures (steroid treatments) the susceptibility of older mice to i.p. infection can be reduced to the same level as that of newborn mice (12, 13, 14).

It has also been shown that immunostimulation can have the opposite effect (15, 16). However the possibility that it is not lymphoid components in the immunological system which are essentially involved in the peripheral steps in pathogenesis is suggested by the failure of thymic ablation, either in neonates or in adults even when combined with irradiation, to effect the disease in any measurable way (17, 18).

What,then. can be established about the properties or identity of these cells which are engaged in the peripheral sequences of scrapie pathogenesis. Anatomically they are part of the lymphoreticular and haemopoietic systems (LRS), such as lymph node and spleen

although not prominently in bone marrow (19, 20). A potential list of possible cell types in the LRS having to be considered as primary contenders in peripheral pathogenesis has been made (13). It is important to recognise that, despite the actual replication of agent which is proceeding in the LRS, no pathological or functional deficiency has been found outside the CNS. A possible explanation of this is that any pathological consequence of scrapie replication in spleen is such a low frequency event as to go unrecognised and to be at too low a level to interfere with physiological activity. At the plateau phase in spleen the level of infectivity is such that on average only about one cell in a hundred will carry an infectious unit of scrapie; but whether this is concentrated in only very few cells, or is distributed as lower levels of infection in a larger number of cells, is unknown (6). There is therefore a probability that any pathological events are "diluted out" by the enormous numbers of unaffected cells. In addition the high cell turnover in the LRS may also prevent the occurrence of pathological consequences. Whatever their identity it is clear that the cells involved must represent only a very small proportion of the total LRS population. There is growing evidence that it is a cell - type which is mitotically inactive. Whole body irradiation at lethal or sub-lethal levels is without effect on incubation period (21), and it is this, added to the failure of regeneration of the peripheral replication sites over many weeks following splenectomy even of infant mice (18), that suggests that the cells involved in the peripheral sequences of pathogenesis may be stable cells, as they presumably are also in the CNS.

Most of the biochemical changes found in scrapie, such as the increased DNA synthesis or glyosidase activity, occur late in the course of the disease and are probably secondary to degenerative events (22). However, a biochemical alteration which is early enough to be associated with primary changes, such as agent replication,

is the depression in the levels of polyadenylated RNA which occurs in the brain and other tissues early in the incubation period following i.c. or i.p. injections of 139A agent. In the spleen these levels initially increase so that at the time when agent titre is rapidly increasing, at three weeks after injection, the level of polyadenylated RNA is doubled, to fall later and throughout the remainder of the disease to depressed levels (23, 24).

One aspect of the pathogenesis of scrapie remains a matter of speculation and this concerns the mode of spread of infectivity in the body, in particular from the periphery, such as the LRS, to the CNS. Although it seems plausible that such access might involve haematogenous cells, no evidence for this has been found in scrapie (19, 25, 26); in experimental C-J disease in guinea pigs such a mechanism has been suggested, as infectivity was detected in the buffy coat (27). However there is now growing evidence in scrapie in mice that, following peripheral routes of inoculation, infection might gain access to the CNS, particularly the spinal cord, via peripheral nerves (24) as had previously been suggested in the case of TME in mink by Marsh who has been the main advocate of this view. (28).

3. THE PATHOLOGY OF SCRAPIE

The clinical manifestations of scrapie appear to result from a primary dysfunction of the neuroectoderm, without primary dysfunction elsewhere. The lesions which occur are largely degenerative, without inflammation or evidence of demyelination. The lesions are of three main types: 1. a vacuolar degeneration of the neuroparenchyma which sometimes extends to spongiosis, 2. cerebral amyloid; 3. glial reactions. It needs to be emphasised that lesions may be inapparent even at the terminal stage of the disease, as has been identified in one form of experimental scrapie in some Cheviot sheep (29); an almost identical situation has also been described in TME in aged Aleutian mink in which the inessential nature of

vacuolation has been shown so unambiguously (30). Figures 3 and 4 exemplify this lack of histologically evident degeneration in experimental ovine and murine scrapie.

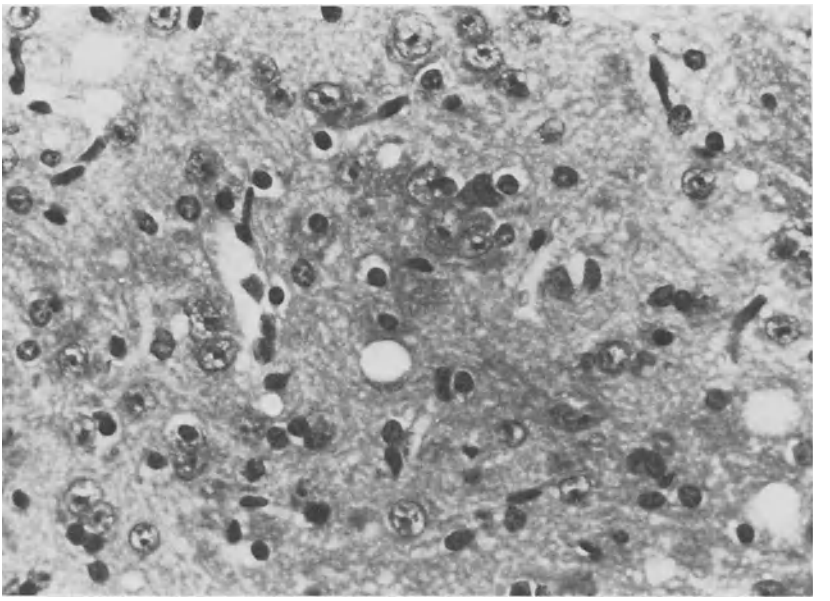


FIGURE 3: Very mild vacuolation in thalamus of a scrapie-affected RIII mouse, with the '22P' scrapie agent, which is undergoing identification. No other site in the brain in routine sections of the whole brain was more severely affected than this. The incubation period was 474 days. Haematoxylin and eosin, x 750.

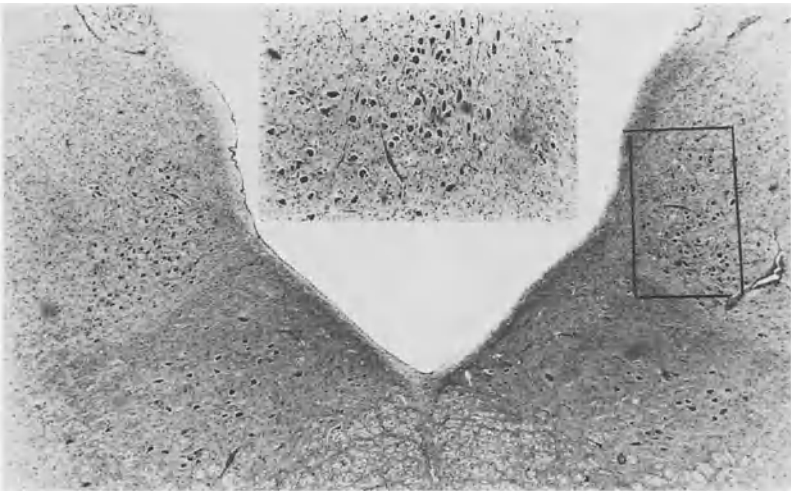


FIGURE 4: Virtual absence of vacuolation in the brain stem of a scrapie-affected Cheviot sheep with the SSBP/ scrapie source, following subcutaneous injection. No other site in the brain in routine sections was more severely affected than this. The incubation period was 203 days. Haematoxylin and eosin, x 40. Inset shows the medial vestibular nucleus, x 160.

In many types of scrapie the most prominent lesion is a degenerative vacuolar change of the grey and sometimes the white matter. This is generally diagnostic of scrapie, but is undoubtedly a secondary morphological consequence of some unknown primary biochemical defect. Ultrastructural studies have tended to identify the basis of this lesion in the body and processes of the neurons, at least in the grey matter (31, 32, 33). What are lacking are systematic ultrastructural studies of lesions from their inception in different regions of grey and white matter, in order to identify a putative common morphological index of a primary molecular pathology. Any such study would need to include a variety of different combinations of agent and host, and include

peripheral and intracerebral inoculation methods with different dose inputs. A major objective of such a study would be to identify the developmental pathology of early changes, in grey and white matter, in young, middle-aged and old animals, and thereby discriminate the early pathological changes specific for scrapie from the somewhat similar changes which occur with increasing intensity as animals age (34, 35, 36). It seems unlikely that pathological studies on their own can identify the elusive "cause" or this enigmatic degenerative process, but carefully conducted studies on the lines suggested here, concentrating on the early lesions, will narrow the options for the primary biochemical deficit. Although it appears likely that a common defect may link the wide array of different forms and patterns of degeneration in scrapie and the related encephalopathies, leaving open the questions of the basis of the variation, a possibility that different primary defects may occur ought not to be discounted. For example it has been shown that a similar depression of choline acetyltransferase occurs in scrapie and in Alzheimer's disease, but in scrapie this depression has been found to be much greater in mice affected with 79A scrapie, than with other agent combinations, including those which represent the closest histopathological analogues of Alzheimer's disease (1, 37).

It has not been possible so far to identify a discrete entity in scrapie tissue or homogenates which might represent the infectious agent itself, although a variety of different particles, all of unknown significance, have been found. Several workers have identified aggregated tubulo-filamentous particles, frequently in post-synaptic locations and with approximately similar estimates of size, 20-50nm diameter, and up to 75nm in length. These have been identified both in experimental murine scrapie and in the natural disease in sheep (31, 32, 38, 39 40, 41, 42, 43, 44, 45). What are probably the same structures have been identified as spherical particles (21-36nm diameter) in dilated

post-synaptic processes, either as random aggregates, as vermicular stacks or in overlapping hexagonal patterns in crystalline array (33). As these aggregated particles appear to be the only consistent observation in so many peoples experience, it is essential that their specific association with scrapie, and their absence from old animals and from animals with unrelated neurological degeneration be confirmed unequivocally by the examination of properly coded controls.

The vacuolar lesion in grey and white matter at the level of the optical microscope, shows great diversity in the patterns of distribution and intensity, and the two major variables responsible for this variation are the host genotype and the strain of the agent. A system for quantifying these differences has been developed which has given rise to the establishment of "lesion profiles" whose major value is that they represent an independent index for agent identification (21, 29, 46, 47).

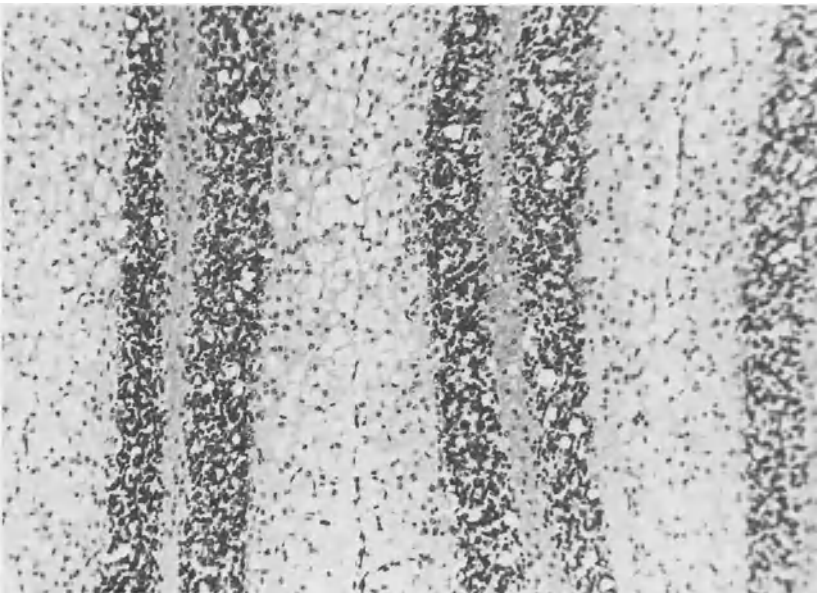


FIGURE 5: Spongiosis in the cerebellar cortex in an RIII mouse with 22L scrapie agent. The incubation period was 215 days. Haematoxylin and eosin, x 220.

There are complex interactions between the three variables of agent strain, host genotype and route of inoculation, but a lesion profile for any particular combination is constant over a wide dose range. When all other factors are held constant, an agent can often be identified on the basis of its lesion profile calculated as an average from a group of only 10 mice of a defined genotype. The vacuolation in different regions of the brain, both within and between the grey and white matter, vary independently of one another. However there are quite big quantitative differences in the overall balance between grey and white matter involvement between agents. 87V, 87A and 22C represent agents in which the white matter is virtually unaffected, whereas in 79A and 139A it is prominently affected. In addition however, mouse strains differ in their tendency to suffer severe white matter degeneration with different agents. For example, ME7 is

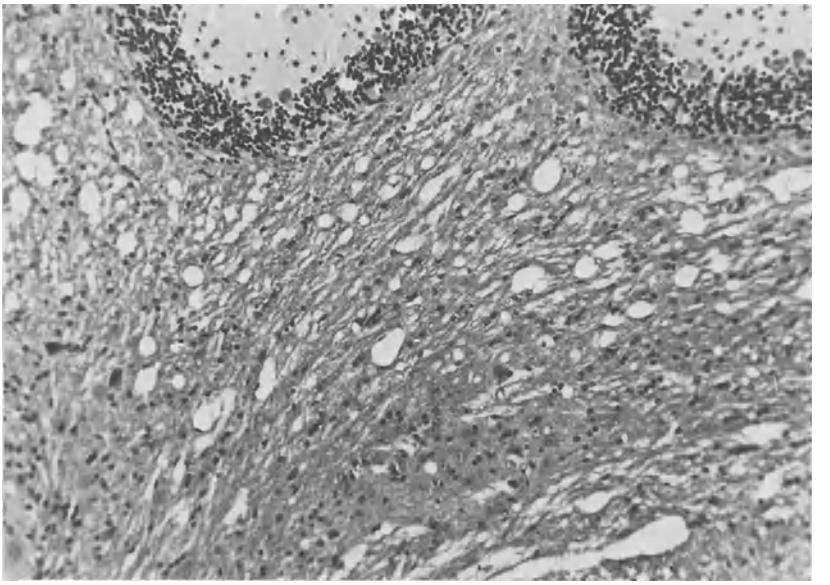


FIGURE 6: Spongiosis in the cerebellar white matter in a VM mouse with 22L scrapie agent. The cortex is spared. The incubation period was 224 days. Haematoxylin and eosin, x 220.

an agent which does not cause this type of pathological change in most genotypes of mice, but does so in the A2G and BALB/c strains (29, 48). An analogous situation occurs with another agent, 22L. A characteristic feature of 22L is that it induces a widespread and severe vacuolation, frequently even spongiform, throughout the molecular and granular layers of the cerebellar cortex, in all the *Sinc*^{s7} mice which have been tested, and this lesion is particularly severe in RIII mice (Figure 5). However in VM mice this lesion is absent, but instead a severe vacuolar degeneration of white matter occurs which is absent in *Sinc*^{s7} mice (46)(Figure 6). In a study of the cerebellar pathology of 22L agent in C57BL's, VM's and their F_1 crosses, it has been found that the F_1's incurred intermediate damage (Figure 7). Two important conclusions drawn from this independence of vacuolation in white and grey matter is that the white matter change is not a secondary degeneration resulting from neuronal loss, and that the lesion in both situations probably reflects a similar primary defect manifesting itself in different locations under different circumstances.

As a rule the distribution of vacuolation, although varying in intensity rostro-caudally, is similar between the two sides of the CNS, and up to a decade ago it was widely held that the lesions of scrapie were bilaterally symmetrical. However, it is now recognised that some agents give rise to a high frequency of *asymmetrical* lesions (Figure 8) (21, 29, 46, 48). These particular isolates are ones which induce cerebral amyloid plaques (see below) as a conspicuous feature of their pathology, although the basis of this association is unknown. An understanding of asymmetry may be crucial for elucidating some of the questions surrounding agent transport and targetting, and lesion distribution differences. The agents (e.g. 87A) associated with asymmetrical lesions are also ones which readily break down, yielding agents (e.g. 7D from 87A) with greatly altered properties - a phenomenon designated 'Class III

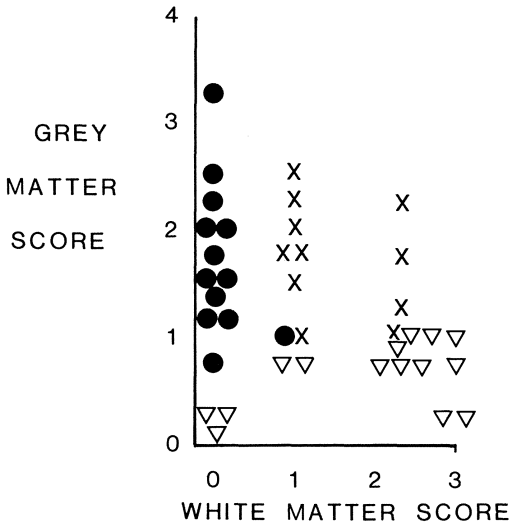


FIGURE 7: Score of spongiosis in individual VM (▽), C57BL (●) and their F_1 cross (X) mice, in grey and white matter of the cerebellum following i.c. injection of 22L scrapie agent. The score in grey matter is the average of the four scores, on a scale 0-5, in: 1. position 2 (cerebellar cortex adjacent to fourth ventricle) as in the routine for lesion profiles (8,29,47), and, at the same transverse levels, the entire : 2. cerebellar cortex, 3. granular layer, 4. molecular layer. The score in white matter, on a scale 0-3, as in the routine method for lesion profiles (21, 29). All specimens were coded and scored 'blind'.

stability' (8,49). One of the most plausible suggestions explaining the association between asymmetry and breakdown is that it results from 'mutation', with loss of some agent coded information, occurring locally in certain regions of neuroparenchyma thereby yielding an altered agent (49). An alternative is that the asymmetrical foci result from minor and 'faster' component

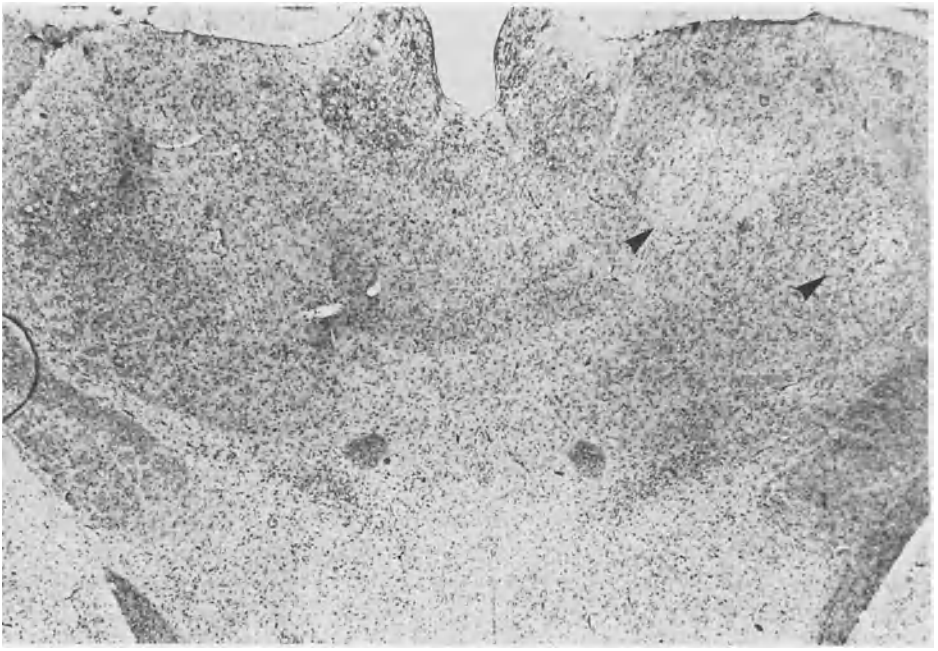


FIGURE 8: Two foci (arrows) of asymmetrical vacuolation in the thalamus in a VL mouse with 87A scrapie agent. The incubation period was 444 days. Haematoxylin and eosin, x 50.

in a mixture of agents (21, 46). The slower and major component replicates in restricted regions of the brain such as brain stem and mid-line regions of the tegmentum and hypothalamus, although it is bound with higher priority than the minor component, to sites throughout the rest of the brain. If the major component leaves some sites unoccupied, the minor and potentially faster component has an opportunity to replicate and induce foci of degeneration. In this case it is necessary to postulate a mechanism whereby the relative proportions of both components are maintained in a stable balance throughout subpassage, and a failure of this mechanism would result in the faster component becoming dominant.

It has been further suggested that asymmetrical foci of vacuolation may involve transport of agent along nerve pathways peripherally or centrally (21). This could account for asymmetrical lesions following either i.c. or peripheral injections. Asymmetrical localisation of agent replication could also be a consequence of possible functional asymmetries (50, 51), and this could help to explain the sidedness of asymmetrical lesions following i.c. and peripheral inoculations (46). The agents which give rise to asymmetry, in the forebrain, thalamus and cortices, produce symmetrical lesions in the brain stem and tegmentum. The asymmetrical foci can to some extent to be localised to the side of i.c.injection, and this suggests some localisation of the inoculum. Breakdown might be more likely to occur in regions where the concentration of agent is high, early in pathogenesis, and this provides some rational basis for the finding of asymmetrical lesions in regions of the brain where 7D produces widespread lesions, namely on the mesencephalic tectum, thalamus, hippocampus, and cerebral cortex, all regions in which 87A produces virtually no lesions.

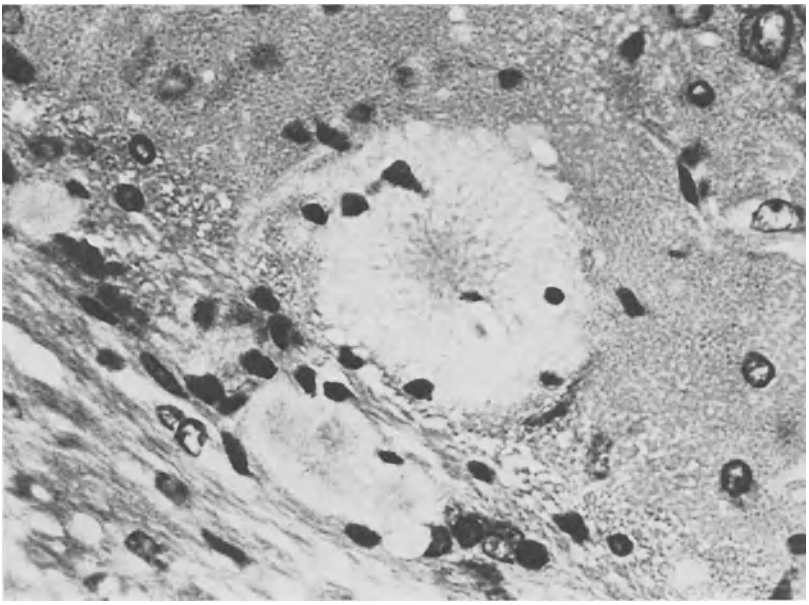


FIGURE 9: Amyloid plaques in the hippocampus (below) and corpus callosum (above) in a VM mouse with 87V scrapie agent. The incubation period was 273 days. Haematoxylin and eosin, x 900.

Some agents cause severe cerebral amyloidosis and amyloid plaques in all mice, while with other agents this lesion is absent. Some agents occupy an intermediate position, with a moderate plaque incidence in some mouse genotypes only (52). On the basis of this criterion some agents can be described as "high" plaque producers in contrast with most strains which either produce none or produce a high incidence in some mouse genotypes only (1). It has been established that there is great variety in the morphology of the cerebral amyloid in murine scrapie, and some of the amyloid plaques are closely analogous to the argyrophilic or senile (neuritic) plaques of Alzheimer's disease (53, 54) (Figure 9). Also this lesion can appear indistinguishable from

certain forms of diffuse or perivascular amyloidosis found in atypical Alzheimer's disease. (55) (Figure 10). In addition to these examples it has been shown that some of these forms in murine scrapie are identical to the amyloid plaques or "kuru-plaques" of kuru and C-J disease in humans (53, 56). The origin and identity of the cerebral amyloid in murine scrapie remain unknown, as is also the case in the human disorders. The most crucial questions concerning the origin of cerebral amyloid are firstly whether it depends for its formation on systemic events with circulating precursors or co-factors, or is entirely a local neuroparenchymatous phenomenon, and

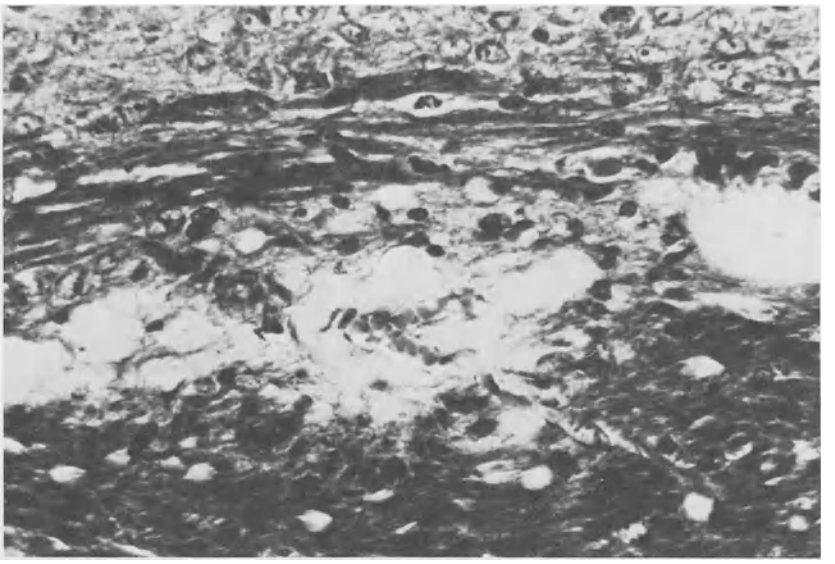


FIGURE 10: Perivascular cerebral amyloid, associated with small vessels, in the corpus callosum in a VM mouse with 87A scrapie agent, after an incubation period of 613 days. The amyloid is unstained with phosphotungstic acid haematoxylin, x 750.

secondly whether it is related to an abortive immunological response. There are two simple alternatives for the origin of the amyloid protein (46). It could be encoded in the genetic information of the agent itself.

The loss of amyloid induction after breakdown i.e. from 87A to 7D (49), might reflect a loss of a region of the agent code. The second more likely possibility is that the cerebral amyloid is a host-coded protein produced in response to a part of the agent itself or agent coded products or degeneration products. In this case the loss of amyloid induction after break down could be due to a loss of part of the agent code responsible for the host stimulus. In either case the amyloid could have a local or systemic origin. The murine scrapie model provides the only available research tool for an eventual elucidation of these, and related questions (46).

There is one final aspect of the pathology which needs to be discussed as it represents a major gap in our knowledge of the pathology of this disease. There are

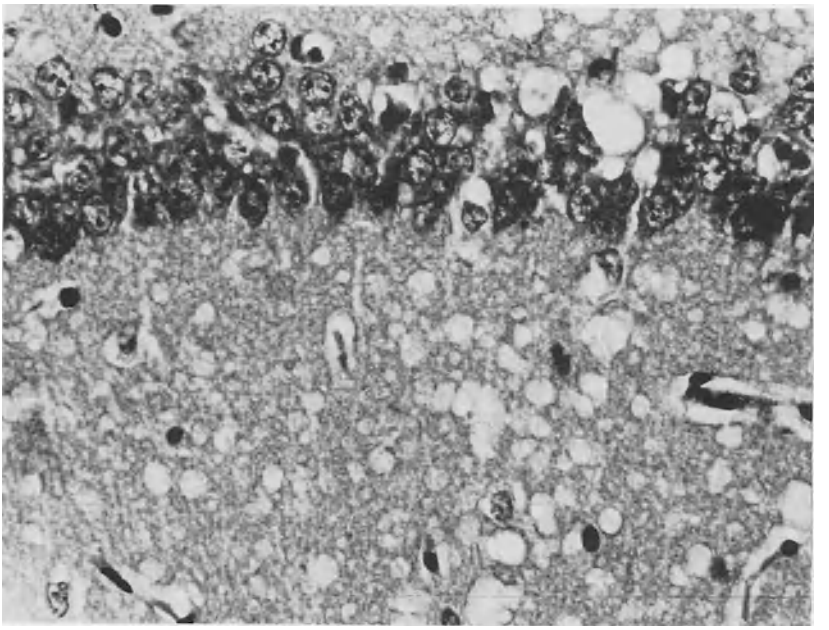


FIGURE 11: Severe spongiosis, without neuron loss or glial response, in the hippocampus of a C3H mouse injected intraperitoneally with ME7 scrapie agent. The incubation period was 325 days. Haematoxylin and eosin, x 750.

numerous unsubstantiated assertions concerning either a loss or an increase of different cell types of the neuroectoderm. The reason that this is an important area is because it relates to the questions raised earlier concerning the potential for reversibility of the degenerative process - if nerve cells die they cannot be replaced. With the agents which induce vacuolation in the forebrain it has been shown that in the hippocampus a severe vacuolation, with intense spongiosis, without evidence of a proliferative or infiltrative glial reaction can be produced following i.p. or other peripheral inoculation (Figure 11). However under as yet ill-defined circumstances, but only in a small proportion of cases following intracerebral injection, a complete loss of the granule cell population of the hippocampus, accompanied by a profound glial response, microgliosis and moderate astrocytic proliferation can occur in a small proportion of cases (Figure 12).

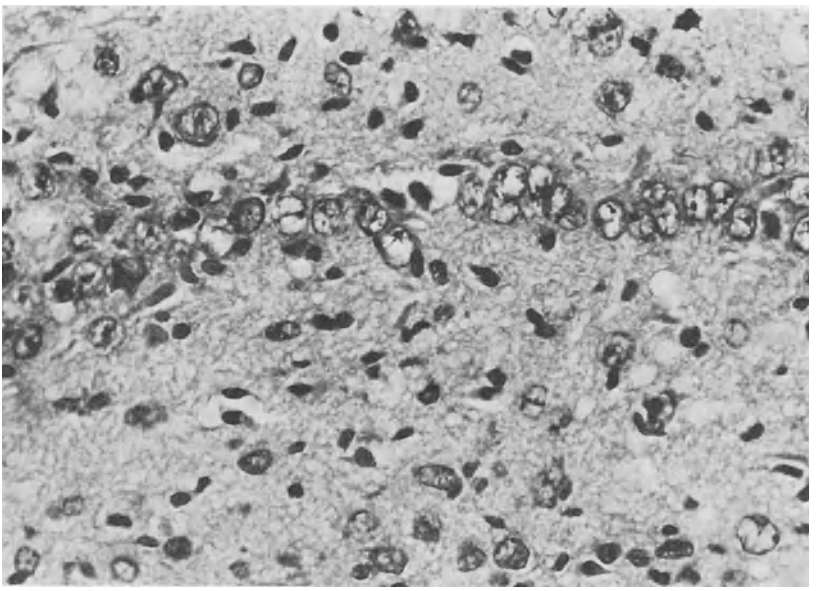


FIGURE 12: Glial response in the hippocampus of a VM mouse injected intracerebrally with 22A scrapie agent. The incubation period was 240 days. Haematoxylin and eosin, x 750.

This pattern of degeneration in the hippocampus bears a striking resemblance to the degenerative pathology in the hippocampus in a high proportion of cases of Alzheimer's disease (57). A similar type of degenerative pathology, with localised nerve cell loss accompanied by an intense glial response occurs in the thalamus in a small proportion of cases following i.c. injection with a number of agents (21, 24, 46). An important future objective will be to distinguish the intermediate situations between the two extremes, namely the stage when the vacuolar degeneration still represents a theoretically reversible stage, from the stage when the degeneration has progressed to irreversible cell death. The considerable technical and interpretative difficulties of defining the quantitative pathology of scrapie and the related diseases, in terms of putative neuron loss or astocytosis, have been discussed in detail elsewhere (21, 46).

4. CONCLUDING COMMENT

In considering any future pharmacological developments which may become available there would appear to be two major objectives: firstly to reverse the deterioration in the biochemical integrity of the neuroparenchyma before the degeneration has progressed to an irreversible stage of cell death; and secondly to halt the biochemical steps involved in agent replication in the LRS and CNS. The present prospects for thereapeutic advances do not seem hopeful. One series of experiments which may offer a gleam of hope are those involving the site-looking action of the pathogenesis of a "fast" scrapie agent by the prior injection of a "slow" agent (6, 8, 58, 59). These findings suggest an eventual possibility of mimicking this site-blocking with non-pathogenic molecules. However these experiments have shown that the blocking agent must undergo replication for blocking to be effective, which may mean that receptor site turnover occurs, with resultant elimination of any bound blocking agent. To be

effective any non-replicating blocking molecule may therefore have to be introducted to the body on a continued basis.

It is important that future work in these areas take account of the wide range of differences in scrapie models, and of the associated different scrapie agents, and not be confined to single and possibly atypical agents or to deviant agents which, during multiple passage in laboratory animals have lost the full expression of their biological capabilities. The clinical and pathological variety in the disease with the various agents makes it highly likely that there will be a wide spectrum of biochemical defects, each with different prominence, and resulting in the different patterns of neurological disturbance.

REFERENCES

1. Dickinson AG, Fraser H, Bruce ME: Animal models for dementias. In: Alzheimer's disease, early recognition of potentially reversible defects, Glen AIM, Whalley LJ (eds), Edinburgh, Churchill-Livingstone, 1979 (in press).

2. Kimberlin RH, Walker CA: Characteristics of a short incubation model of scrapie in the golden hamster. J Gen Virol 34: 295-304, 1977.

3. Dickinson AG: Scrapie in sheep and goats. Chapter 10 in: Slow virus diseases of animals and man, Kimberlin RH (ed), Amsterdam, North-Holland, 1976.

4. Dickinson AG, Fraser H, Outram GW: Scrapie incubation time can exceed natural life span. Nature 253: 732 -733, 1975.

5. Kimberlin RH, Walker CA: Evidence that the transmiss--ion of one source of scrapie agent to hamsters involves separation of agent strains from a mixture. J Gen Virol 39: 487-496, 1978.

6. Dickinson AG, Outram GW: The scrapie replication site hypothesis and its implications for pathogenesis. In: Slow transmissible diseases of the nervous system, Hadlow WJ, Prusiner SB (eds), Academic Press 1979 (in press).

7. Dickinson AG, Fraser H: An assessment of the genetics of scrapie in sheep and mice. In: Slow transmissible diseases of the nervous system, Hadlow WJ, Prusiner SB (eds), Academic Press, 1979 (in press).

8. Kimberlin RH: this volume, Chapter 2.

9. Outram GW: The pathogenesis of scrapie in mice. Chapter 14 in: Slow virus diseases of animals and man, Kimberlin RH (ed), Amsterdam, North-Holland, 1976.

10. Fraser H, Dickinson AG: Pathogenesis of scrapie in the mouse: the role of the spleen. Nature 226: 462-463, 1970.

11. Kimberlin RH, Walker CA: Pathogenesis of scrapie: agent multiplication in brain at first and second passage of hamster scrapie in mice. J Gen Virol 42: 107-117, 1979.

12. Outram GW, Dickinson AG, Fraser H: Developmental maturation of susceptibility to scrapie in mice. Nature 241: 536-537, 1973.

13. Outram GW, Dickinson AG, Fraser H: Reduced susceptibility to scrapie in mice after steroid administration. Nature 249: 855-856, 1974.

14. Outram GW, Dickinson AG, Fraser H: Slow encephalo--pathies, inflammatory responses, and arachis oil. Lancet i: 198-200, 1975.

15. Dickinson AG, Fraser H, McConnell I, Outram GW: Mitogenic stimulation of the host enhances susceptibility to scrapie. Nature 272: 54-55, 1978.

16. Kimberlin RH, Cunnington PG: Reduction of scrapie incubation time in mice and hamsters by a single injection of methanol extracted residue of BCG. FEBS Microbiol Lett 3: 169-172, 1978.

17. McFarlin DE, Raff MC, Simpson E, Nehlsen SH: Scrapie in immunologically deficient mice. Nature 233: 336, 1971.

18. Fraser H, Dickinson AG: Studies of the lymphoreticular system in the pathogenesis of scrapie: the role of the spleen and thymus.J comp Pathol 88: 563-573, 1978.

19. Eklund CM, Kennedy RC, Hadlow WJ: Pathogenesis of scrapie virus infection in the mouse. J Infect Dis 117: 15-22, 1967.

20. Dickinson AG, Fraser H: Genetical control of the concentration of ME7 scrapie agent in mouse spleen. J Comp Pathol 79: 363-366, 1969.

21. Fraser H: Scrapie: a transmissible degenerative CNS disease. In: Progress in Neurological Research, Behan PO, Clifford Rose F (eds), London, Pitman, 1979 (in press).

22. Kimberlin RH: Experimental scrapie in the mouse: a review of an important model disease. Science Progress, Oxford 63: 461-481. 1976.

23. Corp CR, Kimberlin RH: Changes in the metabolism of polyadenylated RNA commencing early in the incubation period of scrapie in the mouse. Vet Microbiol 2: 193-204, 1977.

24. Kimberlin RH: Early events in the pathogenesis of scrapie in mice: biological and biochemical studies. In: Slow transmissible diseases of the nervous system, Hadlow WJ, Prusiner SB (eds) Academic Press, 1979 (in press).

25. Dickinson AG, Meikle VMH, Fraser H: Genetical control of the concentration of ME7 scrapie agent in the brain of mice. J Comp Pathol 79: 15-22, 1969.

26. Clarke MC, Haig DA: Presence of the transmissible agent of scrapie in the serum of affected mice and rats. The Vet Rec 80: 504, 1967.

27. Manuelides EE, Gorgacz EJ, Manuelides L: Viraemia in experimental Creutzfeldt-Jakob disease. Science 200: 1069-1071, 1978.

28. Marsh RF, Miller JM, Harrison RP: Transmissible mink encephalopathy: studies on the peripheral lymphocyte. Infect Immun 7: 352-355, 1973.

29. Fraser H: The pathology of natural and experimental scrapie. Chapter 12 in: Slow virus diseases of animals and man, Kimberlin RH (ed), Amsterdam, North-Holland, 1976.

30. Marsh RF, Sipe JC, Morse SS, Hanson RP: Transmissible mink encephalopathy, reduced spongi--form degeneration in aged mink of the Chediak-Higashi genotype. Lab Invest 34: 381-386, 1976.

31. Lampert P, Hooks J, Gibbs CJ, Gajdusek DC: Altered plasma membranes in experimental scrapie. Acta Neuropathol (Berl) 19: 81-93, 1971.

32. Lampert P, Gajdusek DC, Gibbs CJ: Subacute spongiform virus encephalopathies. Scrapie, Kuru and Creutzfeldt-Jakob disease: a review. Am J Pathol 68: 626-646, 1972.

33. Barringer JR, Prusiner SB: Experimental scrapie in mice, ultrastructural observations. Ann Neurol 4: 205-211, 1978.

34. Field EJ: The significance of astroglial hypertrophy in scrapie, kuru, multiple sclerosis and old age together with a note on the possible nature of the scrapie agent. Dtsche Z Nervenheilk 192: 265-274, 1967.

35. Fraser H: Comparative morphology of ageing and scrapie. In: Proceedings of the VIth International Congress of Neuropathology: p.897, Paris, Masson and Cie, 1970.

36. Gajdusek DC: Slow virus infection and activation of latent infections in ageing. Adv Gerontol Res 4: 201-218, 1972.

37. McDermott JR, Fraser H, Dickinson AG: Reduced choline acetyltransferase activity in scrapie mouse brain. Lancet ii: 318-319, 1978.

38. David-Ferrieira JF, David-Ferrieira KL, Gibss CJ, Morris JA: Scrapie in mice: ultrastructural observations in the cerebral cortex. Proc Soc Exp Biol Med 127: 313-320, 1968.

39. Bignami A, Parry HB: Aggregations of 35-nanometer particles associated with neuronal cytopathic changes in natural scrapie. Science 117: 389-390, 1971.

40. Bignami A, Parry HB: Electron microscopic studies of the brain of sheep with natural scrapie. Brain 95: 319-326.

41. Narang HK: Virus-like particles in natural scrapie of sheep. Res Vet Sci 14: 108-110, 1973.

42. Narang HK: Ruthenium red and lanthanum nitrate, a possible tracer and negative stain for scrapie "particles". Acta Neuropathol (Berl) 29: 37-43, 1974.

43. Narang HK: An electron microscopic study of the scrapie mouse and rat: further observations on virus-like particles with ruthenium red and lanthanum nitrate as a possible trace and negative stain. Neurobiology 4: 349-363, 1974.

44. Lamar CH, Gustafson DP, Krusovich M, Hinsman EJ: Ultra structural studies of spleen, brains and brain cell culture of mice with scrapie. Vet Pathol 11: 13-19.

45. ZnRhein GM, Varakis I: cited by Marsh RF: The subacute spongiform encephalopathies. Chapter 15 in: Slow virus diseases of animals and man, Kimberlin RH (ed), Amsterdam, North-Holland, 1976.

46. Fraser H: Neuropathology of scrapie: the precision of lesions and their diversity. In: Slow transmissible diseases of the nervous system, Hadlow WJ, Prusiner SB (eds) Academic Press, 1979 (in press).

47. Fraser H, Dickinson AG: Agent-strain differences in the vacuolation and intensity of grey matter vacuolation. J Comp Pathol 83: 29-40, 1973.

48. Fraser H, Bruce ME, Dickinson AG: Quantitative pathology for understanding the nature and pathogenesis of scrapie, using different strains of agent. In: Proceedings of the VIII the International Congress of Neuropathology: p.277, Amsterdam, Excerpta Medica, 1975.

49. Bruce ME, Dickinson AG: Biological stability of different classes of scrapie agent. In: Slow transmissible diseases of the nervous system, Hadlow WJ, Prusiner SB (eds) Academic Press, 1979 (in press).

50. Galaburda AM, Le May M, Kemper TL, Geschwind N,: Right-left asymmetries in the brain. Science 199: 852-856, 1978.

51. Kinsbourne M: Asymmetrical function of the brain. Cambridge and London, Cambridge University Press, 1978.

52. Bruce ME, Dickinson AG, Fraser H: Cerebral amyloidosis in scrapie in the mouse: effect of agent strain and mouse genotype. Neuropathology and Applied Neurobiology 2: 471-478, 1976.

53. Fraser H, Bruce ME: Argyrophilic plaques in mice inoculated with scrapie from particular sources. Lancet i: 617, 1973.

54. Wisniewski HM, Bruce ME, Fraser H: Infectious etiology of neuritic (Senile) plaques in mice. Science 190: 1108-1110,1975.

55. Corsellis JAN, Brierley JB: An unusual type of pre-senile dementia (atypical Alzheimer's disease with amyloid change). Brain 77: 571-587, 1954.

56. Bruce ME, Fraser H: Amyloid plaques in the brains of mice infected with scrapie: morphological variation and staining properties. Neuropathology and Applied Neurobiology 1: 189-202,1975.

57. Corsellis JAN: The limbic areas in Alzheimer's disease and in other conditions associated with dementia. Chapter In: Alzheimer's disease, a Ciba Foundation Symposium, Wolstenholme GEW, O'Connor M (eds) London, Churchill, 1970, p37-45.

58. Dickinson AG, Fraser H, Meikle VMH, Outram GW: Competition between different scrapie agents in mice. Nature, New Biology 237: 244-245, 1972.

59. Dickinson AG, Fraser H, McConnell I, Outram GW, Sales DI, Taylor DM: Extraneural competition between different scrapie agents leading to loss of infectivity. Nature 253: 556, 1975.

DISCUSSIONS OF PAPERS BY R.H. KIMBERLIN AND H. FRASER

This began with a consideration of the mode of spread of scrapie in vivo. With the exception of one report of the presence of Creutzfeldt-Jacob disease agent in the buffy coat of guinea-pigs shortly after inoculation, there was no evidence of the presence of this type of agent in the peripheral blood. It was later speculated that infection of a small subpopulation of lymphocytes (e.g. B lymphocytes) might explain some of the features of scrapie; but hard facts were not available. Infection with scrapie probably spread from spleen to brain; splenectomy apparently removed replication sites but there were others in the reticulo-endothelial system and the zero phase was not prolonged. In tissue culture experiments, using the SMB cell line (persistently infected with scrapie), replication appeared to be associated with membranes and endoplasmic reticulum.

A question was asked concerning the role of interferon and whether it influenced infectivity; the answer was, broadly, no. There was no relation between the production of amyloid in scrapie-infected mice and the depression of choline acetyl transferase activity; strains which produced such a depression did not necessarily produce amyloid and there was no correlation between a long incubation period and a high incidence of amyloid. It was not known whether scrapie amyloid contained Ig-like chains. Reports of an inhibition of immune response in scrapied animals have been made but they are only just statistically significant and limited to one system; consequently, it seemed more likely that this phenomenon was non-specific.

Vertical transmission had not been detected and, indeed, immunological competence was necesaary for scrapie infection. Day old mice were less readily infected, or the incubation period was longer than weaned mice but, otherwise, age dependence was minimal. This neonatal immunological incompetence was an important difference between sheep and mice. Kuru was not transmitted vertically but (Dr Cathala) the situation might be different in Creutzfeldt-Jacob disease.

Professor Sanger drew attention to the alleged resemblance of the alterations of the plasmalemma (plasmalemmesomes) in plants with viroid infection and their resemblance to similar changes in scrapie

(vacuoles). Such a comparison was fallacious as plasmalemmasomes are seen also in quite healthy plants.

The nature of scrapie and its relation to kuru and to Creutzfeldt-Jacob disease were discussed at length. Although the pathology of these agents differed in mice, the basic vacuolation, gliosis and plaques remained. Trans-species transmission was sometimes possible but not invariably so (e.g. the failure to transmit transmissible mink encephalopathy in mice). The unusual nature of the scrapie agent was highlighted by its unusual response to UV irradiation. Dr Kimberlin felt that scrapie nucleic acid used host components which fitted (much as a hermit crab) and, together these components formed a fully infectious agent. It seemed much more likely that scrapie was an unusual virus than a viroid and Dr Kimberlin closed by quoting Dr Dickinson who had coined the neologism 'Virelo' for agents such as scrapie.

SUBACUTE SCLEROSING PANENCEPHALITIS: CHARACTERIZATION OF THE ETIOLOGICAL AGENT AND ITS RELATIONSHIP TO THE MORBILLI VIRUSES

R. STEPHENSON AND V. TER MEULEN

1. INTRODUCTION

Subacute sclerosing panencephalitis (SSPE) is a slow virus infection of the central nervous system (CNS) affecting children and young adults (1,2). The clinical picture reflects a variable and wide spread involvement of the CNS. The disease usually starts with mental and behavioural changes, which after a period of weeks or months are followed by characteristic neurological symptoms. They are characterized by various disturbances of motor function, myoclonic jerks and epileptic seizures. At a later stage of the disease progressive cerebral degeneration with symptoms and signs of decerebration occurs leading always to death. Neuropathologically the disease process is characterized by perivascular cuffing, increase in hypertrophic astrocytes, proliferation of microglia as well as demyelination and the presence of intranuclear Cowdry type A and B inclusion bodies. This latter finding, which was already described in 1933 (3) has always been interpreted as footprints of a viral infection, but the first direct evidence of virus involvement was reported in 1965 by Bouteille et al. (4) who observed tubular structures resembling paramyxovirus nucleocapsids in brain tissue of SSPE patients. These structures were soon identified as measles virus nucleocapsids as infectious measles virus (referred to as SSPE virus) was isolated from biopsy material. Although these findings fulfil Koch's first postulate namely the occurrence of the 'parasite in every case of the disease in question' and incriminates measles virus as the causative agent in this disease, the pathogenesis is still not understood. If measles virus is involved, then additional factors,

either host or virus derived, must play a pathogenetic role, since rarity and rural prevalence of this disease cannot be correlated to a ubiquituous measles infection.

This paper summarized the virological findings in SSPE from recent investigations, compares the SSPE virus characteristics with those of related viruses and discusses the possible pathogenetic mechanisms underlying this slow virus infection.

2. VIROLOGICAL AND IMMUNOLOGICAL EVIDENCE FOR MEASLES VIRUS INFECTION IN SSPE

2.1. *Presence of measles virus antigen and nucleocapsids in brain cells*

Neurological investigations have shown that in diseased brain areas of SSPE patients intranuclear and intracytoplasmic eosinophilic inclusion bodies occur in neurons, astrocytes and oligodendroglia cells (1). These inclusion bodies contain RNA by cytochemical staining and consists of paramyxovirus-like nucleocapsids of diameter 17 - 23 nm and of length up to 500 nm. The nucleocapsids are found in both nucleus and cytoplasm of brain tissue culture cells, and also in cells persistently or lytically infected with virus isolated from SSPE patients. The nuclear nucleocapsids have always a 'smooth' appearance and those in the cytoplasm a rough appearance. This distinction between nuclear and cytoplasmic morphology is constant whether autopsy material is observed or that from lytically or persistently infected cells is examined. Only the rough nucleocapsids appear in released virus, whatever the source. By immunofluorescent staining these intranuclear inclusions clearly stain with measles antibodies. Moreover measles antigen can be detected in cell processes, suggesting the spread of viral material along this route.

2.2. Isolation of SSPE virus

All standard methods for the isolation of SSPE virus from SSPE brain material failed to recover infectious virus. Only after cocultivation of SSPE brain tissue culture with cultures susceptible to measles virus

replication was SSPE virus isolated. However, it is important to emphasize that the isolation of SSPE virus is an exception rather than the rule (5). Virus has been recovered not only from biopsy and necropsy material, but also in one instance from lymph nodes (6). The characteristics of these virus isolates vary from a virus which produces c.p.e. and free infectious virus after only a few subcultures to those who need many subcultures for complete expression. Other isolates may establish a persistent infection with no free virus produced and mainly subgenomic nucleocapsids and viral antigens being detected (7,8).

2.3. Immuneresponse to measles virus

One of the main diagnostic criteria in SSPE is the presence of high titre measles antibodies in serum and C.S.F. specimens. All patients exhibit a strong hyperimmuneresponse against all biological active measles virus antigens. Measles specific IgG antibodies represent 10 % to 20 % of the total serum IgG and about 75 % of the total C.S.F. IgG (9). Moreover, the antibodies in the C.S.F. are of oligoclonal nature and reveal restricted variability of the V-region of Kappa and Lambda light chains and restricted specificities. This finding together with a low ratio of serum to C.S.F. antibodies suggest a local production of measles antibodies by invading lymphocyte clones. These antibodies neutralize infectious measles virus, lyse measles infected target cells in the presence of complement and induce antigen capping in cell lines persistently infected with measles virus. However, our analysis of antibody activities against all measles virus structural proteins by immunprecipitation in combination with P.A.G.E. revealed that the hyperimmune reaction in SSPE is only directed against structural proteins H, P, N and F and not against the M or L proteins (Fig.1). Similar findings are obtained with convalescent sera from cases of simple of complicated measles. Probably these two proteins are either poor immunogens, not produced in sufficient quantities or not released from infected cells to be available for the immune

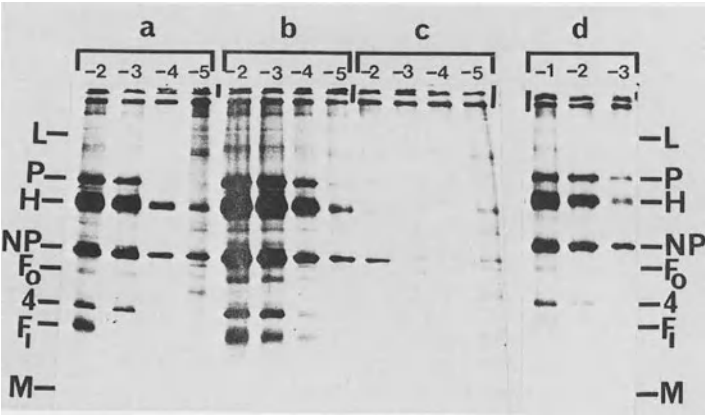


Figure 1

Immune precipitation of measles-specific polypeptides by sera from human patients. Vero cells were infected with the LEC strain of SSPE virus and labelled with ^{35}S -methionine. Cells were lysed in buffer containing 0.15 M NaCl, 10 mM Tris pH 7.4, 1 mM EDTA, 1 % NP40, 0.1 % Na Azide, 2 mg/ml PMSF and 1,000 units/ml Aprotinin. Cells were frozen, thawed, sonicated and clarified at 100,000 g for 30 min. 200 µl of lysate were reacted with 5 µl of sera at the appropriate dilution. The precipitates were incubated with fixed Staphylococcus aureus bacteria, washed and boiled in sample buffer (5M Urea, 1 % SDS, 0.1 % BME). Samples were run on 15 % discontinuous gels containing 1 % SDS and 5 M Urea. Polypeptides designated as described previously (19).

a) Sera from a classical SSPE patient
b) Sera from a patient with viral meningitis
c) Sera from a 6 months old infant with no known measles infection or contact.
d) Convalescent sera 5 months after acute measles.

Tracks are designated by the indice of sera concentration.

competent cells.

In contrast to this humoral hyperimmune reaction no generalized defect of cell-mediated immunity (CMI) has been found in SSPE patients except for a specific unresponsiveness to measles virus on skin testing (2). Inoculation of measles virus antigen does not lead to a positive skin reaction which has been interpreted as a possible counterpart to the transient tuberculin anergy observed in measles disease. Otherwise blastogeneic and lymphokine responses are unimpaired after stimulation with mitogens or antigens unrelated to measles virus. Moreover, skin allografts are rejected in a normal fashion. However, controversial results are reported by using measles virus antigen in assays for CMI. Both normal and abnormal responses have been repeatedly found, which may indicate that a measles specific CMI defect could exist in SSPE (2). However, until the functional state of T lymphocyte in SSPE is defined, no definite answer to the role of the immune response in relation to this chronic disease process can be given (11).

3. PHYSICO-CHEMICAL PROPERTIES OF MEASLES VIRUS

3.1. *Morphology*

Measles virus is a member of the morbillivirus group which include canine distemper (CDV), rinderpest virus (RV) and pest des petits ruminants virus (PRV) and form a subgroup of the genus Paramyxoviridae (ICNV cytptogram: R/1 : 4-8/1 : S/E : v/o) (12). These viruses have a pleomorphic structure with a diameter of 100 - 300 nm. The outer layer of these viruses consists of a lipid bilayer from which protrude spikes of 9 - 15 nm in length. This heterogeneity in the size of the spikes may be due to more than one envelope protein being present. The lipid bilayer surrounds a coiled hollow nucleocapsid similar in appearance to that found in other paramyxoviruses (13).

Reported values for the buoyant density of the purified virus particle in either CsCl or sucrose vary between 1.24 and 1.22 gm/cc for infectious virus, and drops to 1.20 gm/cc for virus with a reduced infectivity

(14,15). This variation in density and the lack of any data on sedimentation values is probably due to significant and variable amounts of host protein in the virus preparations analysed. Also the presence of a fusion protein in the outer membrane of the virus results in decreased rigidity of the viral envelope and makes biophysical examination difficult. Nucleocapsids isolated from purified virus have a density of 1.30 - 1.31 gm/cc and sediment between 100 and 200 S. When examined by electron microscopy these nucleocapsids are hollow, have an external diameter of 15 - 20 nm, an internal diameter of 7 nm, a pitch of 6.6 nm and lengths up to 1,000 nm, although shorter species predominate: rarely circular and branched forms can be seen. Nucleocapsids from purified measles virus and the cytoplasm of infected cells have a 'rough' appearance when examined by electron microscopy, whereas those from the nucleus have a 'smooth' texture. This subcellular difference in nucleocapsid morphology is invarient whether the specimen comes from lytically infected cells, persistently infected cells or from fresh autopsy material (8,16). The nucleocapsid contains about 5 % by weight of RNA. The RNA from the purified virus has a minor component sedimenting at 50S and a major component sedimenting from 12 - 30S (17).

So far isolates of SSPE virus show no gross morphological differences from that described above for measles virus except that in both persistent and lytic infections with SSPE, the nuclear form of the nucleocapsid predominates, whereas with measles it is the cytoplasmic form which is most common (2).

3.2. Molecular structure of the virion

Although there is some discussion in the literature as to the precise number and molecular weight of morbillivirus polypeptides, the genome of this group of viruses appears to specify six primary gene products. The molecular weights of these proteins show some similarity among different members of the group but are not identical in every case (15,18). The largest polypeptide has an apparent molecular weight of between 100 - 150 K. There are two

glycoproteins which by analogy to Sendai virus are assumed to be the Haemagglutinin (HA) and the haemolysin or fusion (F) protein (19). However, the corresponding biological activities have been reported only for measles virus (MV) in spite of many attempts to detect them in canine distemper and Rinderpest. The haemagglutinin of these viruses has a molecular weight of about 80,000 daltons.The fusion protein is found as a glycosylated precursor of molecular weight 60,000 in infected cells but is only found in the cleaved form in the virus. The cleavage products have molecular weights of 40,000 and 18,000 and only the latter is glycosylated (19,20). The nucleocapsid contains two proteins, a minor species of 70,000 daltons and a major species of 62,000 daltons. The smallest major virion polypeptide has a molecular weight of 34,000 to 37,000 daltons and from its structural similarities to Sendai virus M protein, is assumed to be the protein lining the inner membrane of the virion (21). A further polypeptide of molecular weight 55 K has been reported in MV infected cells (18,19). Its function has not yet been elucidated although a similar protein in Sendai virus has been shown to be derived from the major nucleocapsid protein (22).

SSPE viruses reveal, in general, the same properties. However, by comparison of the RNAs of measles and SSPE viruses by hybridization, some differences were noted. One study showed that SSPE 50S RNA shared 60 % homology with measles 18S RNA, although no attempt was made to determine the role played by negative stranded subgenomic defective RNAs (23). A more detailed analysis using oligo dT isolated mRNA and competition hybridization suggested that SSPE mRNA had a higher genetic complexity than measles mRNA (24). These authors have also reported differences in migration on SDS P.A.G.E. of at least one mRNA from SSPE and measles viruses (25). Similar small differences in apparent molecular weight have been reported for the M and P proteins for several strains of SSPE (26). However such variation also appears to occur between measles isolates as well (27). Recently the 50S RNA of 2 measles and one SSPE isolate from the cytoplasm

of AMD-treated infected cells have been compared by analysis of the oligonucleotides from a T_1 digest (28). These data show considerable similarity but not identity between the three isolates. However, at present it is not possible to show that any differences are specific for SSPE virus.

4. BIOLOGICAL PROPERTIES

4.1. *Comparative Antigenicity*

4.1.1. Antigenic relationships between measles and SSPE virus

In general, little difference has been found in the antigenicity of these viruses when assayed by neutralisation, haemolysin inhibition, haemagglutinin inhibition (2) and indirect radio-immunoassay (21). Moreover, all the virus-specific polypeptides present in cells infected with an SSPE virus can be precipitated by antisera against measles virus (Fig. 2). However, when antigenic studies were carried out on purified M protein from one SSPE and one Measles isolate, they revealed antigenic differences between the M proteins of these two viruses (25). The immunological differentiation between these proteins was achieved by using rabbit sera after a short-term immunization with purified protein.

4.1.2. Antigenic relationships between measles virus and canine distemper virus.

It has been known for some time that human measles convalescent sera cross-react with CDV (29). However, sera taken during the acute phase of MV infection showed no heterologous activity. Experiments on animals infected with measles virus gave similar results (30. Sato and co-workers (31) reported that sera from SSPE patients cross-react to a much higher degree with CDV than do either patients with acute atypical measles, convalescent vaccinees, or hyperimmune animals. However, it is difficult to

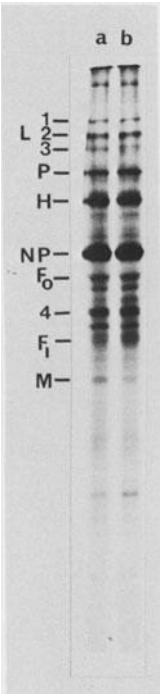


Figure 2
Immune precipitation by rabbit sera of virus-specific polypeptides from cells infected with SSPE virus (LEC isolate). Immune precipitation and electrophoresis of radio-labelled cell lysates were performed as in Figure 1.
a) Sera raised against Measles virus (Edmonston)
b) Sera raised against SSPE virus (LEC)
Polypeptides designated as for Figure 1.

---

compare different studies in a quantitative fashion as sera of widely varying titres are used. Data on the reverse process, i.e. the MV neutralization activity of CDV antisera is equivocal. In general, CDV antiserum contains little or no neutralizing activity to measles. The above studies on virus neutralization presumably only detect surface antigens responsible for attachment and penetration; other studies on infected cells, involving immune fluorescence (32) and electron microscopy of ferritin tagged antibody show a much higher degree of cross-reactivity, presumably because reactions with many of the internal antigens are detected. Similar high levels of cross-reactivity have been observed using radio-immune assay directed against whole disrupted virions (21). Studies on antigenic similarities between individual virus components reveal that the nucleocapsid of MV and CDV are identical antigenically and that sera raised against CDV have a high level of antibody against MV Haemolysin and low activities against MV Haemagglutinin (33).

4.2. Host range and growth kinetics

Although several reports are available which show differences in tissue culture, host range and growth kinetics between measles virus and SSPE virus, no parameter has been found to distinguish SSPE virus from measles

virus per se. It has been observed that some SSPE isolates have a wider, others a narrower host range, when compared with wild-type measles virus (34). Moreover, primary human brain cultures can easily be infected with measles virus but not with SSPE virus (34,35). In similar studies using hamster dorsal root ganglia one of three measles isolates and one of two SSPE isolates were infectious (36). Other workers have compared virulence, HA production and intracellular antigens by FA staining of SSPE and measles isolates, and found that, whereas the SSPE isolates differed from each other, they each shared different properties with Measles virus (37). In addition, the growth kinetics of two SSPE isolates differed from those of two wild-type measles isolates (35) and in both cases, the SSPE viruses grew slower than did the measles isolates. However, it must be emphasized that the passage history and isolation procedure of each isolate under test is seldom comparable, and thus the relative degree of adaptation for each isolate is not known.

5. PATHOGENETIC ASPECTS

The virological findings of measles virus structures and antigens in SSPE brain material as well as the humoral hyperimmune response of these patients to measles virus has led to many hypotheses in the attempts to explain etiology and pathogenesis of this CNS disorder (38). Based on epidemiological observations of a preceeding acute measles infection years before onset of SSPE it is assumed that at this stage measles virus enter the CNS and a persistent infection is established. This event probably marks the beginning of this slow virus infection. However, before a clinically recognizable disease develops this infection is silent for an average of 5 - 6 years. No direct and definite information is available about the virus host relationship during this incubation period and the mechanisms which activate virus latency. Under normal conditions, infectious measles virus entering a sero negative host

induces acute measles which can be overcome by the host defense mechanisms, if the cellular immune system (CMI) is unimpaired. Clinical experiences have revealed that individuals with congenital, acquired or iatrogenic deficiency of thymus dependent lymphocytes usually develop severe often fatal acute measles whereas children with hypogammaglobulinaemia develop normal measles without complications and acquire lifelong immunity (39). Based on these observations Burnet suggested that SSPE is the consequence of a measles specific T cell defect which prevents successful elimination of this virus and therefore allows the virus to establish persistency in the host (40). So far, no major immune defect has been found in SSPE which would explain the rarity, rural prevalence and pathogenesis of this disease or would support Burnet's hypothesis. Obviously the virus-host relationship underlying this disease process plays a major role in SSPE and from a virological point of view the mechanism of measles virus persistency in brain cells has to be explained. Virological studies have shown that isolation procedures to rescue infectious measles virus from SSPE brain material are only successful if infected brain cells are cocultivated with tissue culture cells susceptible to measles virus replication, since infectious virus cannot be found directly (2). The observation of a state of measles virus defectiveness in brain cells is supported by in vitro studies of persistently infected SSPE brain tissue cultures. The biological and biochemical characterization of these tissue culture cells revealed the presence of measles antigen, nucleocapsids and salt dependent haemagglutin in the absence of infectious virus. All attempts to initiate reactivation of virus synthesis were unsuccessful (7,8). In addition, biochemical analysis of intracellular RNA demonstrated that the majority of the virus RNA produced was defective or subgenomic and that the 50S RNA normally associated with infectious virus was only detectable in small amounts. These findings indicate an impaired state of virus replication which may represent the one responsible for virus persistency in SSPE brain. By what

mechanisms such persistency is induced and maintained is not known, but in comparison to other virus groups it could be related to the presence of defective interfering (DI) particles. It has been shown for VSV that DI particles not only interfere with the replication of infectious virus in tissue culture, but are also capable of modifying an acute infection to a more subacute disease process (41).

On the other hand, the recorded biochemical and immunological differences between measles and SSPE virus suggest that SSPE virus may possess additional properties which favour persistency. The observed differences between the M proteins of these viruses indicate one possible site at the replication cycle, where interference can occur. If SSPE virus has been derived from measles virus by mutation it is conceivable that the gene region coding for the M protein is affected and a non-functioning M protein could be produced which may block virus assembly. This could result in the initiation and maintainance of a non-productive persistent infection in which no virus particles are formed. This hypothesis is compatible with the accumulation of nucleocapsids found in infected brain cells and the failure to detect virus budding.

Future virological investigations will have to analyse in detail the virus-host relationship in SSPE. It will be necessary to further characterize the virus RNA from SSPE brain material to obtain direct information about the state of viral defectiveness in brain cells. Moreover, one should attempt to define the virus specific proteins present in brain cells and if possible to identify those in the cell membranes. A detailed analysis of the immune response to measles virus structural proteins in SSPE patients could provide valuable information about the synthesis of these proteins and indirectly their role in the disease process. In addition, studies in measles infected tissue cultures have shown that extra cellular measles virus antibodies block membrane-incorporated viral proteins and thus prevent the immune surveillance system from eliminating these infected cells (42). This event has been postulated as one possible mechanism by which

measles virus persistency in the central nervous system is maintained. Moreover, measles antibodies may also interfere by membrane signals with intracellular virus assembly which could result in a state of virus defectiveness. An investigation of these virological questions may lead to a better understanding of the pathogenetic mechanism responsible for this chronic CNS infection.

Acknowledgements

The experimental work presented is supported by Deutsche Forschungsgemeinschaft. We wish to thank Magdalene Pföhler for excellent technical assistance and Helga Schneider for typing the manuscript.

REFERENCES

1. Meulen, V ter, M Katz, D Müller: Subacute sclerosing panencephalitis. In: Current Topics in Microbiology and Immunology, Arber et al (eds), Berlin, Heidelberg, New York: Springer Verlag, 1972, 57, p1-38.
2. Agnarsdottir, G: Subacute sclerosing panencephalitis. In: Recent Advances in Clinical Virology, AP Waterson (ed) Edinburgh, London, New York: Churchill Livingstone, 1977, p21-49.
3. Dawson, JR: Cellular inclusions in cerebral lesions of lethargic encephalitis. American Journal of Pathology 9: 7-16, 1933.
4. Bouteille, M, C Fontaine, C Vedrenne, J Delarue: Sur un cas d'encéphalite subaigue à inclusions. Étude anatomoclinique et ultrastructurale. Revue Neurologique 113: 454-458, 1965.
5. Katz, M, H Koprowski: The significance of failure to isolate infectious viruses in cases of subacute sclerosing panencephalitis. Archiv für die gesamte Virusforschung 41: 390-393, 1973.
6. Horta-Barbosa, L, R Hamilton, B Wittig, DA Fuccillo, JL Sever, ML Vernon: Subacute sclerosing panencephalitis: isolation of suppressed measles virus from lymph node biopsies. Science 173: 840-841, 1971.
7. Doi, Y, T Samse, M Nakajima, S Okawa, T Katoh, H Itoh, T Sato, K Oguchi, T Kumanishi, T Tsubaki: Properties of a cytopathic agent isolated from a patient with subacute sclerosing panencephalitis in Japan. Japanese Journal of Medical Science and Biology 25: 321-333, 1972.
8. Kratzsch, V, WW Hall, K Nagashima, V ter Meulen: Biological and biochemical characterization of a latent subacute sclerosing panencephalitis virus infection in tissue cultures. Journal of Medical Virology 1: 139-154, 1977.

9. Mehta, PD, A Kane, H Thormar: Quantitation of measles virus-specific immunoglobulins in serum, CSF and brain extract from patients with subacute sclerosing panencephalitis. Journal of Immunology 118: 2254-2261,1977.
10. Vandvik, B, E Norrby: Oligoclonal IgG antibody response in the central nervous system to different measles virus antigens in subacute sclerosing panencephalitis. Proceedings of the National Academy of Sciences of the United States of America 70: 1060-1063, 1973.
11. Kreth, HW, H Pabst: Recent Findings on Cell-Mediated Immune Reactions in Acute Measles and SSPE (this publication).
12. Kingsbury, DW, MA Bratt, PW Choppin, RP Hanson, Y Hosaka, V ter Meulen, E Norrby, W Plowright, R Rott, WH Wunner: Paramyxoviridae. Intervirology 10: 137-152, 1978.
13. Waterson, AP: Measles virus. Archiv für die gesamte Virusforschung 16: 57-80, 1965.
14. Norrby, E: Separation of Measles virus components. Archiv für die gesamte Virusforschung 14: 306-318, 1964
15. Underwood, B, F Brown: Physico-chemical characterization of Rinderpest Virus. Medical Microbiology and Immunology 160: 125-132, 1974.
16. Oyanagi, S, V ter Meulen, M Katz, H Koprowski: Comparison of subacute sclerosing panencephalitis and measles viruses: an electron microscope study. Journal of Virology 7: 176-187, 1971.
17. Schluederberg, A: Measles Virus RNA. Biochem biophys Res Commun 42: 1012-1015, 1971
18. Mountcastle, WE, PW Choppin: A Comparison of the Polypeptides of Four Measles Virus Strains. Virology 78: 463-474, 1977.
19. Graves, MC, SM Silver, PW Choppin: Measles virus polypeptide synthesis in Infected Cells. Virology 86: 254-263, 1978.
20. Tyrrell, DLJ, E Norrby: Structural Polypeptides of Measles Virus. J gen Virol 39: 219-230, 1978.
21. Hall, WW, WR Kiessling, V ter Meulen: Biochemical comparison of measles and subacute sclerosing panencephalitis viruses. In: Negative Strand Viruses and the Host Cell, RD Barry, BWJ Mahy (eds), London, New York: Academic Press, 1977,p144-156.
22. Lamb, RW, PW Choppin: The Synthesis of Sendai virus polypeptides in Infected cells. Virology 81:382-397,1977.
23. Yeh, J, Y Iwasaki: Isolation and characterisaion of subacute sclerosing panencephalitis nucleocapsids. Journal of Virology 10: 1220-1227, 1972.
24. Hall, WW, V ter Meulen: RNA homology between subacute sclerosing panencephalitis and measles virus. Nature London 246: 474-477, 1976.
25. Hall, WW, WR Kiessling, V ter Meulen: Studies on the membrane protein of subacute sclerosing panencephalitis and measles viruses. Nature London 272:460-462,1977.
26. Wechsler, SL, BN Fields: Differences between the intracellular polypeptides of measles and subacute sclerosing panencephalitis virus. Nature,London 272:458-460, 1978.

27. Rima, BK, SJ Martin, EA Gould: A Comparison of Polypeptides in Measles and SSPE Virus Stains. J gen Virol 42: 603-608,1979.
28. Stephenson, JR, V ter Meulen: The Virological State in Subacute Sclerosing Panencephalitis. NATO Advanced Study Institute on Humoral Immunity in Neurological Diseases, Plenum Press, 1978.
29. Adams, JM: Comparative study of Canine Distemper and Respiratory disease of Man. Pediatrics 11: 15-27, 1953.
30. Imagawa, DT: Relationships among measles, canine distemper and rinderpest viruses. Progress in Medical Virology 10: 160-193, 1968.
31. Sato, TA, K Yamanouchi, A Shishido: Presence of Neutralizing Antibody to Canine Distemper Virus in Sera of Patients with Subacute Sclerosing Panencephalitis. Archiv für die gesamte Virusforschung 42: 36-41, 1973.
32. Yamanouchi, K, F Kobune, A Fukuda, M Hayami, A Shishido: Comparative Immunofluorescent Studies on Measles, Canine Distemper, and Rinderpest Viruses. Archiv für die gesamte Virusforschung 29: 90-100, 1970.
33. Örvell, C, E Norrby: Further Studies on the Immunologic Relationships among Measles, Distemper, and Rinderpest Viruses. The Journal of Immunology 113: 1850-1858, 1974.
34. Horta-Barbosa, L, DA Fuccillo, R Hamilton, R Traub, A Ley, JL Sever: Some characteristics of SSPE measles virus. Proceedings of the Society for Experimental and Biological Medicine 134: 17-21, 1970.
35. Meulen, V ter, M Katz, YM Käckell, G Barbanti-Brodano, H Koprowski, EH Lennette: Subacute Sclerosing Panencephalitis: In Vitro Characterization of Viruses Isolated from Brain Cells in Culture. The Journal of Infectious Diseases 126: 11-17, 1972.
36. Ecob, MS: PhD thesis, University of Cambridge, 1975.
37. Hamilton, R, L Barbosa, M Dubois: Subacute sclerosing panencephalitis measles virus: study of biological markers. Journal of Virology 12: 632-642, 1973.
38. Meulen, V ter, WW Hall, HW Kreth: Pathogenic aspects of subacute sclerosing panencephalitis. In: Persistent Viruses, CJ Stevens, GJ Todaro, CF Fox (eds), New York: Academic Press, p615-633, 1978.
39. Gatti, JM, RA Good: The immunological deficiency diseases. Medical Clinics of North America 54: 281-307, 1970.
40. Burnet, FM: Measles as an index of immunological function. Lancet ii: 610-613, 1968.
41. Huang, AS: Viral pathogenesis and molecular biology. Bacteriological Reviews 41: 811-821, 1977.
42. Joseph, BS, MBA Oldstone: Immunologic injury in measles virus infection. II. Suppression of immune injury through antigenic modulation. Journal of Experimental Medicine 142: 684-876, 1975.

MEASLES VIRUS-HOST CELL RELATIONSHIPS IN SUBACUTE SCLEROSING PANENCEPHALITIS

K. B. FRASER

SUMMARY

In SSPE measles virus is present as a persistent infection showing varying degrees of incomplete maturation. By manipulation measles virus can be made to display all the biological behaviour of SSPE virus including rapid adaptation to the cell - associated state. The possible origins of the persistence of measles virus are physiological, genetic, infectious or immunological. Animal experiments point to a failure of cell - mediated immunity in the presence of adequate humoral immunity as the most favourable circumstances for chronic infection to begin. This state of affairs could arise from immaturity or from immunosuppression by virus infection.

INTRODUCTION

Subacute sclerosing panencephalitis (SSPE) is a rare and late sequel of measles. By the time that the illness has begun, the numerous infected cells in the brain have overwhelmed or bypassed the immune clearing system much in the same way as a palpable tumour has got beyond the control of a defence which would normally be capable of disposing of a few discrete malignant cells. The immunological relationships are probably equally complex in the late stages of the two illnesses, but the specific immune reactions against measles virus are said to be largely intact at the beginning of the subacute sclerosing panencephalitis (1).

Distemper virus has been suspected on epidemiological grounds (2, 3) as a cause of SSPE alternative to or additional to measles virus but an early and important contribution by Connolly (4) (Table 1) rules out canine distemper as the characteristic antigen in brain during SSPE. The serological tracers demonstrate clearly that, if distemper virus plays any part in the aetiology of SSPE, either independently or in combination with measles virus, at least it is not expressed as fluorescent - staining antigen in the central nervous system.

Table 1.

IMMUNOFLUORESCENCE OF SSPE BRAIN, DISTEMPER BRAIN AND VIRUS - INFECTED CELLS

	FITC - Measles Antisera		FITC - Distemper Antiserum
	Patient	Goat	Horse
SSPE Brain	++	++	-
Healthy Control Brain	-	-	-
Distemper Brain	++	+	++
Control Dog Brain	-	-	-
Measles Infected Cells	++	++	-
Uninfected Cells	-	-	-
Distemper Infected Cells	++	+	++
Uninfected Cells	-	-	-

+ etc. = Intensity of fluorescence. Adapted from Connolly, 1968.

Other viruses have been suspected of being associated with SSPE. Electron microscopy has occasionally demonstrated papova virus - like particles in SSPE brain (5) and herpes - virus - like particles in measles encephalitis (6) giving rise to the suggestion that SSPE is dependent on double infection, but so far only antibody to Epstein Barr virus of the herpes virus group has been reported in addition to measles virus antibody as being significantly associated with SSPE.

Table 2.

NUMBER OF SSPE PATIENTS WITH EBV ANTIBODY

Present In SSPE	Present In Non - SSPE	Ref.
8/8	36/88	43
$35/45^{1}$	39/54	25
$19/41^{2}$	0/20	
$16/16^{1}$	123/140	*
$15/16^{3}$	N.D.	

1 = VCA; 2 = EA; 3 = EBNA - specific antibody; N.D. = not done;
* = Haire & Connolly unpublished.

All patients out of 8 tested had it (7) on one survey and titres are said to be raised above average in another (8) but as antibody to this virus is raised during measles (9) and as both viruses can inhabit lymphocytes, there may be some other explanation for the association than double infection of brain. Our figures in Belfast, showing 15/16 patients with more than one antibody to EB virus

components support these findings (Table 2) (Haire and Connolly, unpublished). Many more data are needed to substantiate these claims and to assess their significance. It seems unlikely that simultaneous double infection by measles virus and another different virus is the explanation for chronic encephalitis.

A PERSISTENT AND DEFECTIVE INFECTION

Persistence - Measles virus or variants of it have now been recovered frequently from SSPE brain. When the presence of measles virus in SSPE became known, the interest of virologists was directed to Rustigian's much neglected contribution to virology. He had shown that measles virus could readily enter into a perpetuating, non - cytocidal infection in human cell culture. Adding measles antibody to the medium not only helped to stabilise the infected culture, but also helped to establish a replicative cycle which made antigen but produced no or very little, infectious virus (10, 11). At that time few virologists were in a position to think of incomplete growth cycles in terms of a limited number of virus structural proteins. In Rustigian's system transmission of infection was vertical at cell division without evidence of spread by cell - fusion. This could not apply to infected neurones, which do not divide.

The next important improvement in our understanding of the pathogenesis of SSPE was the discovery that free measles virus was rarely present *in vivo*, but that culture of live brain cells or co - cultivation with a species of cell susceptible to measles virus regularly showed cell - associated measles virus antigen with or without giant - cell formation and from these cultures free measles virus could sometimes be recovered (12, 13, 14).

Table 3.

SSPE VIRUSES
VARIATION IN STAGE OF RECOVERY OF HAEMAGGLUTININ

Patient	Pass	HA	Ref.
1	1 and 2	+	(12)
2	7	+	
3	16	+	(13)
4	200	0	(18)*
5	55	0	(19)
6	Repeated co-cultivation	0	(20)

+ = recovered; 0 = not recovered at any time; * = quoted by (20).

It was apparent from the beginning that the replication of measles virus was under some form of physiological control from which it took different lengths of time or different numbers of passage in tissue culture before the virus was released to give anything like normal laboratory behaviour (Table 3). This recovery took place long after any immunological inhibition present in the starting material would have been diluted out in culture. The properties of stable SSPE viruses varied as much as do those of laboratory strains of standard measles virus and the existence of a specific SSPE type of measles virus seemed unlikely (15, 16). Later, cultures were recovered which alternated between lytic and non - lytic cycles of infection (17) and several have also been reported as being permanently non - virus - yielding (Table 3) but nevertheless function as cytopathogenic infectious centres and cause virulent encephalitis *in vivo* (18,19,20).

A Defect of Maturation - The nature of the non - productive state has been best revealed by electron microscopy. It is a blocking of maturation at various stages after the synthesis of virus nucleocapsids even in the presence of structural proteins of the virus. There is one reported instance of a culture in which all but 2 structural polypeptides of the measles virus are absent (21) and at the other extreme another showing apparently complete, but non - infectious, virions (22). Normally at the peak of the normal virus growth cycle rough nucleocapsid tubules of measles virus are abundant and generally distributed in the cytoplasm with a noticeable tendency to be aligned at the inner surface of the plasma membrane where spikes of haemagglutinin are beginning to appear. Budding virions are numerous at or near the cell surface. In SSPE and in persistent defective cultures there is little budding and particles are spikeless or devoid of a proper complement of nucleo - capsid. In the cytoplasm nucleocapsid molecules often remain aggregated and are not associated with the sub - membranal surface (23, 24, 25, 26). Reorganisation of nucleocapsid and migration to the sub - membranal area may begin as soon as permissive cells fuse with carrier cells (27). These morphological points are very similar to stable steady - state infections where concentration of antigen in aggregates in the cytoplasm and diminution of virus antigens at the cell surface are characteristic (28, 29). The growth of measles virus in the carrier state is often temperature - sensitive (30, 31, 32).

Following these observations and considerations the virologist is faced with five main questions: (1) Why is the disease rare? (2) What happens initially to start off the persistent virus - cell relationship? (3) What makes the cycle defective? (4) How does defective virus spread from cell to cell in the presence of antibody? (5) Why does cell-mediated immunity not eliminate infected nerve cells? The answers will always depend on reasonable speculation, proof being impossible, but during the last few years much has been discovered about measles virus and its immunology which makes the various possibilities actual rather than theoretical.

GENESIS OF PERSISTENT INFECTION

Epidemiology - Patients who get SSPE have no stigmata of immune deficiency before the onset of illness such as has been associated with giant cell pneumonia (33) so we must conclude that chance or some inate unrealised condition or some unobserved environmental event determines the difference between normal recovery from childhood measles and the origin of a chronic submerged process which surfaces at an average of five years (2 to 20 years) later. Post - vaccine SSPE has a somewhat shorter incubation period, about 3 years (34). In view of the solid immunity that follows measles, SSPE is not likely to be a second measles infection unless strains of SSPE virus are proven to be serologically different from measles. Neither epidemic clustering nor studies of SSPE in twins support the idea of a specially neurotropic strain of SSPE measles virus nor of an inborn defect of resistance to measles virus but point rather to some environmental influence (35).

The one significant piece of epidemiological evidence about which all observers are agreed is that half the patients who get SSPE had measles below the age of 2 years, well below the modal age of infection for their own social group (34, 36). There is a predominance of male patients, but the reason for it is not known.

Physiological Chance - The chance of SSPE beginning may be entirely dependent on a virus - cell relationship and may be physiological or genetic. Persistent infection may be as natural a mode of existence for measles virus as lytic or cytocidal infection. Given alternative pathways of replication in identical cells and in constant environmental conditions, one would expect a virus to have a definite probability, as does lysogenic bacteriophage, of taking one way or the

other. This probability will vary with the strain of host cell. There are no measurements of such a probability with measles virus, but alternative pathways of lysis or persistence certainly exist.

Table 4. GROWTH OF MEASLES VIRUS IN BRAIN CELL CULTURE

Species	Cell Culture	Measles Virus	SSPE Virus	Type of Growth	Ref.
Man	Astrocyte	+	N.D.	Persistent induced	(37)
Man	Astrocytoma	+	N.D.	Persistent induced	
Rat	Schwannoma	+	+	Persistent[1] immediate (ts)	(38)
Rat	Astrocytoma	+	+	Persistent[1] immediate (ts)	

Induced = by selection of survivors of lysed culture;
ts = temperature sensitive replication; 1 = host - controlled.

Brain cells are known to support virus growth readily without lethal results and measles virus is no exception (Table 4). Different lines of brain cells respond differently (37, 38) and persistent infection may be host - controlled and temperature - sensitive (38). Since there is indirect evidence from electroencephalograms that measles virus probably invades the brain of half the children who get measles (39) the opportunities for persistent infection to arise must be many.

Genetic Chance - The other sort of finite chance is mutation and it will apply only if mutants of measles virus exist which normally cause persistent infection. Proof requires plaque purification and that rules out the investigation of defective mutants. Accordingly, some workers have sought for and recovered variants from measles and SSPE viruses which grow poorly *in vitro* and which make plaques of widely different appearance (40) (Table 5). No constant link with SSPE has been demonstrated but it is possible that strains showing a combination of two markers, poor replication and good syncytial formation tend to induce chronic encephalitis readily. Thormar makes an excellent case for syncytial formation in cultured brain cells and non - productive replication being linked to encephalitogenic potency (Table 6) but he himself points out that encephalitic producer strains may be made apparently non - neurotropic as a consequence of rapidly inducing local concentrations of antibody in the central nervous system (41).

Table 5. PLAQUE VARIANTS OF MEASLES VIRUS

Plaque Type	Yield of Virus pfu	Encephalitis in Hamster		Ref.
Measles				
Small	$10^{5.0}$	13/59	(22)	(40)
Large	$5 \times 10^{3.0}$	33/69	(47)	
Small	$7 \times 10^{3.0}$	0/38	(0)	
SSPE-1				
Small	$5 \times 10^{7.0*}$	12/12	(100)	(+)
Large	$1 \times 10^{4.0}$	1/10	(10)	
Points focus	$5 \times 10^{5.0}$	0/10	(0)	

* = assayed by fluorescent antibody; 1 pfu = 1 focus forming unit; + = Gould et al unpublished.

Table 6. SYNCYTIAL FORMATION IN VITRO AND ENCEPHALITIS

Measles-Virus		HAD	Virions (EM)	Syncytia in Vitro (FB)	Encephalitis in Ferret
Wild types	1	+	+	-	-
	2	+	+	-	-
SSPE	1	+	+	-	-
	2	+	+	-	-
	3	+	+	-	-
SSPE	4	+	-	+	+
	5	±	-	+	+
	6	-	-	+	+
	7	-	-	++	++

HAD = haemadsorption; FB = Ferret brain culture (modified from 41).

One unusual feature of two of Gould's variants recovered from a well known strain of SSPE virus (SSPE 1. Horta - Barbosa) is their restricted growth in the absence of methionine which they do not incorporate adequately into virus protein (Fig. 1). How this may be related to defective growth or to intra - cerebral habitat has not yet been investigated, but it is of consequence that the small focus - former is almost undetectable without fluorescent antibody assay and may have been missed in many recorded, non - producing SSPE strains. The same SSPE virus also yielded a productive variant which did incorporate methionine as well as laboratory strains of wild type

virus did. Thus, non - cytocidal variants of measles do exist, can cause encephalitis and, if present, would be expected, even more than wild type virus, to set up persistent infection in the human brain.

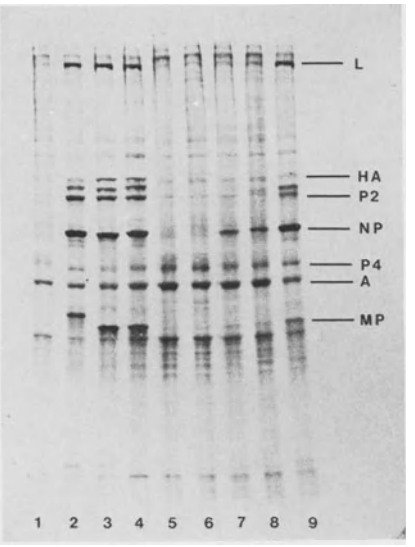


Fig. 1. SDS - PAGE analysis of ^{35}S methionine labelled measles and SSPE virus variants. 1. Uninfected Vero cells. 2. Human measles isolate. 3. Edmonston virus. 4, 5 and 6. Small plaque, Foci - producing and large plaque variants, respectively, of SSPE - 1 virus isolated by Horta - Barbosa. 7, 8 and 9. The dilute large plaque, undilute large plaque and small plaque variants of Edmonston virus described by Chiarini et al (40). HA - haemagglutinin, NP - nucleoprotein, A - actin and MP - membrane protein.

Metabolic Abnormality - The inhibition of maturation of SSPE viruses could be produced by a control of cell metabolism, for the production of infectious measles is recognised to require some unknown function of the cell nucleus (42). Cell fusion is very active in early isolates of SSPE viruses so fusion factor, which is probably virus

haemolysin, must be an important constituent or product of SSPE defective virus strains. It is interesting therefore that when Shirodaria (43) preferentially inhibited the synthesis of measles virus haemolysin by using low concentrations of glucose analogues, either glucosamine or 2 deoxy D glucose (Fig. 2) he and Dr. Dermott observed that maturation of virus ceased and nucleocapsids remained aggregated in the cytoplasm instead of lying adjacent to the differentiating cell membrane. They have also shown that removal of the chemical inhibitor was followed by disaggregation of nucleocapsid clusters and rapid resumption of maturation within 2 hr. (Fig. 3). It cannot be decided yet whether maturation is prevented by lack of haemolysin or whether migration of components in the cell and protein synthesis were both indirectly inhibited by loss of some other metabolic pathway, but the general picture is very like that in replication by defective SSPE measles virus.

Multiple Infections (DI Particles) - It has been shown that most persistent virus infections are associated with large numbers of particles that contain incomplete amounts of RNA and do much to help the establishment of non - cytocidal infection (44, 45). With measles also there is good evidence that a susceptible cell - culture can be infected non - cytocidally by measles virus prepared by undiluted passage (46) and so carrying a high proportion of these DI particles (47). Even after 93 passes, Rima's culture contained no defective or temperature-sensitive virus, which indicates that noncytocidal (46) infection is not dependent on the appearance of poorly - growing or ts variants like those recovered from SSPE virus (Table 5). If this mechanism of interference is responsible for initiating persistent infection in SSPE, some system of multiple infection of brain cells must be postulated. Measles spreads in the patient in mononuclear leukocytes in which it can replicate (48, 49) so that inter - cellular transmission from leukocyte to brain cell is likely to bring in a high multiplicity of genomes. Alternatively, as suggested by Martin (16) the measles virus itself could be polyploid and so produce the same polygenic infection as a virus - loaded white blood cell.

Antigenic Modulation - Persistent infection and defective growth of the virus are both accelerated by the presence of measles virus - specific antibody in the culture medium (10, 11). Recently the consequences of antibody uniting with virus antigen at the cell surface

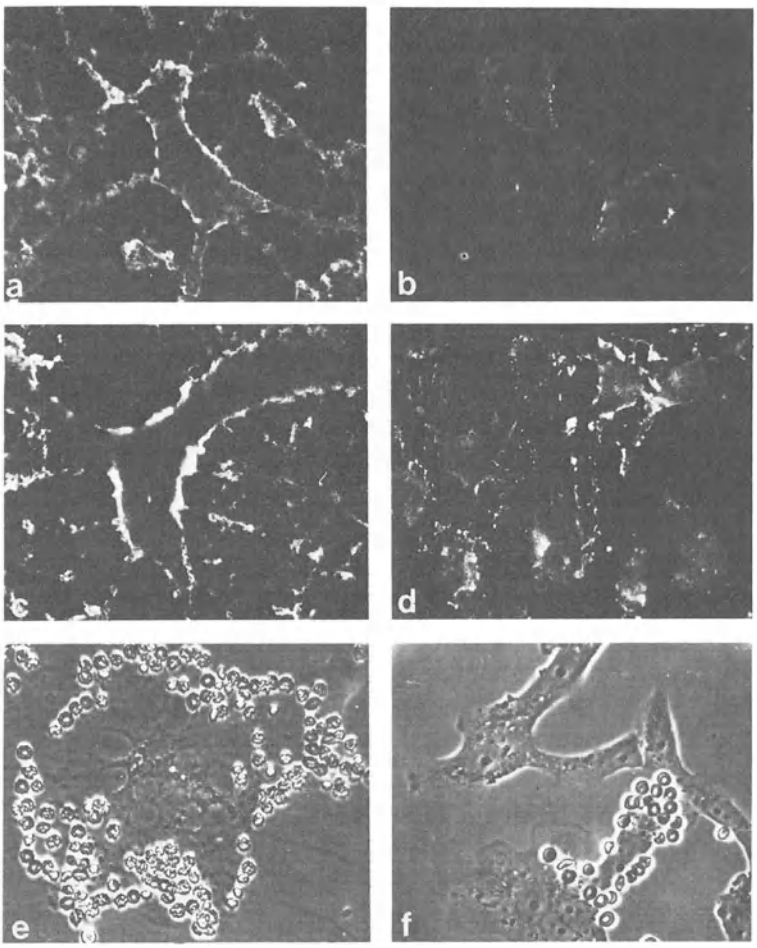


Fig. 2. Immunofluorescence of and haemadsorption by measles virus antigens in unfixed Vero cells infected with strain TC243, incubated without (Left) and with (Right) 4mM 2-deoxy-D-glucose added at the beginning of the incubation period.
a, b - haemolysin; c, d - haemagglutinin; e, f - haemadsorption. Note the loss of antigen, especially haemolysin, loss of syncytial formation and reduction of haemadsorption in presence of 2-deoxy-D-glucose.

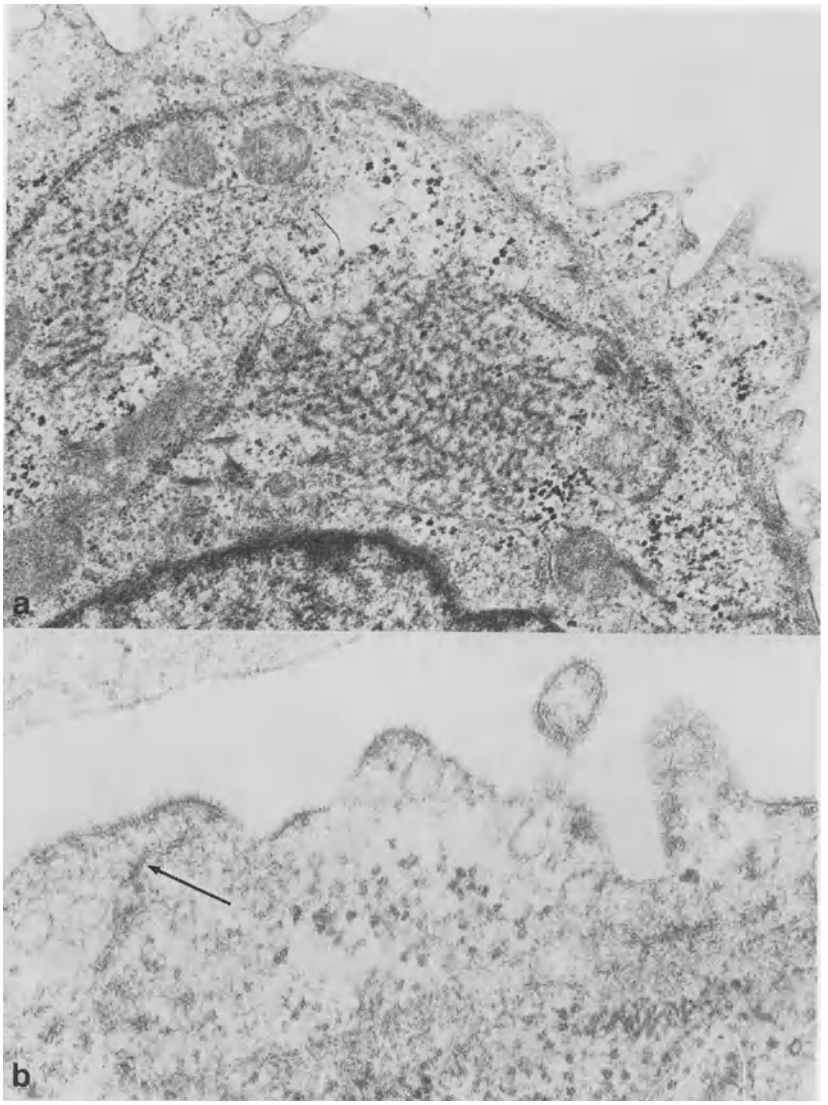


Fig. 3. (a) HEp2 cells in 10mM 2-DG 26 hr. after infection with measles virus. Cytoplasmic inclusion of rough nucleocapsids (NC); no submembranal alignment; no budding, X 40,000; (b) without 2-DG, NC migrates towards cell surface (arrow) where differentiation and budding are seen, X 90,000.

have been described, both as capping, which means moving to a restricted site on the surface of the cell (50) and stripping which leads to loss of antigen from the cell surface. Such antigenic loss or modulation can provide an explanation for incomplete maturation because the surface antigens required by the virus at budding are blocked by antibody (51) or are continually removed and unused nucleocapsid will build up within the cell. The process has also been credited with protection of infected cells which, being stripped of virus antigen, are not subject to attack by measles virus - specific immune processes (52). It should be remembered, however, that reduction of surface antigen ensues in many persistent infections without the intervention of antibody.

So, in addition to inherited virus traits, there are two other mechanisms which result physiologically in the establishment of a persistent infection by measles virus, either multiple infection with D.I. particles or antigen modulation. The latter method does not explain how defectiveness persists after antiserum has been removed from the system; the former method is self - perpetuating because D.I. particles are replicated by each infected cell. It is difficult to explain how either method should select SSPE virus which retains its ability to spread and to cause cell death *in vivo*. The formation of syncytia by SSPE carrier cells *in vitro* is inhibited by measles antiserum. Anatomy should not be neglected. It may be that SSPE virus is selected in situ by its ability to spread entirely through synapses as suggested by patterns of fluorescent antibody staining (53, 21) and the immune system does deal capably with all virus antigens at exposed sites.

ANIMAL EXPERIMENTS AND IMMUNOLOGICAL CONTROL

Animal experiments provide information about multi - cellular relationships. They have been used to show that SSPE viruses can cause chronic encephalitis which is histologically similar to SSPE; they have shown that standard strains of measles virus can in certain circumstances initiate chronic encephalitis remarkably similar to SSPE (Table 7). Most important of all, they have revealed that the onset and course of measles virus - induced encephalitis are firmly controlled by the immune state of the host. (Table 8).

Extra cellular SSPE virus is hardly ever neuro - virulent for adult animals after intra - cerebral inoculation (54). By far the most reliable method of inducing encephalitis in all species of

laboratory animal is to inoculate cell - associated virus in living cells (55, 56, 57). This finding is in accord with the cell - fusing properties of isolated SSPE strains and fusion has actually been demonstrated *in vivo* both in animals and man (58, 59). Measles specific - antibody is not always formed after such inoculation even when encephalitis has followed (60) which indicates that measles - specific antibody is not the mediator for the production of chronic encephalitis nor for its symptoms. Nevertheless the time - course of infection does depend on the virus - immune state as seen, for example, by inoculating neuro - adapted SSPE virus into rhesus monkeys in whom immunity was suppressed by cyclophosphamide (61).

Table 7.

ENCEPHALITIS IN HAMSTERS
SSPE AND MEASLES VIRUSES

Virus	Type of Illness	Inclusion Seen	Ref.
SSPE	Acute	+	(75)
SSPE	Acute	+	(76)
SSPE	Chronic	+	(64)
Vaccine Measles	Chronic	-	(77)
Measles Carrier Cells	Chronic	+	(78)

Table 8.

EFFECT OF IMMUNE STATE ON EXPERIMENTAL
ENCEPHALITIS INDUCED BY MEASLES VIRUS

Immunosuppressed	Virus	Host	Result	Ref.
Cyclophosphamide	Measles Vaccine HNT	Monkey	Susceptible[1] to HNT only	(61)
Cyclophosphamide	Measles Vaccine	Hamster	Reactivation	(77)
Anti-Lymphocyte	SSPE - HBS	Hamster	Susceptible[1]	(62)
Anti-Thymocyte Serum	SSPE - LEC	Hamster	Susceptible[1]	(66)
Neonatal Thymectomy	SSPE - HBS	Hamster	Susceptible[1]	(65)
Hypersensitized (Allergic)	Measles Neurotropic	Rat	Protective[2]	(79)

1 = compared with non - suppressed controls.
2 = compared with non - sensitized controls.

Partial immunosuppression by anti - lymphocyte serum can convert a non - lethal infection with SSPE virus into a lethal one (62) and the morphogenesis of the virus in chronic infection of weanling hamsters is defective as compared with complete maturation in the acute infection of newborn animals (63). Age of the host has an influence on the chronicity of infection by SSPE virus, as it has with laboratory strains of measles virus. Infection was chronic and focal with intranuclear inclusion bodies in twelve week - old hamsters, but acute and inflamed in three week - old animals (64). The same strain of virus was more virulent in adult hamsters which had had a neonatal thymectomy (65) or which had been treated with anti - thymocyte serum (66) than it was in untreated hamsters. Further work on non - immune rhesus monkeys has shown that inoculated extra - cellular SSPE viruses was harmless, but 3 of 4 non - productive SSPE viruses produced severe acute encephalitis. Slightly neuro - adapted non - productive SSPE virus produced a chronic encephalitis in animals that were already immunized against measles (67).

Speed of adaptation to defective growth has been formally demonstrated. A fully lytic, productive and neurotropic derivative of SSPE virus could be shown to produce encephalitis in weanling hamsters. The parent virus could be recovered from inoculated brains up to about the eighth day after inoculation when measles virus - specific antibody began to appear. Thereafter the only means of demonstrating virus infectivity was by co - cultivation of brain cells. Although HA and HL were both inserted into the cell membrane, no infectious virus could be released by freezing and thawing. Adaptation to defectiveness had occurred as early as 8 days after inoculation (68).

Intra - cerebral inoculation is a highly artificial procedure but encephalitis has been shown to follow parenteral inoculation - intraperitoneal in the hamster (69) and intramuscular, using a non - producer strain, in the monkey (70). The last experiments justify our speculation about transmission of infection at high multiplicity by means of leukocytes (p.) since the SSPE virus used could not travel extra - cellularly from muscle to brain.

The slow progress of SSPE may have other causes than immunological constraint. Encephalitis due to laboratory strains of measles virus is greatly reduced in virulence as age of the host increases (71, 53, 72). A non-SSPE, hamster - neurotropic strain of measles virus

which is rapidly restricted in its effects on mice after they are 6 to 7 days old has been found to make much virus antigen at the restrictive age although no virus components are formed; no extension beyond the primarily infected cells is possible (73). The restriction is not due to immune reactivity, to interferon production, to body temperature or to the influence of cellular proteases on the infection (74). If such processes occur in human SSPE the slow progress of virus throughout the brain would be partly explained. A small population of producer cells would gradually fill the blind alleys with non - infectious but lethal, virus antigen.

RECAPITULATION AND ARGUMENT

Subacute sclerosing encephalitis, SSPE, is assumed here to be a sequel of measles and not a reinfection, in conformity with epidemiological observations, but the virus - host relationships discussed could equally well arise as the result of a reinfection that had by - passed normal host defences. There is no evidence for this.

The nature of the encephalitis and the properties of persistent and defective infection with measles virus indicate five main problems in the pathogenesis of the disease. They are the epidemiology, the origin of persistent infection, the development of defective virus reproduction, the means of spread of non - productive but lethal measles virus in brain, and failure of elimination by the immune system.

There is reasonable evidence that the encephalitis is essentially an infection by a single virus, measles virus. There is no consistent evidence that SSPE is a continuing double intracerebral infection with measles virus and another such as distemper virus, papova viruses or herpes viruses. Coincidental but temporary infection in the brain or elsewhere cannot be ruled out as part of the aetiology.

Persistent infections by wild - type measles virus often become defective, especially with the aid of measles virus - specific antibody in the culture medium and the properties of carrier cultures and of virus recovered from them strongly resemble cell - associated and intracellular strains of SSPE virus. In both experimental and in SSPE systems defectiveness is revealed to be a failure of maturation of various degrees of incompleteness but often relieved by fusion with permissive cells.

Persistence of measles virus can arise readily and spontaneously especially in many kinds of brain cells where it is host - controlled at low multiplicity or is rather similar to artificial persistent virus cell systems induced at high multiplicity. Both kinds may show temperature - sensitive synthesis of virus.

Genetic variants of measles virus exist which have a propensity to set up non - cytocidal rather than lytic infection of cells in culture. They tend to be missed in tests for infectious virus and could act like D.I. particles in suppressing maturation of lytic virus. It is also possible to stimulate the physiology and morphology of defective infection by inhibition of normal growth cycle biochemically. There is no known counterpart in SSPE.

Antigenic modulation or stripping of measles antigen from the surface of virus - infected cells is common to experimental persistent infections and perhaps to *in vivo* 'protection' of the infected host - cell from cell - mediated immune clearance. The immune modulation mechanism does not explain why spread by fusion is not prevented *in vivo* when, in fact, antibody *in vitro* inhibits syncytial formation quite well.

Animal experiments have been invaluable in showing that encephalitic infections produced by wild type or laboratory measles viruses and by SSPE virus strains are remarkably alike. Above all they have shown that the genesis and the course of chronic measles encephalitis can be determined by the immune state of the host. This is seen in three sets of conditions: (1) Before specific active cell - mediated immunity exists but when protective antibody is present as in passive maternal immunity. (2) After specific humoral and cell - mediated immunity is present, but cell - mediated immunity is suppressed. (3) In one set of experiments only, where hyperergy to measles virus was established before infection. The first two conditions determine increased susceptibility, the third increased resistance, to encephalitis.

The importance of other factors has been discovered by experiments in animals. One is that adaptation to defectiveness occurs rapidly in the brain and is complete at the time when antibody begins to inhibit cell - to - cell spread of infection. More important still, cell - associated, syncytial - forming virus is the most potent inducer of encephalitis and excites least immune response.

Why measles virus - specific antibody of high titre cannot inhibit the spread of syncytial forming virus *in vivo*, when small amounts do so *in vitro* seems paradoxical. In all probability the facts simply mean that virus spreads through cell synapses and the comparative rarity of giant cells in SSPE means that antibody is effective whenever the virus antigen is exposed at the cell surface.

A second finding is that increasing maturity affects susceptibility of cerebral tissue to measles virus and slowness of spread - the slow virus infection - could be accounted for by the reproduction of relatively minute amounts of complete infectious virus or by the high probability of virus entering a series of blind alleys represented by cells in which virus replication is abortive.

The most significant factor in the epidemiology of SSPE is the early age at which measles had been acquired by at least half the patients. Two attributes of experimental measles encephalitis are related to this. Access to the brain could be easier in the very young or immunological competence to deal with measles virus could be less well developed. Alternatively the leucocytes of very young subjects could be more susceptible to measles virus or could produce more virus than leucocytes of older children, so subjecting the nervous system to a heavier invasion of transported virus. It will never be possible to prove whether one or more of these possible characteristics of immaturity are operating at the time when the mechanisms of SSPE are first set in motion, but they are less applicable to older cases and they do not explain the entire epidemiology of SSPE.

One factor which is environmental, applicable to the age group concerned as well as to older ages and immunologically acceptable, is coincidental infection by a virus which can produce temporary immunodepression of greater or lesser specificity for measles virus. A 'two virus' hypothesis has been favoured amongst the problems of virus - host relationships in multiple sclerosis (80) though without virological or serological supporting evidence. We have referred to the serology in SSPE of Epstein - Barr virus (82, 83) which, amongst others (81) can depress cell - mediated immunity. In the older SSPE patients who presumably have been immunologically mature, some reason for immunodepression should be sought. Coincidental virus infection would be high on the list of such causes and further work on this line of enquiry seems justified.

ACKNOWLEDGEMENTS

I am indebted to the following colleagues for discussion of their work and permission to use their data: J. Connolly, Table 1; M. Haire, Table 2; E. Gould and K. McCullough, Table 5; M. Gharpure and E. Gould, Fig. 1; P. Shirodaria, Fig. 2; and E. Dermott, Fig. 3. I thank Mr. R. Woods for photography.

REFERENCES

1. Agnarsdottir, G: Subacute sclerosing panencephalitis. In: Recent advances in clinical virology, Watson, A. (ed), London and Edinburgh, Churchill Livingstone, 1977, p21-49.

2. Brody, JA, R Detels: Subacute sclerosing pan-encephalitis: A zoonosis following aberrant measles. Lancet 2: 500-501, 1970.

3. Detels, R, JA Brody, J McNen, AH Edgar: Further epidemiological studies of subacute sclerosing panencephalitis. Lancet 2: 11-14, 1973.

4. Connolly, JH: Additional data on measles virus antibody and antigen in subacute sclerosing panencephalitis. Neurology 18: 87-89, 1968.

5. Koprowski, H, G Barbanti-Brodano, M Katz: Interaction between papova-like virus and paramyxovirus in human brain cells - a hypothesis. Nature 275: 1045-1047, 1970.

6. Dayan, AD: Encephalitis due to simultaneous infection by herpes simplex and measles viruses. J. neurol. Sci. 14: 315-323, 1971.

7. Joncas, J, G Geoffrey, B McLaughlin, G Albert, N Lapointe, P David, R Lafontaine, M Granger-Julien: Subacute sclerosing panencephalitis. Elevated Epstein-Barr virus antibody titres and failure of amantadine therapy. J. neurol. Sci. 21: 381-390, 1974.

8. Gotlieb-Stematsky, GT, L Rannon, A Vonsovera: Antibodies to Epstein-Barr virus in subacute sclerosing panencephalitis patients. Europ. Neurol. 13: 418-421, 1975.

9. Gotlieb-Stematsky, T, L Rannon, A Vonsovera, N Varsano: Stimulation of antibodies to Epstein-Barr virus (EBV) in acute viral infections. Arch. Virol. 57: 199-204, 1978.

10. Rustigian, R: A carrier state in Hela cells with measles virus (Edmonston strain) apparently associated with non-infectious virus. Virology 16: 101-104, 1962.

11. Rustigian, R: Persistent infection of cells in culture by measles virus. II. Effect of measles antibody on persistently infected Hela sublines and recovery of a Hela clonal line persistently infected with incomplete virus. J. Bact. 92: 1805-1811, 1966.

12. Horta-Barbosa, L, DA Fuccillo, JL Sever, W Zeman: Subacute sclerosing panencephalitis : Isolation of measles virus from a brain biopsy. Nature (London), 221: 974, 1969.

13. Payne, FE, JV Baublis, HH Itabashi: Isolation of measles virus from cell cultures of brain from a patient with subacute sclerosing panencephalitis. New Eng. J. Med. 281: 585-589, 1969.

14. Degré, M, B Vandvik, T Hovig: Subacute sclerosing panencephalitis : Isolation and ultra-structural characterization of a measles-like virus from brain obtained at autopsy. Acta Path et. Microbiologica Scand. Section B. 80: 713-728, 1972.

15. Hamilton, R, L Barbosa, M Dubois: Subacute sclerosing panencephalitis measles virus : study of biological markers. J. virol. 12: 632-642, 1973.

16. Fraser, KB, SM Martin: Measles virus and its biology, London, Academic Press, 1977.

17. Burnstein, T, LB Jacobsen, N Zeman, T Tsu Chen: Persistent infection of BSC-1 cells by defective measles virus derived from subacute sclerosing panencephalitis. Infect. Immun. 10: 1378-1382, 1974.

18. Doi, Y, T Sampe, M Nakajima, S Okawa, T Katoh, H Itoh, T Sato, K Oguchi, T Kumanschi, T Tsubaki: Properties of a cytopathic agent isolated from a patient with subacute sclerosing panencephalitis in Japan. Jap. J. Med. Sci. Biol. 25: 321-333, 1972.

19. Makino, S, K Sasaki, M Nakagawa et al: Isolation and biological characterization of a measles virus-like agent from the brain of an autopsied case of subacute sclerosing panencephalitis. Jap. Microbiol. Immunol. 21: 193-205, 1977.

20. Mirchamsy, H, S Bahrami, A Shafyi, MS Shahrabady, M Kamaly, P Ahourai, J Razavi, P Nazari, T Derakshan, J Lotfi, K Abassioun: Isolation and characterization of a defective measles virus from brain biopsies of the patients in Iran with subacute sclerosis panencephalitis. Intervirology 9: 106-118, 1978.

21. Albrecht, P: Immune control in experimental subacute sclerosing panencephalitis. Amer. J. clin. Path. Suppl. to Vol. 70 (1): 175-184, 1978.

22. Kratsch, V, WW Hall, K Nagashima, V ter Meulen: Biological and biochemical characterization of a latent subacute sclerosing panencephalitis (SSPE) virus in tissue culture. J. med. virol. 1: 139-154, 1977.

23. Oyanagi, S, V ter Meulen, M Katz, H Koprowski: Comparison of subacute sclerosing panencephalitis and measles viruses: an electron microscope study. J. Virol. 7: 176-187, 1971.

24. Raine, CS, LA Feldman, RD Sheppard, LH Barbosa, MB Burnstein: Subacute sclerosing panencephalitis virus. Observations on a neuroadapted and non-neuroadapted strain in organotypic central nervous system cultures. Lab. Invest. 31: 42-53, 1974.

25. Dubois-Dalcq, M, K Worthington, O Gutenson, LH Barbosa: Immunoperoxidase labeling of subacute sclerosing panencephalitis virus in hamster acute encephalitis. Lab. Invest. 32: 518-526, 1975.

26. Dubois-Dalcq, M, TS Reese, M Murphy, D Fuccillo: Defective bud formation in human cells chronically infected with subacute sclerosing panencephalitis virus. J. Virol. 19: 579-593, 1976.

27. Knight, PR, RG Duff, R Glaser, F Rapp: Characteristics of the release of measles virus from latently infected cells after co-cultivation with BSC-1 cells. Intervirology 2: 287-298, 1973.

28. Walker, DL: The viral carrier state in animal cultures. Progr. med. Virol. 6: 111-148, 1964.

29. Fraser, KB: Defective and delayed myxovirus infections. Brit. Med. Bull. 23: 178-184, 1967.

30. Haspel, MV, PR Knight, RG Duff, F Rapp: Activation of a latent measles virus infection in hamster cells. J. Virol. 12: 690-695, 1973.

31. Gould, EA, PE Linton: The production of a temperature-sensitive persistent measles virus infection. J. gen. Virol. 28: 21-28, 1975.

32. Armen, RC, JF Evermann, AL Truant, LA Laughlin, JV Hallum: Temperature sensitive mutants of measles virus produced from persistently infected Hela cells. Arch. Virol. 53: 121-132, 1977.

33. Enders, JF, K McCarthy, A Mitus, WJ Cheatham: Isolation of measles virus at autopsy in cases of giant cell pneumonia without rash. New Engl. J. Med. 261: 875-881, 1959.

34. Modlin, JF JT Jabbour, JJ Witte, NA Halsey: Epidemiological studies of measles, measles vaccine, and subacute sclerosing panencephalitis. Pediatrics 59: 505-512, 1977.

35. Ch'ien, LT, WH Wilborn, JH Carey, R Ceballos, JW Benton, CA Alford: The simultaneous occurrence of subacute sclerosing panencephalitis in two brothers. I. clinical virologic and histopathologic studies. J. Infect. Dis. 125: 173-178, 1972.

36. Jabbour, JT, DA Duenas, JL Sever, HM Krebs, L Horta-Barbosa: Epidemiology of subacute sclerosing panencephalitis (SSPE). A report of the SSPE registry. J A M A 220: 959-962, 1972.

37. Macintyre, EH, JA Armstrong: Fine structural changes in human astrocyte carrier lines for measles virus. Nature 263: 232-234, 1976.

38. Lucas, A, M Coulter, R Anderson, S Dales, W Flintoff: In Vivo and In Vitro models of demyelinating diseases. II. Persistence and host-regulated thermosensitivity in cells of neural derivation infected with mouse hepatitis and measles viruses. Virology 88: 325-337, 1978.

39. Gibbs, FA, EL Gibbs, PR Carpenter, HW Spies: Electroencephalographic abnormality in 'uncomplicated' childhood diseases. J A M A 171: 1050-1055, 1959.

40. Chiarini, A, A Sinatra, P Ammatuna, R Distefano: Studies on a measles virus variant inducing persistent infections in cultured cells. I. Isolation and characterization of plaque purified virus clone. Arch. Virol. 52: 47-58, 1976.

41. Thormar, H, PD Mehta, HR Brown: Comparison of wild-type and subacute sclerosing panencephalitis strains of measles virus, Neurovirulence in ferrets and biological properties in cell cultures. J. exp. Med. 148: 674-691, 1978.

42. Follett, EAC, CR Pringle, TH Pennington, P Shirodaria: Events following the infection of enucleate cells with measles virus. J. gen. Virol. 32: 163-175, 1976.

43. Shirodaria, PV: In: Measles virus and its biology, Fraser and Martin (eds), London, Academic Press, 1977, p61.

44. Huang, AS, D Baltimore: Defective viral particles and viral disease processes. Nature (London) 226: 325-327, 1970.

45. Holland, JJ, LP Villarreal: Persistent non-cytocidal vesicular Stomatitis virus infections mediated by defective T particles that suppress virion transcriptase. Proc. nat. Acad. Sci. USA. 71: 2956-2960, 1974.

46. Rima, B, B Davidson, SJ Martin: The role of defective interfering particles in persistent infections of Vero cells by measles virus. J. gen. Virol. 35: 89-97, 1976.

47. Hall, WW, SJ Martin, E Gould: Defective interfering particles produced during the replication of measles virus. Med. Microbiol. Immunol. 160: 155-164, 1974.

48. Osunkoya, BO, AR Cooke, O Ayeni, TA Adejumo: Studies on leukocyte cultures in measles: I. Lymphocyte transformation and giant cell formation in leukocyte cultures from clinical cases of measles. Arch. Ges. Virusforsch. 44: 313-322, 1974.

49. Osunkoya, BO, GI Adeleye, TA Adejumo, LS Slimonu: Studies on leukocyte cultures in measles. II. Detection of measles virus antigen in human leukocytes. Arch. Ges. Virusforsch. 44: 323-329, 1974.

50. Joseph, BS, MBA Oldstone: Antibody-induced redistribution of measles antigen on the cell surface. J. Immunol. 113: 1705-1709, 1974.

51. Johnson, KP, P Swoveland: Measles antigen distribution in brains of chronically infected hamsters: An immunoperoxidase study of experimental subacute sclerosing panencephalitis. Lab. Invest. 37: 459-465, 1977.

52. Joseph, BS, MBA Oldstone: Immunologic injury in measles virus infection. II. Suppression of immune injury through antigenic modulation. J. exp. Med. 142: 864-876, 1975.

53. Janda, Z, E Norrby, H Marusyk: Neurotroprism of measles virus variants in hamsters. J. Infect. Dis. 124: 553-564, 1971.

54. Katz, M, H Koprowski: The significance of failure to isolate infectious viruses in cases of subacute sclerosing panencephalitis. Arch. Ges. Virusforsch. 41: 390-393, 1973.

55. Tijl, WFJ, SLH Notermans, M Katz, V ter Meulen, H Koprowsky: SSPE virus encephalitis produced experimentally in young dogs. Clinical and electro-encephalographic aspects. In: Proc. 13th Int. Congress Pediatrics Vienna, 3: 425-429, 1971.

56. Thein, P, A Mayr, V ter Meulen, H Koprowski, MY Kackell, D Müller, R Meyermann: Subacute sclerosing panencephalitis: Transmission of the virus to calves and lambs. Arch. neurol. 27: 540-548, 1972.

57. Notermans, SLH, WFJ Tijl, FTC Willems, JL Sloof: Experimentally-induced subacute sclerosing panencephalitis in young dogs. Neurology 23: 543-553, 1973.

58. Raine, CS, LA Feldman, RD Sheppard, MB Bornstein: Subacute sclerosing panencephalitis virus in cultures of organised central nervous tissue. Lab. Invest. 28: 627-640, 1973.

59. Iwaski, Y, H Koprowski: Cell to cell transmission of virus in the central nervous system. Lab. Invest. 31: 187-196, 1974.

60. ter Meulen, V, M Katz, YM Kackell: Properties of SSPE virus: tissue culture and animal studies. Ann. clin. Res. 5: 293-297, 1973.

61. Albrecht, P, AL Shabo, GC Barns, NM Tauraso: Experimental measles encephalitis in normal and cylophosphamide treated monkey. J. infect. Dis. 126: 154-161, 1972.

62. Byington, DP, KP Johnson: Subacute sclerosing panencephalitis virus in immunosuppressed adult hamsters. Lab. Invest. 32: 91-97, 1975.

63. Raine, CS, DP Byington, KP Johnson: Subacute sclerosing panencephalitis in the hamster: Illustrations of the acute disease in newborns and weanlings. Lab. Invest. 33: 108-116, 1975.

64. Byington, DP, KP Johnson: Experimental subacute sclerosing panencephalitis in the hamster: correlation of age with chronic inclusion cell encephalitis. J. infect. Dis. 126: 18-26. 1972.

65. Johnson, KP, EG Feldman, DP Byington: Effect of neonatal thymectomy on experimental subacute sclerosing panencephalitis in adult hamsters. Infect. Immun. 12: 1464-1469, 1975.

66. Kibler, R, NE Cremer: Anti-thymocyte treatment of hamsters inoculated with a measles virus extracted from a patient with subacute sclerosing panencephalitis. Immunol. Commun. 2: 303-321, 1973.

67. Albrecht, P, T Burnstein, MJ Klutch, JT Hicks, FA Ennis: Subacute sclerosing panencephalitis: experimental infection in primates. Science 195: 64-66, 1977.

68. Johnson, KP, E Norrby: Subacute sclerosing panencephalitis (SSPE) agent in hamsters. III. Induction of defective measles infection in hamster brain. Exp. mol. Path. 21: 166-178, 1974.

69. Carrigan, DR, RR McKendall, KP Johnson: CNS disease following dissemination of SSPE measles virus from intraperitoneal inoculation of suckling hamsters. J. med. Virol. 2: 347-357, 1978.

70. Ueda, S, T Otsuka, Y Okuno: Experimental subacute sclerosing panencephalitis (SSPE) in a monkey by subcutaneous inoculation with a defective SSPE virus. Biken J. 3: 179-181, 1975.

71. Waksman, BH, T Burnstein, RD Adams: Histologic study of the encephalomyelitis produced in hamsters by a neurotropic strain of measles. J. Neuropath. exp. Neurol. 21: 25-49, 1962.

72. Griffin, DE, J Mullinix, O Narayan, RT Johnson: Age dependence of viral expression: Comparative pathogenesis of two rodent-adapted strains of measles virus in mice. Infect. Immun. 9: 690-695, 1974.

73. Herndon, RM, L Rena-Descalzi, DE Griffin, PK Coyle: Age dependence of viral expression. Electron microscopic and immunoperoxidase studies of measles virus replication in mice. Lab. Invest. 33: 544-553, 1975.

74. Roos, RP, DE Griffin, RT Johnson: Determinants of measles virus (Hamster neurotropic strain) replication in mouse brain. J. infect. Dis. 137: 722-727, 1978.

75. Johnson, KP, DP Byington: Subacute sclerosis panencephalitis (SSPE) agent in hamsters. I. Acute giant cell encephalitis in newborn animals. Exp. Mol. Path. 15: 373-379, 1971.

76. Albrecht, P, HP Schumacher: Neurotropic properties of measles virus in hamsters and mice. J. infect. Dis. 124: 86-93, 1971.

77. Wear, DJ, F Rapp: Latent measles virus infection of the hamster central nervous system. J. Immunol. 107: 1593-1598, 1971.

78. Norrby, E, K Kristensson: Subacute encephalitis and hydrocephalus in hamsters caused by measles virus from persistently infected cell cultures. J. Med. Virol. 2: 305-317, 1978.

79. Schumacher, HP, P Albrecht, NM Tauroso: The effect of altered immune reactivity on experimental measles encephalitis in rats. Arch. Ges. Virusforsch. 37: 218-229, 1972.

80. Maugh, TH: Multiple sclerosis: two or more viruses may be involved. Science 195: 768-771, 1977.

81. Notkins, AL, SE Mergenhagen, RJ Howard: Effect of virus infections on the function of the immune system. Ann. Rev. Microbiol. 24: 525-538, 1970.

82. Haider, S, M de L Coutinho, RTD Edmond, RNP Sutton: Tuberculin anergy and infectious mononucleosis. Lancet 2: 74, 1973.

83. Mangi, RJ, MD Niederman, JE Kelleher, JM Dwyer, AS Evans, FS Kantor: Depression of cell-mediated immunity during acute infectious mononucleosis. New Eng. J. Med. 291: 1149-1153, 1974.

DISCUSSIONS OF PAPERS BY V. TER MEULEN AND K. B. FRASER

This discussion opened with some consideration of the role of the virus matrix protein. Attention then shifted to the epidemiology of SSPE. The high incidence in parts of the Middle East, particularly Turkey, was alluded to. The apparent absence of SSPE in Nigeria, where measles is common in early life, was disputed; Dr Cathala recalled that SSPE was, in fact, common in Nigeria where measles epidemics were followed by waves of SSPE. Although SSPE may occur in Kenya and Nigeria, there thus remained some doubt as to whether it was diagnosed as such. Probably, the very high figures quoted from some countries, (e.g. Turkey, Czechoslovakia and Hungary) related to the personal interest of the physician responsible for reporting. The data from the U.S.A. were regarded by Dr Lachmann as incredible and he was sceptical of the apparent increased prevalence in rural areas.

There was little protection by maternal antibody and it might well be that children who developed SSPE had an abnormal immunological response. The activation of other viruses and the development of antibodies to herpes simplex and mumps viruses in the CSF of patients with SSPE might indicate this. In the context of altered immunological surveillance it was mentioned that cell-mediated immunity is commonly depressed in measles.

CANINE DISTEMPER ENCEPHALITIS

E. LUND

The distemper virus is very closely related to measles virus and the spectrum of canine and human diseases show remarkable similarities. In addition to the common acute disease there are occasional development of a perivascular demyelinating disease and the rare occurrence of chronic CNS infection. In man this presumably may occur as subacute sclerosing panencephalitis.

Canine distemper in dogs

Distemper in dogs has been studied for many years. Some dogs develop an acute encephalitis during the late course of disease, and in other dogs long term persistence of virus occurs in CNS and lead to a chronic encephalitis (old dog encephalitis). Gillespie and Richard pointed out already in 1956 (4) that the basic factors influencing distemper virus to produce encephalitis are poorly understood, and that a major difficulty is the failure to produce nervous manifestations regularly. They demonstrated that a series of intracerebral inoculations in dog of a strain, designated Snyder Hill, resulted in a material that could produce neurological signs regularly. Raine (15) reported a total of eight dogs which suffered between 10 days to 12 weeks of acute or chronic natural disease, which could be segregated according to the lesions found

by histological manifestation. Viral inclusions were found only within areas showing pathological changes, but active multiplication of virus was not demonstrated. Perivascular cuffing was frequently observed and edema. Eventually only fibrous astrocytes remained. The pattern seemed similar to the one of experimental allergic encephalomyelitis with mononuclear cells invading the myelin sheath.

It has been reported (11) that the chance to get a demyelinating process depends very much on the strain of virus employed, and that an especially well suited strain has been found. It has, however, not been available outside the group. The same group (9) has suggested that the inability to produce antibodies to envelope antigens may be a crucial factor in the establishment of a persistent infection with canine distemper virus.

As pointed out by Johnson and Weiner (7) there has for many years been controversy regarding the pathogenesis of the acute demyelinating CNS disease, essentially whether it is a direct viral effect or an immune-mediated myelin destruction. It is not stated that after all both mechanisms might be involved at the same time. They point out that essentially no experimental model is available to study the human post-infectious encephalomyelitis in the laboratory. In a number of countries measles immunization has successfully eliminated the disease in man, so that clinical material is not available any more.

Møller (12) has performed post mortems on around 5000 dogs in the period before distemper vaccine of good quality was generally available. In the cases of acute distemper he demonstrated essentially inclusions in the cytoplasm. In the cases of chronic, i.e. old dog encephalitis, he found intranuclear inclusions in cells of the central nervous systems. It has been strongly suggested (1), that there is a possible relationship of old dog encephalitis to multiple sclerosis, subacute sclerosing panencephalitis and neuromyelitis optica,and that old dog encephalitis is a valuable model for further study of severe demyelinating diseases of dogs and man. In the same publication it is,

however, admitted, that accurate diagnosis in the living animal is still difficult, and that demyelination in old dog encephalitis is usually diffuse.

Raine et al (16) described measles virus (Edmonston strain) grown in central nervous system tissue, and how the intranuclear presence of nucleocapsid became gradually more abundant.

Koestner et al (8) have worked with the Lederle (vaccine) strain in explant cultures of canine cerebellum, where they found inclusions and a number of viral changes. As they can demonstrate demyelination in the cerebellum cultures, they suggest the use of such cultures as an uppropriate model for certain parts of the in vivo process.

It seems that the most significant study to explain part of the pathogenesis is the one of Nakai, Shand and Howatson (13). They described the development of measles virus in vitro by electron microscopy and otherwise. They found that nucleocapsids produced in the cytoplasm budded off from the cellular membranes and became infectious virions, but that the nucleocapsids of the nuclei apparently was a dead end not resulting in infectious virions. This observation together with the intranuclear inclusions of the old dog encephalitis seem potentially very important as part of an hypothesis for measles-distemper encephalitis.

Distemper virus infection of mink

In all the literature dealing with measles and distemper encephalitis and the search for a relevant model for the human infections it seems overlooked, that not only Canines, but also Mustelidae may become infected with distemper virus, and that central nervous system involvment in mink is at least as common as in canines. This has been very obvious in Denmark, where epidemics of distemper have occurred since commercial breeding started around 1940 and became especially troublesome in the late 60'es. Mink farms have been placed quite close, so that the density of mink may be more than 100000 animals per 50 square kilometer, and the yearly production is and has for several years been several millions of animals. In the dense areas the morta-

lity of distemper could be up to 60-70 per cent. The mortality depends on a number of factors like genotypes of mink and virus strain and very much on age. The kits are born within a short period from late April and are except the breeder animals pelted in December. In spite of this the epidemics occurred very often in the autumn. Certain genotypes, like the pastels, had a higher morbidity and mortality than other genotypes, like the standards. Ferrets are more sensitive than mink. The ability to form antibodies does not depend on the genotypes (5, 6).

The epidemics varied in intensity, but also so that the respiratory and conjunctivitis symptoms dominated in some years and the hard-pad disease in other years. The infection is very contagious. All animals become infected. It seems that convulsions and other signs of CNS involvment were more frequent in years with hard-pad disease. Convulsions may occur in animals which have been ill for some time. They scream suddenly in a special high-pitched way and die in a strong convulsion. By the end of an epidemic other animals have sometimes fatal convulsions without any previous signs of disease. Animals which survive the epidemics may much later die suddenly, e.g. during mating. Fatal convulsions in connection with noise from low flying airplanes have also been connected with a previous history of a distemper epidemic. Although the Danish Fur Breeders Association kept a very careful record of the number of distemper cases, there is unfortunately no information on the frequency of nervous disorders in the epidemics.

A study of encephalitis caused by distemper in mink

With the purpose of studying the pathogenesis of distemper encephalitis in mink and possibly setting up an experimental model for the corresponding syndrom in man a group in Copenhagen decided in 1976 to start a series of experiments employing mink. We were aware of a number of difficulties: 1) The lack of knowledge of the normal anatomy of the mink brain, 2) The surely quite different qualities of the mink blood, especially compared to human

blood, [3] the limited resources, [4] the lack of a conveniently situated experimental farm, [5] the lack of an optimal strain of distemper virus.

It seems that the literature on the influence of immunological factors on distemper encephalitis contains quite conflicting points of view. We had expected that natural distemper would occur again and give us material to study, but that has not been the case.

The factors that might influence the development of neurological symptoms are the genotypes and histocompatibility type, the virus strain and dose, immunological factors and other infections like plasmacytosis ("Aleutian disease") and virus enteritis.

The decision was to look into the effect of immunological factors. We tried the treatments indicated in Table 1.

Table 1

Group	Treatment	Mortality in percentage
1	Immunosuppressive treatment employing cyclophosphamide starting one day before virus inoculation	35
2	Niridazol treatment starting one day before virus inoculation	70
3	Niridazol treatment starting 7 days after virus inoculation	75
4	Specific immunoglobulin starting one day before virus inoculation	72
5	Levamisol treatment starting one day before treatment	61
6	Levamisol treatment starting 7 days after virus inoculation	75
7	Heat inactivated vaccine	60

8	Proper vaccination employing live vaccines	0
9	Control receiving only virus inoculation.	30

Each group consisted of 20 animals, 4 months old and without pastel genes. They came from a farm that never had distemper outbreaks and thus were seronegative in neutralization test. Unfortunately a high number became plasmacytosis positive during the experiment. This could be avoided now through the better testing available.

ad 1) The broad, non-specific suppression of cyclophosphamide treatment has been valuable in connection with plasmacytosis antigen production in mink and has influence e.g. Marek disease in chickens (10).

ad 2 and 3) Niridazole, an anthelminthic drug, has been shown to be a potent, long-acting suppressant of cell-mediated immune responses (14).

ad 5 and 6) Levamisole (tetrahydro phenylimidazothiazole hydrochloride) is an anthelminthic drug, which apparently stimulates T-cell functions. It seems that the effect is functional especially in connection with subnormally functioning cells.

ad 7) We have previously seen (5) that such an antigen could cause formation of neutralizing antibodies, but not protection against distemper.

Three weeks after vaccination of group 5 and 6 all the animals were inoculated on Sept. 9 with a Snyder Hill strain of virus. The final result in terms of mortality was registered on Dec. 27 with the result given in Table 1. Only few (around eight) of the animals had signs of centralnervous system affection like ataxi, strange behaviour, excessive saliva, and the mortality of the control group was low. On 94 animals a preliminary post mortem was carried out immediately after death or at the end of the experiment. Very few cases of diffuse lesions in the brain with mononuclear cells perivascularly and no regular demyelination were observed. Plasmacytosis was partly inhi-

bitory for the proper evaluation. It was the intention to make a more thorough examination of the material, but the pathologist, professor Møller, died quite suddenly. In 18 mink some neurological changes were found. None of these were animals of the control group or the vaccinated ones, but the findings were perhaps not significant at all. Determination of neutralizing antibodies and a preliminary study of leucocyte migration inhibition were carried out. Virus isolations were carried out on throat and rectal swabs and determinations of virus antigens by means of indirect immunoperoxidase testing. No mink had demonstrable virus excretion one week after inoculation, but by day 22 all animals were excreting virus, also the vaccinated ones. No virus was demonstrated after 5 weeks. There was no correlation between antibody titre and course of infection. Titres between 25 and 625 were found in all groups and very few outside that range.

In the following year we performed a new study with only two groups: Levamisol treated animals and control animals. The Snyder Hill strain was this time received from Behringwerke. The impression from previous vaccination trials was that this strain gave a higher frequency of neurological symptoms than the strain employed the year before.

The plan was to take out brain material for the establishing of cell cultures and for histological examination from animals dying or with clear neurological symptoms. Leucocyte migration test (2) and lymphocyte cultures (3) for a blasttransformation test were attempted in a number of modifications, but very often it proved to be difficult to obtain a proper number of free white blood cells, and no conclusions can be drawn from the results. Mink blood differs in many ways from that of other species, and clumping and destruction of the desired cells often occurred. In addition the wrong antigen, a vaccine virus was used, which according to Schultz (17) would not suppress the lymphocytes.

Distemper virus was demonstrated from most of the

cultivated brain tissues of the animals dying during the first 4 weeks after inoculation. In most cases the cells had to be transplanted to form new cultures, before cell degeneration and cytoplasmic inclusions could be seen.

The Levamisol treated animals had convulsions, sometimes fatal, more often than the controls (8/30 against 2/30). The animals died faster: 17 were left out of the 30 Levamisol treated on the 15th day after virus inoculation, but 22 out of the 30 controls. One week later 10 remained in both groups.

After 3 months the Levamisol treatment was repeated. In this case some animals died in convulsions soon after inoculation (1 ml of an 1/5 dilution given intramuscularly). Consequently it cannot be excluded that the substance was toxic to the animals, rather than enhanced the effect of the virus. The brain cultures set up after 3 months did not contain viral inclusions or show CPE. Electron microscopy did not reveal changes related to distemper virus.

Unfortunately no histological examinations have been carried out on the brain materials so far. Consequently it is not yet possible to draw any conclusions from the experiments.

In spite of a number of practical difficulties encountered with the experiments we have so far carried out, I would like to suggest that mink potentially are very useful animals for distemper experiments, much to be preferred to dogs.

REFERENCES

1. Adams, JM, WJ Brown, HD Snow, SD Lincoln, AW Sears, jr., M Barenfus, TA Holliday, NE Cremer and EH Lennette: Old dog encephalitis and demyelinating diseases in man. Vet Pathol 12, 220-226, 1975.
2. Clausen, JE: Comparison between capillary tube and agarose migration technique in the study of human peripheral blood leukocytes. Acta Allergol 28, 145-158, 1973.
3. Gerber, JD and AE Marron: Cell-mediated immunity and age at vaccination associated with measles inoculation and protection of dogs against canine distemper. Am J Vet Res 37, 133-138, 1976.
4. Gillespie,JH and CG Richard: Encephalitis in dogs produced by distemper virus. Am J Vet Res 17, 103-108, 1956.
5. Hansen, M and E Lund: The protecting capacity of neutralizing antibodies by distemper virus infections in mink. Acta path microbiol scand 80, 795-800, 1972.
6. Hansen, M and E Lund: Comparative distemper vaccine titrations in ferrets and mink. Nord Vet-Med 25, 1-7, 1973.
7. Johnson, RT and LP Weiner: The role of viral infections in demyelinating diseases In: Multiple Sclerosis, Wolfgram, F, GW Ellison, JG Stevens and JM Andrews (eds), UCLA Forum in Medical Sciences No 16, New York AP 1972, p 245-264.
8. Koestner, A and JF Long: Ultrastructure of canine distemper virus in explant tissue cultures of canine cerebellum. Lab Invest 23, 196-201, 1970.

9. Krakowka, S, R Olsen, A Confer, A Koestner and B McCullough: Serologic response to canine distemper viral antigens in gnotobiotic dogs infected with canine distemper virus. J Infect Dis 132, 384-393,1976.
10. Lu, Y-Sh, V Kermani-Arab and T Moll: Cyclophosphamide-induced amelioration of Marek's disease in Marek's susceptible chickens. Am J Vet Res 37, 687-692, 1976.
11. McCullough, B, S Krakowka, A Koestner and J Shaddock: Demyelinating activity of canine distemper virus isolates in gnotobiotic dogs. J Infect Dis 130, 343-350, 1974.
12. Møller, T, personal communications.
13. Nakai, T, FL Shand and AF Howatson: Development of measles virus in vitro. Virology 38, 50-67, 1969.
14. Pelley, RP, RJ Pelley, AB Stavitsky, AAF Mahmoud and KS Warren: Niridazole, a potent long-acting suppressant of cellular hypersensitivity. J Immun 115, 1477-1482, 1975.
15. Raine, CS: Viral infections of nervous tissue and their relevance to multiple sclerosis. In: Multiple Sclerosis, Wolfgram, F, GW Ellison, JG Stevens and JM Andrews (eds), UCLA Forum in Medical Sciences No 16, New York, AP 1972, p 91-118.
16. Raine, CS, LA Feldman, RD Sheppard and MB Bornstein: Ultrastructure of measles virus in cultures of hamster cerebellum. J Virology 4, 169-181, 1969.
17. Schultz: Failure of attenuated canine distemper virus (Rockborn strain) to suppress lymphocyte blastogenesis in dogs. Cornell Vet 66, 27-31, 1976.

DISCUSSIONS OF PAPER BY E. LUND

The provision of suitable animal models for the study of SSPE provoked much comment. Thus, in hamsters and ferrets, an SSPE-like condition can be induced by the intracerebral inoculation of non-productive virus. Doubts were expressed as to whether distemper in dogs or in mink resembled SSPE clinically; Dr Lund replied that illnesses following an acute course and also a more prolonged course were found in the natural disease. Passage of the presumably infectious agent in 'old-dog encephalitis', where inclusions are found in brain tissue, had not been successful.

Dr Norrby summarised the model systems available and considered that the best one consisted of the inoculation of cell-associated measles-SSPE virus into ferrets. In hamsters, older animals (3 weeks of age) could be used. In newborn hamsters, inoculation with defective ts virus mutants from persistently infected tissue cultures led to persistent infections. In the rat model, the newborn animals had maternal antibody.

THE IMMUNE RESPONSE IN SUBACUTE SCLEROSING PANENCEPHALITIS

P. J. LACHMANN, N. T. GORMAN, J. HABICHT, P. W. EWAN, G. AGNARSDOTTIR AND H. VALDIMARSSON

1. INTRODUCTION

The idea that some rare, and not obviously contagious, human diseases may result from an unusual immunological response to commonly encountered infectious agents has attracted increasing attention in recent years. While much of this interest was stimulated by studies in mice, for example on chronic LCM disease (1) it is nevertheless probable that the clearest example of a disease where this view of pathogenesis is correct is subacute sclerosing panencephalitis (SSPE). The disease is extremely rare while the virus causing it is virtually universally encountered. Thus, in the United States before the advent of immunisation against measles it was estimated that the incidence of SSPE was one case for every million cases of measles although in the Middle East it seems to be significantly commoner (see 2). The disease is not contagious in as much as there are no reports of anyone having caught SSPE or even measles from an SSPE patient. Finally, there is no doubt that the immunological response to the measles virus in SSPE is unusual and it is the nature of this response which is the topic of this paper.

2. THE IMMUNE RESPONSE TO MEASLES VIRUS IN SSPE

Measles virus contains six major polypeptides (3,4,5). These are shown with their molecular weights (and with the comparable proteins of canine distemper virus) in Table 1. By using immunoprecipitation techniques followed by polyacrylamide gel electrophoresis (PAGE) analysis it is possible to detect in serum, antibodies to all six of these polypeptides (6). However, this has been relatively infrequently done and most of the work on the antibody response to measles virus has used other techniques: haemagglutination inhibition which measures antibodies to the haemagglutinin; haemolysis inhibition which measures antibodies to the fusion factor or haemolysin; neutralisation which may measure both the two previous antigens; and

Table 1 Comparison between measles and canine distemper viral proteins

			M.V.	C.D.V.	Present on membrane of infected cell
L	-	Large	200K	200K	0
H	-	Haemagglutinin	80K	76K	+
P	-	Phosphorylated	70K	66K	0
NP	-	Nucleocapsid	62K	58K	0
F_o	-	Fusion factor or haemolysin	55K	60K	+
M	-	Matrix protein	37K	34K	??
F_o	=	F_1 + F_2(on reduction)			
		F_1	40K	40K	+
		F_2	15K	20K	+

complement fixation which measures antibodies principally to the nucleocapsid. In considering the pathogenesis of virus infections, particular interest attaches to antibodies towards those viral proteins that are represented on the membrane of the infected cell. In the case of the measles virus these are the haemagglutinin and fusion factor. The possibility that the matrix protein may be present on the membrane cannot be wholly excluded (7) though in our experiments we have not been able to detect it on cell membranes by the technique of cell membrane radio-labelling followed by immunoprecipitation and PAGE analysis (8).

2.1. The antibody response to the measles virus in SSPE

Connolly et al (9) first reported that patients with SSPE had very high levels of antibodies to measles virus both in serum and in CSF. This has been borne out in all subsequent studies. The levels can be really extremely high. Thus 10 - 20% of the IgG in one of the sera that we have studied could be bound to the surface of persistently measles virus infected cells. This measures only antibody to the haemagglutinin (H) and the fusion factor (F) and it therefore seems likely that probably as much as 20% of the IgG in this patients serum may have been antibodies to the measles virus. This sort of antibody level is comparable to what can be achieved in a hyperimmunised rabbit! The high levels of measles antibody can be detected by any of the standard techniques as well as by immunoprecipitation and PAGE analysis. Using the last named technique Hall et al (6) have claimed that antibody titres in SSPE are raised to all the measles virus polypeptides except one. Antibodies to the matrix protein were found at only low levels in a number of SSPE sera that they studied. This selective relative failure to make antibody to the M protein was characteristic of SSPE sera in their study. They suggest that this may reflect a failure of measles virus in SSPE to produce adequate a mounts of M protein and they believe that this may form an integral part of the pathogenesis of the disease.

Besides having high levels of anti-measles antibodies in their sera patients with SSPE also show high levels in the CSF. The antibody response in the CSF is characteristically oligoclonal and it has been shown by Vandvik et al (10) that individual oligoclonal bands

within the CSF represent individual measles virus antigens. Oligoclonal Ig bands in the CSF are probably a marker of antibody that is synthesised within the CNS and it is believed that this reflects the fact that the number of precursor cells within the CNS is small, and that this does not allow the development of the high degree of polyclonality that is seen in the serum when antibody synthesis is initiated by a much larger number of precursor cells.

2.1.1. Antibody affinity

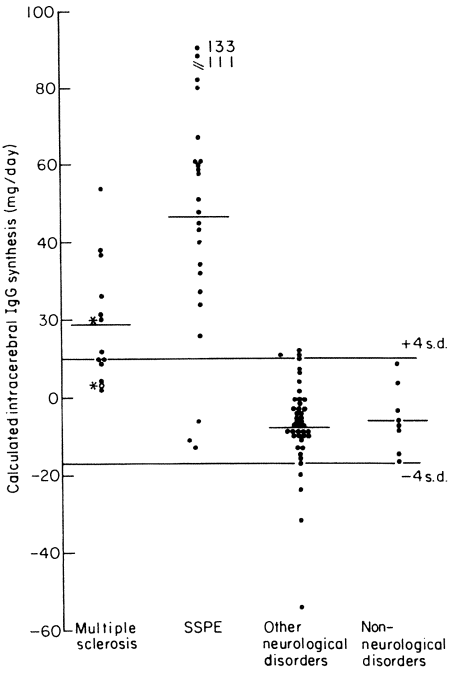
There have been no substantive studies on the affinity of the anti-measles antibodies in SSPE. Some preliminary studies were carried out some years ago (Lachmann & Fazekas de St. Groth unpublished observations) which appeared to show that in the SSPE sera there was a mixture of low affinity with a smaller concentration of antibodies of a high affinity.

2.2 Immunoglobulin and antibody synthesis in the CNS as measured by the Tourtellotte technique

Tourtellotte (12, 13) described a relatively non-invasive method for determining the extent to which immunoglobulin is synthesised within the CNS. This depends upon the simultaneous measurement of albumin and IgG levels in serum and CSF. From the albumin levels an expected value of the IgG level can be calculated, this being made up of Ig that is transuded across a normal blood brain barrier plus a quantity that is exuded across a leaky blood brain barrier. Although there is some doubt about the assumptions underlying the calculation of the exuded IgG (see 11) and the confidence intervals of the calculations are therefore wider in patients with leaky blood brain barriers, there is no doubt that a reasonable estimate of the IgG synthesised in the CNS can be obtained by subtracting from the observed IgG level that calculated from the albumin levels as derived from plasma. We have used the Tourtellotte technique for measuring IgG synthesis in patients with SSPE as well as with multiple sclerosis (11,14) and the results are shown in Figure 1. Here it can be seen that the IgG synthesis levels in SSPE are remarkably high even compared to those found in MS.

The principle underlying the Tourtellotte technique is of course equally applicable to the measurement of individual antibodies providing that these can be accurately quantitatively measured in this way (14, 15). In order to obtain an adequately quantitative

Figure 1



Intracerebral IgG synthesis in MS, SSPE, and two control groups. * Indicates results in one MS patient studied on two occasions. The normal range indicated (mean ± 4 s.d.) is taken from Tourtellotte's data obtained in normal subjects. Horizontal bars indicate mean for each group. The differences between MS and the neurological control group, and SSPE and the neurological control group are statistically significant ($P < 0{\cdot}001$ for both by Student's t-test).

From Ewan & Lachmann (1979)

assay the antibodies were measured by competitive radioimmunoassay using as antigen fixed HeLa cells persistently infected with measles virus. This assay is likely to measure predominantly antibodies to haemagglutinin and to the fusion factor. There is a problem in the use of competitive radioimmunoassay for this purpose since this measures the antibody in inhibitory units compared to the standard preparation of SSPE serum and since the affinity of the antibody as well as its concentration plays a part in this inhibitory activity the values obtained will contain errors if the affinity of antibody in serum and CSF are not the same. Table 2 shows the results of the measles antibody synthesis experiments in a group of SSPE patients compared with two groups of MS patients: one showing elevated IgG synthesis and the other not so doing and with a group of normal patients. It can be seen that there is indeed a very marked hyper-synthesis of anti-measles virus antibody within the CNS. The amount detected by this technique was about 20% of the total IgG synthesis as measured. Since there are antibodies to at least several other measles virus polypeptides that are not detected by this assay these findings are compatible with the great majority of the antibody in the CNS being anti-measles virus. The same is not true for the MS patients where the amount of anti-measles IgG synthesis found is independent of whether or not any overall IgG synthesis is found and where it is little more than is found in a control group. It is interesting that low levels of antibody synthesis are detected by this technique even when no overall IgG synthesis can be found. It remains unclear whether this is an artefact of the measuring technique and depends on the fact that the antibody formed in the CNS is of particularly high affinity. It is certainly true, however, that the ratio of antibody between serum and CSF is much lower than is found for the total IgG level and that in our hands it is not markedly different in SSPE from the ratio found in normals or in patients with MS. This finding was unexpected since it is at variance with what has previously been published (16). However it is possible to use this data to make a rough calculation of what proportion of the body's anti-measles antibody is being made within the CNS. These calculat-ions are shown in Table 2, and it can be seen that the 8% found in SSPE is not significantly higher than in normals. This shows that

Table 2 Measles virus antibody synthesis in CNS

	SSPE	MS(1) +ve IgG synth.	MS(2) -ve IgG synth.	Other
Number	4	6	4	6
Blood brain barrier	292	290	184	273
I/c IgG synthesis mg/day	31.8	16.4	-2.3	-8.4
Measles antibody levels				
Serum µg/ml	529	27	15.4	16.5
CSF µg/ml	13.4	0.9	1.1	0.5
Ratio serum/CSF	39.5	30.3	14.7	33
I/c measles antibody synthesis mg/day	6	0.4	0.5	0.2
I/c measles antibody synthesis as % of i/c IgG synthesis	18.9%	2.3%	-	-
I/c measles antibody synthesis as % of total measles antibody synthesis in body	8%	5%	12%	6%
Total measles antibody synthesis in body (approx.) mg/day *	101	6	4	4
I/c measles antibody synthesis as % of total measles antibody synthesis in body	8%	5%	12%	6%

* Calculation assumes plasma volume of 5 litres and further ECF volume of 10 litres (with antibody concentration one half that of plasma) for adults; (for children with SSPE 80% of these volumes were assumed); and assumes half life of IgG to be 21 days.

Then total anti-measles IgG(mg) = 10 x plasma conc. (µg/ml)

and synthesis rate in mg/day $= \frac{\text{total Ab}}{42}$

$= \frac{\text{plasma conc. (µg/ml)}}{4.2}$

although there is undoubted and considerable hypersynthesis of anti-measles antibody (about 6mg/day compared with 0.2mg/day in normals) within the CNS there is an entirely comparable degree of hyper-synthesis in the rest of the body (about 100mg/day cf 4mg in normals). The amount of these particular anti-measles virus antibodies made is much greater than the total intracerebral IgG synthesis and it is therefore impossible that a large proportion of it could be made within the CNS. The degree of systemic hyperreactivity in SSPE is not generally taken full account of in discussing theories of the pathogenesis of the disease. The abnormality is not situated wholly within the brain although persistent leakage of antigen from CNS into the systemic circulation may be responsible for the chronic hyper-immunisation. The data also shows that ratios of serum/CSF antibody titres are an unsatisfactory way of showing intracerebral synthesis.

Comparable studies have been done using distemper virus (14). This was done largely in view of the interest in the possibility that distemper may be implicated in the pathogenesis of multiple sclerosis, but the SSPE patients are an interesting control group for this purpose since it is clear that their disease is due to the measles virus. The distemper data are shown in Table 3. It can be seen that the levels of antibody found are very much lower than those detected against the measles virus and the somewhat elevated level in SSPE is probably a sign of cross reactivity. It is again of interest how low the ratios of serum to CSF antibody are for this virus in all groups of patients. In fact the lowest values are found among the groups of normals and those MS patients showing no IgG synthesis. Again, taking all the reservations about these techniques into account it would appear that a substantial proportion of anti-distemper antibody found is made within the central nervous system in normals. The ratio of measles antibody to distemper antibody in both serum and CSF is much the same in normals and in both MS groups but is much elevated in SSPE as might be expected. These findings lend little support to the idea that there is a significant immune response to the distemper virus in multiple sclerosis but they do show that the techniques can distinguish the measles related response in SSPE from a distemper virus cross-reacting response.

Table 3 Distemper virus antibody synthesis in CNS

	SSPE	MS(1) +ve IgG synth.	MS(2) -ve IgG synth.	Other
Number	4	6	4	6
Blood brain barrier	292	290	184	273
IgG synthesis mg/day	31.8	16.4	-2.3	-8.4
Distemper antibody levels				
Serum µg/ml	3.6	1.6	1.0	0.8
CSF µg/ml	0.2	0.1	0.3	0.1
Ratio serum/CSF	18	12.2	3.9	5.4
I/c distemper antibody synthesis mg/day	.13	.05	.1	.05
I/c distemper antibody synthesis as % of i/c IgG synthesis	0.4%	0.3%	-	-
Total distemper antibody synthesis in body (approx.) mg/day	.7	.4	.2	.2
I/c distemper antibody synthesis as % of total distemper antibody synthesis in body*	25%	11%	47%	23%

* As in Table 2

2.3. The cellular immune response to the measles virus in SSPE

There are considerable problems attending the studies of cell-mediated immune responses in patients with measles and to the measles virus in particular. These are related firstly to the well known property of the measles virus in producing anergy in delayed hypersensitivity reactions (17). Furthermore the virus binds to human T lymphocytes, not by specific anti-measles virus receptors but by some receptor on the T cell as a class that has affinity for the measles virus (18). There are technical problems with lymphocyte transformation tests using measles virus preparations as antigen and the techniques that have been mainly used are (i) delayed hypersensitivity skin tests (ii) the production of lymphokines usually macrophage migration inhibitory factor and (iii) cytotoxic reactions killing measles virus infected cells. In vivo skin testing with measles virus preparations is almost invariably negative in patients with SSPE (19, 20). The in vitro tests using migration inhibition are, however, generally positive providing that they are done in the presence of normal and not SSPE serum (21). This blocking effect of SSPE which can be demonstrated both on lymphokine production and in cytotoxicity assays is believed to be due to immune complexes (22) although these have not so far been adequately studied by modern techniques.

2.3.1 Cytotoxic reactions using measles virus infected cells

This topic will be treated only briefly since it is to be discussed by Dr. Kreth in the next paper. The mechanism by which lymphocytes kill measles virus infected cells remains a matter of some controversy. The reason is almost certainly that a number of mechanisms can come into play (Table 4) and that the extent to which each one is detected depends upon the exact experimental system used. The antigens expressed on the cell membrane are likely to be important and there is reason to believe that acutely infected cells are better targets at least for some cytotoxic mechanisms than are persistently infected cells (8).

It was shown by Valdimarsson et al (18) that embyronic lung cells persistently infected with measles virus could be killed by lymphocytes in the absence of antibody. Such killing was given even by the lymphocytes of children who had never encountered the measles

Table 4 Mechanisms for killing measles virus infected cells

	Killer cell		Target cell				
	Fc receptor	From immune donor	Infected with virus	Needs antibody	HLA preference		Found by
1	+	0 (normal in SSPE)	+	+	0	K cell	All groups
2	0	0 or +	+	0	0	'NK' cell	Valdimarsson(1975)
3	0	+ (?low in SSPE)	+ (acutely inf.)	0 (typically inhibits)	0	? T cell ? NK cell	Ewan & Lachmann (1977; 1979)
c.p.	+	+ Mumps virus + only to induce cytotoxic cell		0	0	NK cell	Harfast et al(1978)
4	0	+ (acute measles only)	+	0	+	T cell	Kreth et al(1979)

virus although the cells of adults who had encountered the virus gave stronger killing. This 'non-allergic cell-mediated killing' given by the cells of measles-negative children would presumably now be referred to as NK killing. NK killing of virus infected cells has been studied by Harfast et al (23) using mumps virus who showed that the role of the virus is to activate the effector cell which can then kill non-specifically. The effector cell in the studies of Harfast et al is an Fc positive lymphocyte showing T cell markers. It may be that in the case of the measles virus where attachment of T cells occurs spontaneously that cytotoxicity may be given by a wider selection of T lymphocytes. The killing of measles virus infected cells by the K cell mechanism is generally agreed to occur (24,25,26,27). It requires the presence of antibody on the infected cell and is mediated by an Fc receptor positive lymphocyte. All groups also agree that K cell activity is normal in SSPE. Although easily demonstrable in vitro it remains a question of some doubt whether this mechanism is effective in vivo since it is inhibited not only by physiological concentrations of immunoglobulin but is readily inhibited by small concentrations of immune complex. A third type of cytotoxicity to measles virus infected cells was described by Ewan & Lachmann (27). This was mediated by populations of lymphocytes depleted of Fc receptored cells - a population which is largely T cell in nature - and was not potentiated by the presence of antibody on the cell. In fact it is typically inhibited by anti-measles antibodies in the test system. The killing produced by these cells does not show HLA preference and the killing is now known to occur much more readily on acutely infected than on persistently infected cells (8). This type of killing is found variably among normal adults and is present generally only weakly in patients with multiple sclerosis. The two patients with SSPE who were tested also showed low levels of this type of killing. It remains to be shown whether this type of T cell killing is mediated by the T cell antigen binding receptor or whether it is a variant of the NK type of cytotoxicity. T cell cytotoxicity which does show HLA preference has been shown by Kreth et al (28) to be demonstrable if the cells are taken from children with acute measles. This form of killing is therefore more likely to represent T cell killing through its antigen binding receptor. There seems to be no clear evidence of any defect in SSPE patients in their capacity to kill measles virus infected cells.

The antibodies in SSPE serum are also highly effective in sensitising measles virus infected cells for K cell killing. There is therefore no reason to believe that the ability to kill measles virus infected cells is intrinsically abnormal in SSPE. The extent to which such killing could be inhibited by immune complexes is difficult to assess. There have been some demonstrations of blocking factors in serum and CSF (18, 22) but also others who have failed to show it (24, 26). The amount of antigen expressed on the target cell appears to influence how easily the reaction is inhibited (20). Neither the serum nor even the lumbar CSF however, is wholly representative of the microenvironment of the neurone where it seems quite likely that immune complexes are being formed. Immune complexes involving measles virus have been detected in the kidney (29) so they presumably circulate in the systemic circulation from time to time.

3. IMMUNE RESPONSE TO ANTIGENS OTHER THAN MEASLES VIRUS

The other prominent immunological abnormality in SSPE especially in advanced cases is anergy on skin testing with a variety of other antigens, for example DNCB, streptokinase-streptodornase, candida and tuberculin (19). This, it is believed, reflected persistent infection with the measles virus. It is interesting that lymphocyte transformation tests to the same antigens are usually normal. The cutaneous anergy is relatively readily reversible by the administration of transfer factor (19) though it is very doubtful whether this treatment has any effect upon the progress of the disease. Other parameters of the immune response in SSPE (e.g. immunoglobulin levels and lymphocyte populations) have generally been found to be normal. It has recently, however, been found by H. Valdimarsson & G. Agnarsdottir (personal communication) that in rapidly progressive SSPE there may be some reduction in the number of circulating T cells as measured by rosetting with sheep erythrocytes.

3.1. HLA association

In all diseases with a putative immunological pathogenesis associations with particular HLA antigens are looked for. In SSPE these have so far been unsuccessful. There was one claim (30) that HLA W29 was associated with the disease but this association was not maintained

when large numbers of patients were studied (2). The discordant occurrence of SSPE in identical twins (who presumably caught their measles at the same time) also make it unlikely that there is a major genetic determinant in the disease. However two pairs of siblings with SSPE have been seen in London in the last five years.

4. DISCUSSION

The immune response in children with SSPE is dominated by their high level of antibodies to measles virus protein. With the exception of the report by Hall et al (6) that there is a relative failure to make antibody to the matrix protein this hyperreactivity extends to all the polypeptides in the measles virus and the antibodies have not been shown to have any differences in specificity from those found in patients convalescent from measles.

It was suggested many years ago that SSPE might represent an example of immune deviation there being a failure of T cell responsiveness to the measles virus with corresponding hyperreactivity of the B cell system. Put into more modern terminology one might say that a failure of T suppressor cells allows an excessive antibody response. There is so far no good data on suppressor cell function in SSPE but if there is a defect it must be specifically towards the measles virus since other antibody titres are not obviously abnormal and nor indeed is there any clear evidence of a failure of T cell immunity even towards the measles virus. This subject is however complicated by the capacity of measles virus infection to produce anergy and skin tests to a variety of antigens are negative. On the other hand in vitro tests, such as they are, usually fail to show any marked abnormality in the SSPE children. It is by no means clear whether the persistence of the measles virus infection in the brain is a consequence of the abnormal immunological response to the virus; or whether the abnormal immunological response to the virus is due to the persistence of the infection of the brain; or whether the two interact in a cyclical fashion. Perhaps the best suggestion for the pathogenesis of SSPE remains that of Joseph & Oldstone (31) who showed that when measles virus infected cells are grown in the presence of antibody but in the absence of an intact complement system the cells become modulated so that the surface antigens are no longer expressed on the cell membrane. The cells are then no longer

able to be destroyed either by antibody and complement or by lymphocytes and the virus is effectively sequestered intracellularly. In such a situation one can envisage the virus infection persisting, occasionally releasing virions (complete or incomplete) or occasionally spreading to other cells by direct cell fusion.

What factors allow modulation and persistence rather than elimination of the virus remains obscure. It is unlikely to be only lack of complement since SSPE has never been described in association with genetic complement deficiencies.

5. SUMMARY

The outstanding feature of the immune response in SSPE is the greatly exaggerated antibody response to the measles virus. This is found, to a similar extent, both within the CNS and elsewhere in the body. Antibody is made to all the measles virus antigens but it is claimed that antibodies to the matrix protein are under-represented. There is no convincingly demonstrated failure of T cell reactivity.

Whether hyperimmunisation with measles virus is the cause of the persistent infection or its result remains to be determined.

REFERENCES

1. Oldstone, MBA, FJ Dixon: Pathogenesis of chronic disease associated with persistent lymphocytic choriomeningitis viral infection. J. Exp. Med. 129:483-505, 1969.
2. Lachmann,PJ, H Valdimarsson, G Agnarsdottir: The aetiology of subacute sclerosing panencephalitis. Immunopathology VII, ed. PA Miescher, Schwabe, Basel p. 148, 1977.
3. Wechsler, SL, BN Fields: Intracellular synthesis of measles virus specified polypeptides. J. Virol. 25: 285-297, 1978.
4. Graves, MC, SM Silver, PW Choppin: Measles virus polypeptide synthesis in infected cells. Virology 86: 254-263, 1978.
5. Tyrell, DLJ, E Norrby: Structural polypeptides of measles virus. J. Gen. Virol. 39: 219-229, 1978.
6. Hall, WW, RA Lamb, PW Choppin: Measles and SSPE virus proteins: Lack of antibodies to the M protein in patients with subacute sclerosing panencephalitis
7. Fraser, KB, M Gharpure, PV Shirodania, MA Armstrong, A Mave, E Dermott: In: Negative Strand Viruses and the Host Cell. Eds. BWJ Mahy, RD Barry, Academic press, pp.771-780, 1978.
8. Ewan, PW, PJ Lachmann: in preparation, 1979
9. Connolly, JH, I Allen, IJ Hurwitz, JHD Millar: Measles virus antibody and antigen in subacute sclerosing panencephalitis. Lancet 1: 542-544, 1967.
10. Vandvik, B, E Norrby, HJ Nordal, M Degre: Oligoclonal measles virus specific IgG antibodies isolated from cerebrospinal fluids, brain extracts and sera from patients with subacute sclerosing panencephalitis and multiple sclerosis. Scand. J. Immunol.5: 979-992, 1976.
11. Ewan, P, PJ Lachmann: IgG synthesis within the brain in multiple sclerosis and subacute sclerosing panencephalitis. Clin. exp. immunol. 35: 227-235, 1979.
12. Tourtellotte, WW: On cerebrospinal fluid immunoglobulin-G (IgG) quotients in multiple sclerosis and other diseases. A review and a new formula to estimate the amount of IgG synthesised per day by the central nervous system. J. Neurol. Sci. 10: 279-304, 1970.

13. Tourtellotte, WW: What is multiple sclerosis? Laboratory criteria for diagnosis. Multiple sclerosis Research ed. AN Davison, JH Humphrey, AL Liversedge, WI McDonald, JS Porterfield. H.M.S.O. Lond. 1975.

14. Gorman, NT, PJ Lachmann, J Habicht: in preparation, 1979.

15. Lachmann, PJ, J Habicht: Synthesis of IgG and of anti-measles antibodies within the central nervous system in patients with subacute sclerosing panencephalitis and with multiple sclerosis. In: Progress in Neurological Research. Eds. P Behan, F Clifford Rose, Pitman, in press.

16. Norrby, E, H Link, JE Olsson: Measles virus antibodies in multiple sclerosis. Arch. Neurol. (Chicago) 30: 285-292, 1974.

17. von Pirquet, C: Das Verhalten der kutanen Tuberkulinreaktion während der masern. Dtsch.med.Wschr. 34: 1297, 1908.

18. Valdimarsson, H, G Agnarsdottir, PJ Lachmann: Measles virus receptor on human T-lymphocytes. Nature 255, 554-556, 1975.

19. Valdimarsson, H, G Agnarsdottir, PJ Lachmann: Cellular immunity in subacute sclerosing panencephalitis. Proc. Roy. Soc. Med. 67: 1125-1129, 1974.

20. Valdimarsson, H, G Agnarsdottir, PJ Lachmann: Subacute sclerosing panencephalitis. In: Clinical Neuroimmunology. Ed. F. Clifford Rose, Blackwell Scientific Publications, pp. 406-418, 1979.

21. Thurman, GB, A Ahmed, DM Strong, RC Knudsen, WR Grace, KW Sell: Lymphocyte activation in subacute sclerosing panencephalitis virus and cytomegalovirs infections. J. Exp. Med. 138: 839-846, 1973.

22. Ahmed, A, DM Strong, KW Sell, GB Thurman, RC Knudsen, R Wistar, WR Grace: Demonstration of a blocking factor in the plasma and and spinal fluids of patients with subacute sclerosing panencephalitis. J. Exp. Med. 139:902-924, 1974.

23. Härfast, B, T Andersson, P Perlmann: Immunoglobulin-independent natural cytotoxicity of Fc receptor-bearing human blood lymphocytes to mumps virus-infected target cells. J. Immunol. 121: 755-761, 1978.

24. Perrin, LH, A Tishon, MBA Oldstone: Immunologic injury in measles virus infection. III. Presence and carahcterisation of human cytotoxic lymphocytes. J. Immunol. 118: 282-290, 1977.

25. Kreth, HW, V ter Meulen: Cell-mediated cytotoxicity against measles virus in SSPE. 1. Enhancement by antibody. J. Immunol. 118:

291-295, 1977.

26. Kreth, HW, G Wiegand : Cell-mediated cytotoxicity against measles virus in SSPE. II. Analysis of cytotoxic effector cells. J. Immunol. 118: 296-301, 1977.

27. Ewan, PW PJ Lachmann: Demonstration of T-cell and K-cell cytotoxicity against measles-infected cells in normal subjects, multiple sclerosis and subacute sclerosing panencephalitis. Clin. exp. immunol. 30: 22-31, 1977.

28. Kreth, HW, V ter Meulen, G Eckert: Demonstration of HLA restricted killer cells in patients with acute measles. Med. Microbiol. Immunol. 165: 203-214, 1979.

29. Dayan, AD, MI Stokes: Immune complexes and visceral deposits of measles antigens in subacute sclerosing panencephalitis. Brit. Med. J. ii: 374-376, 1972.

30. Kurent, JE, JL Sever, PI Terasaki: HLA W29 and subacute sclerosing panencephalitis. Lancet 1: 927-928, 1975.

31. Joseph, BS, MBA Oldstone: Antibody induced redistribution of measles virus antigens on the cell surface. J. Immunol. 113: 1205-1209, 1974.

RECENT FINDINGS ON CELL-MEDIATED IMMUNE REACTIONS IN ACUTE MEASLES AND SSPE

W. KRETH AND F. PABST

1. INTRODUCTION

It is now more than 10 years that BURNET published his hypothesis on the pathogenesis of SSPE (1). BURNET assumed that the underlying defect was a specific unresponsiveness at the level of T cells. Despite a large body of experimental data (reviewed in (2)) this hypothesis cannot be conclusively answered at present. It is also evident that the mode of action of specific T cells during acute measles itself is incompletely understood. This may be at least partly due to the difficulties in designing appropriate in vitro experiments which explore conclusively specific T cell immunity in man.

2. HETEROGENEITY OF T CELL EFFECTOR FUNCTIONS

Most of the information on T cells has been derived from experiments in mice. By means of antigenic and functional markers 4 different subpopulations of T cells can now be recognized in mice (3): T helper cells (T^{H}), cytotoxic T lymphocytes (T^{CTL}), T cells involved in delayed type hypersensitivity (T^{DTH}), and T cells endowed with regulatory functions.

In SSPE, a deficient T cell response against measles antigens could extend to all T cell subpopulations. However, this is rather unlikely. Patients with SSPE produce extremely high amounts of specific antibody of IgG type (2). Also, the response to paramyxoviruses has been shown to be T cell-dependent in nude mice (4). Both lines of evidence strongly suggest that specific T helper cells must be functionally unimpaired in patients with SSPE.

As has been demonstrated in animal models, cytotoxic

T lymphocytes might play a major role in elimination of host cells with altered surface moieties, such as tumor and virus-infected cells. This was the reason why over the last 5 years many investigators concentrated on lymphocyte-mediated cytotoxicity against measles virus-infected target cells in SSPE (5,6,7,8,9). Peripheral SSPE lymphocytes were indeed found to be effective killer cells. Since it was assumed that these killer cells belonged to the T cell lineage it was generally concluded that specific T cell mediated immunity was intact in patients with SSPE. Today these conclusions can no longer be maintained. It is the scope of this communication to critically analyze lymphocyte-mediated killing in acute measles and SSPE.

3. SPONTANEOUS LYMPHOCYTE-MEDIATED CYTOTOXICITY IN SSPE: INVOLVEMENT OF NATURAL KILLER CELLS

The experimental design for testing peripheral lymphocyte killing has been similar in different laboratories: Fibroblasts or Hela cells carrying a persistent measles virus infection are cocultivated with an excess of freshly isolated lymphocytes for 12 - 18 hrs without addition of specific antibodies. The degree of cytotoxic activity is determined by standard Cr-51 release. We would like to summarize our experimental evidence using persistently measles virus-infected human embryonic lung fibroblasts (10).

As demonstrated in Figure 1, cytolytic activity is low at 4 hrs and increases in a linear fashion over the next 12 hrs. This suggests that a population of killer cells might be generated by prolonged contact with measles virus-infected cells. The time course of killer cell activation is almost identical for patients with SSPE, measles sero-positive adults and measles non-immune children. Cell separation experiments revealed that cytotoxicity was dependent on lymphoid cells carrying receptors for the Fc part of IgG (FcR γ) (8,11). By further cell separation the bulk of cytotoxicity was found to reside in the non-T cell fraction, while a

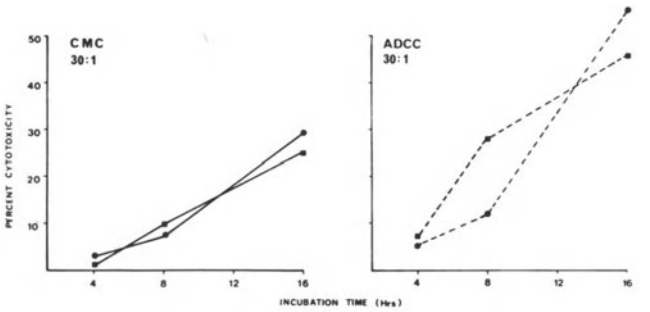


Figure 1. Generation of killer cells against persistently measles virus infected allogeneic target cells. A 30 x excess of either normal ( ) or SSPE ( ) peripheral blood lymphoid cells were incubated with Cr-51 labeled, persistently measles virus-infected human embryonic lung fibroblasts (10). CMC=cell mediated cytotoxicity in the presence of 10 % fetal calf serum. ADCC=antibody-dependent cellular cytotoxicity in the presence of 10 % fetal calf serum plus 2,5 % heat inactivated measles convalescence serum (HI titer 1:512). Percent cytotoxicity calculated according to (11).

---

smaller portion belonged to the T_γ cell population (Figure 2). T_μ cells which comprise ca. 60 - 80 % of total T cells were found not to be cytotoxic.

It was originally speculated that the cytotoxicity observed might be due to K cells and a small amount of measles-specific antibody either locally produced in culture or otherwise passively transferred with lymphoid cells (11). It now seems that the cytotoxic effect in this system might actually be due to natural killer cells (NK cells). This conclusion is based on 3 lines of evidence: 1) Cytotoxicity is not restricted by the immune history of lymphocyte donors; 2) similar generation of cytotoxic potential can be achieved by cocultivation of peripheral lymphocytes with a number of tumor or other virus-infected cells (12, 13, 14); 3) the distribution of natural killer cells as

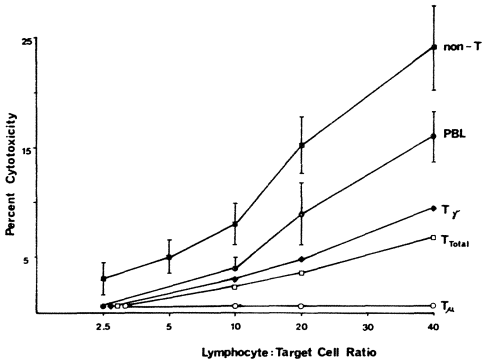


Figure 2. Cytotoxic activity of lymphocyte subpopulations against persistently measles virus-infected human embryonic lung fibroblasts. SSPE peripheral blood lymphocytes were separated into T and non-T cells by rosetting with neuraminidase-treated sheep red blood cells (25). T cells were further fractionated into T γ and Tμ cells as described by MORETTA et al. (26). Lymphocyte subpopulations were tested for cytotoxic activity for 18 hrs in the absence of anti-measles antibodies. Means of triplicates $\pm$ 1 SD or means of duplicate determinations.

---

defined by cytotoxicity against the K 562 cell is also restricted to non-T and T γ cells (15).

It should be stressed that up to now nothing is known about the biological role of natural killer cells in vivo. This type of effector cell must be clearly differentiated from virus-specific cytotoxic T lymphocytes.

4. CTL RESPONSE LIMITED TO THE ACUTE PHASE OF MEASLES

In mice, cytotoxic T lymphocytes are functionally restricted by the H-2 complex. Lysis will only occur if effector and target cells share either H-2D or H-2K gene products (16). It might be argued that in the experiments cited above with human lymphocytes, virus-specific CTL had remained undetectable because tests were always

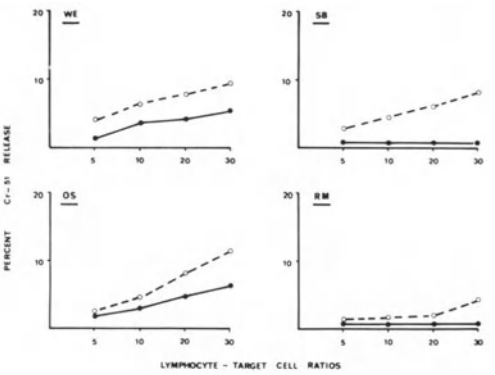


Figure 3. Peripheral lymphocyte killing in SSPE is not restricted by HLA. Peripheral blood lymphoid cells from 4 patients with SSPE were tested on either autologous (●——●) or allogeneic incompatible (○- - -○) measles virus-infected blast cells in 4 hr Cr-51 release assays. Autologous and allogeneic target cells contained almost the same percentages of virus-infected cells as revealed by the fluorescent antibody technique.

---

done against allogeneic target cells. The system was therefore modified to allow cytotoxic interactions to take place under histocompatible conditions by using measles virus-infected phytohemagglutinin-induced lymphoblasts with known HLA determinants as target cells in 4 hr Cr-51 release assays. There are several advantages in working with PHA-stimulated lymphoblasts: once activated, lymphocytes are permissive to measles virus infection regardless of the immune history of the donor, and these cells are a poor target for natural killer cells which ensures a lower background of Cr-51 release.

Freshly isolated lymphocytes from patients with SSPE were definitely not restricted by HLA when tested on autologous or allogeneic histoincompatible measles virus-infected PHA blasts (Figure 3). Of particular interest is the small but consistently higher cytotoxicity on allogeneic incompatible target cells by SSPE lymphocytes. This phenomenon can also be observed with effector cells from

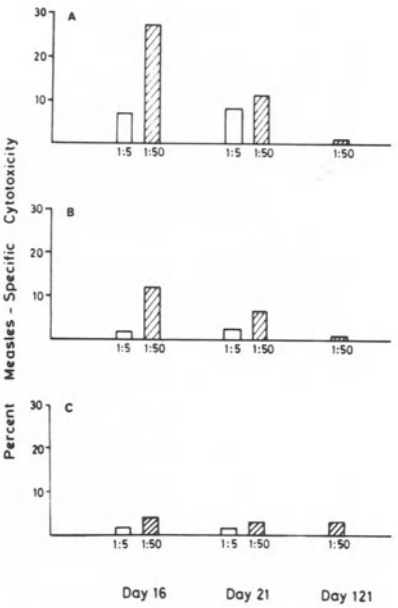


Figure 4. Kinetics and HLA dependency of killer cells in acute measles. Peripheral blood lymphoid cells were isolated from a child (HLA phenotype A1, AW24, B8, BW 44) at day 16, day 21 and day 121 after exposure to measles virus. Onset of measles rash was on day 14. Cells were cryopreserved in the presence of 11 % DMSO and 20 % FCS and stored in liquid nitrogen (17). After thawing, these cells were simultaneously tested in 4 hr Cr-51 release assays against uninfected and measles virus-infected PHA blasts bearing different HLA determinants. A: Autologous target cells prepared from day 121 lymphocytes. B: Semi-allogeneic target cells, HLA type A2, AW31, B8, BW42. C: Allogeneic incompatible target cells, HLA type A2, A26, B15, BW22. Tests were run at effector-target cell ratios of 1:5 and 1:50. Background killing of uninfected PHA blasts was in the range of 1 - 2 %.

normals. The reason for this "allogeneic preference" by natural killer cells is not known.

Markedly different results were obtained with peripheral blood lymphocytes from patients with acute measles(17). As demonstrated by one representative example (Figure 4) cytotoxicity depends on 2 important variables: 1) the stage of acute disease when effector lymphocytes are obtained and 2) the extent of histocompatibility between effector and target cells. As shown in Figure 4A, cytolytic activity of lymphocytes obtained 3 days after measles rash (day 16) is much higher than that of cells collected 5 days later (day 21). A final blood sample donated more than 3 months later (day 121) was devoid of killer cells. These data which could be confirmed in other patients suggest a rapid decline of cytotoxic cells during early convalescence. The speed with which cytotoxicity declines may differ from one patient to another and sampling time seems crucial. This might be the reason why others (18) working in a similar system could not detect any cytolytic activity by cells from acute measles though testing was done under HLA defined conditions.

By fluorescent antibody technique and antibody and complement mediated lysis all target cells were found to contain almost the same percentages of cells bearing surface-bound viral antigens, however, not all virus-infected blast cells were susceptible to lysis. Cytolytic activity was clearly dependent on the degree of histocompatibility between effector and target cells in a hierarchal pattern: Highest cytolysis was found on autologous target cells (Figure 4A), while lysis was minimal on allogeneic incompatible target cells (Figure 4C). Sharing of a single antigen between effector and target cells (e.g. HLA B8 in Figure 4B) elicited an intermediate reaction. It seems that HLA dependent killing in measles is associated both with HLA A and B determinants. Effective cytolysis was observed when effector and target cells shared HLA A2, A3, AW24, A28, B5, B8, B12, BW35.

HLA dependent killer cells from patients with acute

measles cannot be adsorbed onto plastic-bound IgG immune complexes (Figure 5). These cells therefore belong to the FcR γ negative pool of lymphocytes. Similar properties have been reported for virus-specific and alloreactive cytotoxic T cells in mouse and man (19,20,21). It should be noted that killing by SSPE peripheral lymphocytes of autologous virus-infected target cells is totally abrogated by a similar adsorption procedure (Figure 5, lower half).

In summary, killer cells in acute measles have 3 characteristic features: 1) Dependence on the acute stage of the disease, 2) functional restriction by the HLA system, and 3) absence of Fc γ receptors. We think that these cells represent genuine virus-specific T lymphocytes with strikingly similar properties to CTL in other species (16).

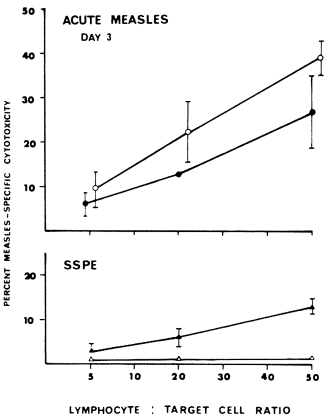


Figure 5. HLA dependent killer cells in acute measles belong to the FcR γ negative cell population. Lymphocytes were tested either unfractionated (●—●, ▲—▲) or after removal of FcR γ bearing lymphoid cells by adsorption to solid IgG immune complexes (○—○, △—△). 4 hr Cr-51 release assays were run on autologous measles virus-infected and uninfected PHA blasts. Background killing of uninfected target cells was in the range of 1 - 2 %.

5. GUIDELINES FOR FUTURE RESEARCH

With measles-specific cytotoxic T lymphocytes confined to acute measles the question is then raised on how to pursue studies on cell-mediated immunity in SSPE. Again, experimental clues can be derived from animal studies. Experiments in mice have shown that although cytotoxic T cells disappear from lymphoid tissues within 20 days after virus infection, memory is still retained. These memory T cells can be reactivated by appropriate antigenic stimulation *in vitro* into secondary populations of cytotoxic T cells (22).

Thus efforts should also be made in man to reinduce measles-specific cytotoxic T lymphocytes *in vitro*. As has been demonstrated for influenza virus in man, virus-specific memory T cells can be found within the pool of recirculating lymphocytes (23,24). Such indirect cytotoxicity tests with measles convalescent and SSPE lymphocytes would be the crucial challenge to BURNET's hypothesis (1). If BURNET's assumption of a specific T cell deficiency is correct, memory for T cell cytotoxicity should not exist in SSPE.

ACKNOWLEDGEMENTS

These studies were supported by grant Kr 376/8 from the Deutsche Forschungsgemeinschaft. H. Pabst is a recipient of the Thyssen Foundation stipend. We wish to thank Lore Kress for excellent technical assistance and Helga Schneider for typing the manuscript.

REFERENCES

1. Burnet, FM: Measles as an index of immunological function. Lancet ii: 610-613, 1968.
2. Agnarsdottir, G: Subacute sclerosing panencephalitis. Recent Adv Clin Virol 1: 21-49, 1977.
3. Simpson, E, PC Beverley: T cell subpopulations. In: Progress in Immunology, III, Amsterdam, North-Holland, 1977, p 206-214.
4. Burns, WH, LC Billups, AL Notkins: Thymus dependence of viral antigens. Nature 256: 654-655, 1975.
5. Valdimarsson, H, G Agnarsdottir, PJ Lachmann: Cellular immunity in subacute sclerosing panencephalitis. Proc Roy Soc Med 67: 1125-1129, 1974.
6. Kreth, HW, YM Kaeckell, V ter Meulen: Cellular immunity in SSPE patients. Med Microbiol Immunol 160: 191-199, 1974.
7. Steele, WR, DA Fuccillo, SA Hensen, MM Vincent, JA Bellanti: Specific inhibitory factors of cellular immunity in children with subacute panencephalitis. J Pediat 88: 56-62, 1976.
8. Perrin, LM, A Tishon, MBA Oldstone: Immunologic injury in measles virus infection. III. Presence and characterization of human cytotoxic lymphocytes. J Immunol 118: 282-290, 1977.
9. Ewan, PW, PJ Lachmann: Demonstration of T cell and K cell cytotoxicity against measles-infected cells in normal subjects, multiple sclerosis and subacute sclerosing panencephalitis. Clin Exp Immunol 30: 22-31, 1977.
10. Norrby, E: A carrier cell line of measles virus in Lu 106 cells. Arch ges Virusforsch 20: 215-224, 1967.
11. Kreth, HW, G Wiegand: Cell-mediated cytotoxicity against measles virus in SSPE. II. Analysis of cytotoxic effector cells. J Immunol 118: 296-301, 1977.
12. Morales, A, GD Bonnard, M Dean, JH Herbermann: In vitro augmentation of natural killer (NK) and antibody-dependent killer (K) cell activities by co-cultivation with mumps virus. (Abstract) Fed Proc 36: 1325, 1977.
13. Trinchieri, G, D Santoli: Anti-viral activity induced by lymphocytes with tumor-derived or virus-transformed cells. Enhancement of human natural killer cell activity by interferon and antagonistic inhibition of susceptibility of target cells to lysis. J exp Med 147: 1314-1333.

14. Santoli, D, G Trinchieri, FS Lief: Cell-mediated cytotoxicity against virus-infected target cells in humans. I. Characterization of the effector lymphocyte. J Immunol 121: 526-531, 1978.
15. Kall, MA, HS Koren: Heterogeneity of natural killer cell populations. Cell Immunol 40: 58-68, 1978.
16. Doherty, PC, RV Blanden, RM Zinkernagel: Specificity of virus-immune effector T cells for H-2K or H-2D compatible interactions. Implication for H2-antigen diversity. Transpl Rev 29: 89-124, 1975.
17. Kreth, HW, V ter Meulen, G Eckert: Demonstration of HLA restricted killer cells in patients with acute measles. Med Microbiol Immunol 165: 203-214, 1979.
18. Galama, JMD, CT Lucas, A Vos: Lymphocyte-mediated cytotoxicity to cells infected with measles virus. I. Use of in vitro infected leukocytes as autologous target cells; absence of virus-specific cytotoxic lymphocytes. Cell Immunol 38: 365-377, 1978.
19. Kedar, E, M Ortiz de Landazuri, B Bonavida: Cellular immunoadsorbents: A simplified technique for separation of lymphoid cell populations. J Immunol 112: 1231-1243, 1974.
20. Ertl, M, M Koszinowski: Cell-mediated cytotoxicity against Sendai-virus-infected cells. Z Immun Forsch 152: 128-140, 1975.
21. Eijsvoogel, VP, TA Schellekens, MJGJ Du Bois, WP Zeijlemaker: Human cytotoxic lymphocytes after alloimmunization in vitro. Transplant Rev 29: 125-145, 1975.
22. Dunlop, MBC, RV Blanden: Secondary cytotoxic cell response to lymphocytic choriomeningitis. I. Kinetics of induction in vitro and yields of effector cells. Immunol 31: 171-180, 1976.
23. McMichael, AJ, B Askonas: Influenza virus-specific cytotoxic T cells in man: induction and properties of the cytotoxic cell. Europ J Immunol 8: 711-715, 1978.
24. Biddison, WE, S Shaw, DL Nelson: Virus specificity of human influenza virus-immune cytotoxic T cells. J Immunol 122: 660-664, 1979.
25. Gmelig-Meyling, F, RE Ballieux: Simplified procedure for the separation of human T and non-T cells. Vox Sang 33: 5-8, 1977.
26. Moretta, L, M Ferrarini, MC Mingari, A Moretta, SR Webb: Subpopulations of human T cells identified by receptors for immunoglobulins and mitogen responsiveness. J Immunol 117: 2171-2174, 1976.

DISCUSSIONS OF PAPERS BY P.J. LACHMANN ET AL. AND H.W. KRETH

This began with a query as to whether the blood-brain barrier was altered in SSPE and, if so, whether this was a selective lesion,as in malignant meningitis or the nephrotic syndrome, or not. The question was also put as to whether immunoglobulins could be synthesised in the central nervous system. In MS and SSPE, this was almost certainly so, as the IgG excess was calculable and had to come from within the CNS. The evidence suggested that oligoclonal immunoglobulin was produced in the CSF; bands were found in CSF protein which were not found in serum but bands found in serum were usually also present in the CSF. It was possible that some of the immunoglobulin was formed inside the CNS, and some outside. The amount of antigen which was synthesised in LCM and measles was considered and it was suggested that probably only a small amount was synthesised outside the CNS in SSPE. There was no idea how much antigen was required to stimulate hyper-immune reactions like those that occur in LCM and SSPE.

The question of HLA subtypes and their relationship to SSPE was raised. A prospective study of measles in children might be productive and there are some data on survivors of the congenital rubella syndrome who have a somewhat similar syndrome to SSPE. Professor ter Meulen mentioned one child in his experience who, one year before the onset of SSPE, was tested for an entirely different reason and was found to have a very high level of measles antibody. Dr Weiss commented that siblings of children with SSPE were less often affected than might be expected; Dr Kreth, in reply, wondered whether HLA subtypes were involved in the host response. Following further discussion on the nature of the immune reaction in SSPE, possible modification of the disease process by immunological stimulation was suggested by Professor ter Meulen. In reply, Dr Lachmann mentioned that Dr Valdemarsson had used transfer factor in some patients; this might have slowed down the disease process but had no real effect.

A question was asked concerning the possible blocking of the T cell response by infected cells being coated with antibody; this did not occur.

Dr Norrby summed up the pathogenesis of SSPE as he saw it. Following measles infection in early childhood (under the age of two

years), a persistent infection in a few target cells in the CNS arose in some individuals, usually boys, where there was an immature immune system which could not eliminate such an infection. At the cellular level, viral antigen was not exposed at the cell surface and, associated with deficient virus matrix protein synthesis, antigen accumulated within the cell. By cell to cell contact, over a period of five to seven years, infection of more cells occurred and a larger amount of antigen accumulated. This was released, leading to hyper-immunisation, break-down of CNS tissue and the precipitation of the clinical features of SSPE. In the persistent infection described, changes in the matrix protein might occur, possibly affecting glyco-protein synthesis and the expression of glycoprotein at the cell surface.

THE BIOLOGY OF RNA TUMOUR VIRUSES

R. A. WEISS

1. INTRODUCTION

RNA tumour viruses, or oncoviruses as they are now called, belong to the family Retroviridae, comprising the retroviruses which also include foamy virus (*Spumavirinae*) and Maedi/Visna virus (*Lentivirinae*). Oncoviruses are divided according to a morphological classification into Type B, C and D viruses (1). Type C viruses have been isolated from or identified in numerous vertebrate hosts ranging from fish to mammals, and also from mosquitos. Oncoviruses are known to cause a variety of neoplasms in their natural host species. Thus the lymphomatous leukoses of cattle, cats, mice and chickens are typically caused by Type C oncoviruses, whereas Type B and D oncoviruses are associated with mammary carcinomas. Rare, acute neoplasms, such as sarcomas, and erythroid and myeloid leukaemias, are also recognised to result from retrovirus infection, as well as non-malignant diseases, such as osteopetrosis in chickens, anaemia in cats, and possibly autoimmune and paralytic diseases in mice. The problem of identifying retroviruses with neoplastic potential in humans remains equivocal, although tantalising items of evidence continue to be thrown up, as exemplified by Thiry's contribution to this volume. While much of the impetus and funding for research in viral oncology is motivated by the search for human retroviruses, retrovirus research today is proving most useful in providing conceptual models of oncogenesis and experimental systems for probing the molecular and cell biology of neoplasia.

2. TRANSMISSION OF RETROVIRUSES

Although the genetic information of retrovirus particles is contained in RNA molecules, upon infection of the host cell this information is transcribed by the viral enzyme, RNA-directed DNA polymerase (reverse transcriptase) into a double-stranded DNA provirus (2). This provirus, like the

genomes of DNA tumour viruses, becomes inserted into host chromosomal DNA, so that the "integrated" viral genes become adopted by the host as extra genetic information. Integration is probably not the oncogenic event itself, although the insertion of new DNA sequences at inappropriate sites could very conceivably cause disruption of cellular regulatory mechanisms. Nevertheless, integration is the means by which viral genes may be heritably transmitted to daughter cells. Furthermore, during the evolution of retrovirus-host relations, retrovirus genomes have on occasion become integrated into cells of the host germ line, with the result that the viral genes are now inherited from one generation to the next as host Mendelian factors. Such stable, inherited viral genomes (called endogenous viruses), with the exception of certain inbred strains of mice, are not known to be oncogenic, but may give rise to oncogenic agents on reactivation to viral form, and may become recombined with other DNA sequences to form new genetic elements that are potentially oncogenic.

Thus retroviruses can persist by masquerading as host genetic information. These endogenous viral genes may be unexpressed for many host generations, or some viral antigens may be synthesised in certain types of host cell. On occasion, complete virus may be activated, either spontaneously, or by treatment of the host cell with ionizing radiation or chemical carcinogens and mutagens. The reactivation phenomenon led to an hypothesis that all cases of oncogenesis by diverse agents might be accounted for by activation of endogenous viruses (3). This now seems unlikely, and the most efficient virus-inducing agents, such as halogenated pyrimidines, have little carcinogenetic potential. The latency and inheritance of retrovirus genomes considerably complicates any analysis of epidemiology, not least because some of the newly activated viruses frequently cannot reinfect cells of the species in which they are inherited, but may be infectious for foreign species, a phenomenon called xenotropism (4).

Leukaemogenic retroviruses, with the important exception of murine leukaemia viruses, are typically transmitted as infectious agents. Thus leukosis is a contagious disease in cats and cattle which is spread horizontally by close contact with infected individuals. Horizontal infection of chickens results most frequently in effective viral immunity, but 'vertical' infection of eggs leads to immunological tolerance and perhaps as a consequence of a persistently high viral load, such congenital infection typically causes a bursal lymphoid leukaemia. Congenital retrovirus infection also occurs in several mammalian species via either the placenta or the milk, and activation of endogenous virus in mouse embryos can even lead to a 'reverse vertical' infection of a non-viraemic mother.

The evolutionary origin of retroviruses is often obscure. The exogenous retrovirus causing bovine leukaemia appeared as a new enzootic agent in Danish cattle some years ago and might have been transmitted from another species. Endogenous viral genomes have frequently been present in the germ line of their hosts for many million years, showing divergence of genetic sequences alongside host gene divergence during evolution. On the other hand, some endogenous viruses have obviously been acquired by exogenous infection emanating from unrelated host species in recent evolutionary times. Thus the endogenous virus of cats has been acquired from ancestors of modern baboons (5). The endogenous virus of chickens comes from an unknown source; it is present in the feral, undomesticated form, the Red Junglefowl, but not in other Junglefowl species (6). Exogenous chicken leukosis viruses are very closely related genetically to the endogenous chicken virus, but differ in host range and leukaemogenicity; presumably they are derived from the endogenous virus. On the other hand, the exogenous feline leukaemia virus strains are quite unrelated to the endogenous feline virus, and may be derived from endogenous rodent viruses. Clearly there has been much hopping in and out of host genomes of different species in the evolution

and spread of retroviruses, and it is possible that these viruses act as vectors of parasexual genetic exchange between phylogenetically distant host species. Mendelian inheritance of retrovirus genomes is the ultimate form of persistant infection, so that viral genes may become subject to natural selection acting on the host species if they are enrolled to perform host functions. For instance, endogenous retroviral glycoproteins in mice may play a role in the maturation of lymphocytes (7) and in the function of certain secretions (8).

3. RETROVIRUS GENES

The proteins of retroviruses are, of course, antigenic, and the preparation of specific antisera for radioimmunoassays and other immunological techniques has been of great use for studying viral gene expression and virus relationships. The other major analytical tool has been nucleic acid hybridisation. With the preparation of specific radioactive probes, the presence and expression of retroviruses and of single retrovirus genes can be accurately monitored, and the evolutionary relationships between retroviruses in different host species can be assessed.

Non-defective retroviruses have a simple unit genome comprising three well-defined genes coding for virion proteins (2). The *gag* gene encodes a large, precursor polypeptide which becomes proteolytically cleaved to generate the internal or core antigens of the virion. These proteins are named according to their estimated molecular weight, e.g. murine p30 denotes the major core protein of 30,000 daltons of murine leukaemia virus (MuLV). Precursor polypeptides are similarly labelled pr65, or pr90, etc. The *env* gene encodes the proteins located in the envelope of the virion which is derived by budding from the plasma membrane of the host cell; thus murine gp70 denotes the glycosylated envelope protein of MuLV of approximately 70,000 daltons. The *pol* gene encodes the polymerase (reverse transcriptase).

The three genes are ordered in the genomic RNA molecule in the sequence 5'-*gag-pol-env*-3'; apart from some nucleotide seqeunces at the 5' end of the molecule, they appear to be translated from separately transcribed mRNA species.

4. ONCOGENESIS

Retroviruses can be roughly divided into three groups on the basis of oncogenicity, 'non-transforming', weakly transforming' and 'strongly transforming' viruses. Those that cause acute neoplasms with short latent periods between infection and the appearance of the tumour are called strongly transforming viruses. In most cases these viruses will also transform appropriate target cells in culture. Weakly transforming viruses cause tumours only after long latent periods, i.e., months rather than days in mice and chickens, the most closely studied host species, and *in vitro* transformation systems have not to date been devised for these viruses. Some endogenous C-type retroviruses such as those of cats and chickens, may be regarded as non-transforming, but this classification may have to be modified when they are studied in more detail.

4.1. *Strongly transforming viruses*

These viruses rarely occur in nature, but their recognition, isolation and experimental use has led to major advances in our understanding of viral oncogenesis. The best known and most venerable example of a strongly transforming virus is the Rous sarcoma virus (RSV) of chickens (9), others are the avian myeloblastosis and erythroblastosis viruses, and the murine Friend erythroleukaemia, Abelson lymphoma and Moloney, Kirsten and Harvey sarcoma viruses. RSV has a gene, designated *src* for sarcoma induction, in addition to the three genes essential for viral replication. Studies of deletion mutants and temperature-sensitive mutants have shown that the *src* gene is essential for fibroblast transformation and for sarcomagenesis, but is not required for viral replication. Recent data indicate that the *src* gene product is a cytoplasmic protein of 60,000 daltons that possesses protein kinase activity (10). Precisely how this

protein causes cell transformation and what are the crucial targets in the cell for phosphorylation remains to be determined. Nevertheless it is a remarkable advance in experimental oncogenesis that an enzyme has been identified with an oncogene.

In most strains of RSV, the *src* gene is carried as an extra gene to the viral genes, in the order 5'-*gag*-*pol*-*env*-*src*-3'. All other well studied strongly transforming viruses are defective for replication, that is, new genetic information specifying neoplastic transformation (*onc* genes) appears to be inserted in the viral genome in place of essential genes for replication. A part of the *gag* gene and the 3' end of the viral genome are usually maintained, giving a typical structure 5'-*ga*-*onc*-3'. The infectivity of such defective viruses relies on the presence of replication-competent 'helper' viruses, and the disease spectrum caused by such viruses depends on the properties of this complex virus population. In several strains of defective virus, the polypeptide coded by the supposed *onc* region of the viral genome actually starts in what remains of the *gag* gene. Since the polypeptide therefore bears some *gag* antigens, it can be identified by immunoprecipitation with anti-*gag* anti-sera from lysates of transformed cells. Such *gag*-*onc* 'polyproteins' have been detected in cells transformed by avian myelocytoma and erythroleukaemia viruses, murine Abelson leukaemia cells, and cells transformed by feline sarcoma virus. These proteins are not related to the *src* protein of RSV or to each other, and each may have individual functions resulting in neoplastic transformation. This would account for the high degree of specificity of the target cell for transformation, as each virus causes a specific type of cancer or leukaemia.

Oncogenes appear to originate from the host, as genetic elements related by molecular hybridisation to viral oncogenes are found in the host genome, though not linked to endogenous viral elements (13). Possibly the natural host sequences code for normal proteins important in the

function or differentiation of particular cell types (15). When they are picked up and modified by viral genomes, and reinserted into appropriate target cells, they may cause disruption to regulatory cell functions, blocking or even reversing the normal pathway of differentiation. Further analysis of the oncogenes of strongly transforming viruses should illuminate much about differentiation and neoplasia.

4.2. *Weakly transforming viruses*

These viruses do not appear to carry oncogenes distinguishable from the three viral genes, *gag*, *pol* and *env*. Commonly occurring weakly transforming viruses are the murine mammary carcinoma virus, murine thymic lymphoma viruses, avian bursal leukosis viruses, and the leukaemia viruses of cats and cattle. In contrast to the strongly transforming viruses, the tumours they cause appear after long latent periods and only very few of the cells that become infected subsequently give rise to tumours. The tumours are probably clonal in origin, whereas with strongly transforming viruses such as RSV the tumours grow as quickly by infection and transformation of new target cells, as by mitosis of the originally transformed cell. There is growing evidence for the murine viruses which induce thymic lymphomas that genetic recombination involving the *env* gene takes place, often between xenotropic and mouse-tropic endogenous viruses, giving rise to new virus variants which may interact with and transform different cell types than those transformed by the parental viruses (15). The recombinant *env*-coded glycoproteins may play a dual role, both by allowing the virus to recognise and infect specific target cells bearing appropriate receptors for the glycoproteins and by acting as a perpetual mitogenic stimulus to such cells.

In summary, oncoviruses persist so intimately with their hosts, that genetic exchange in both directions has taken place. On the one hand, viral genomes have become adopted and perhaps exploited as new host genes; on the other hand, host genetic sequences have been incorporated into viral genomes, which in some cases become highly oncogenic.

References

1. Tooze, J: The molecular biology of tumour viruses, Cold Spring Harbor Laboratory 1973.

2. Baltimore, D: Tumor Viruses, Cold Spring Harbor Symp. Quant. Biol. 39:1187-1200, 1974.

3. Todaro, GD: and RJ Huebner: The viral oncogene hypothesis: new evidence. Proc. Nat. Acad. Sci. U.S.A. 69:1009-1015, 1972.

4. Weiss, RA: Receptors for RNA tumor viruses. In: Cell membrane receptors for viruses, antigens and antibodies, polypeptide hormones, and small molecules, Beers, RF, EG Bassett (eds), Raven Press, New York, 1976, p237-251.

5. Todaro, GJ: RNA tumour virus genes and transforming genes: patterns of transmission. Br. J. Cancer 37: 139-158, 1978.

6. Frisby, DP, RA Weiss, M Roussel, D Stehelin: The distribution of endogenous chicken retrovirus sequences in the DNA of galliform birds does not coincide with avian phylogenetic relationships. Cell, in press.

7. Moroni, C, G Schumann: Are endogenous C-type viruses involved in the immune system? Nature 269:600-601, 1977.

8. Elder, JH, FC Jensen, ML Bryant, RA Lerner: Polymorphism of the major envelope glycoprotein (gp70) of murine C-type viruses: Virion associated and differentiation antigens encoded by a multi-gene family. Nature 267:23-28, 1977.

9. Rous, P: A sarcoma of the fowl transmissible by an agent from the tumor cells. J. Exp. Med. 13:397-402, 1911.

10. Collett, MS, RL Erikson: Protein kinase activity associated with the avian sarcoma virus src gene product. Proc. Nat. Acad. Sci. U.S.A. 75:2021-2024, 1978.

11. Bister, K, MJ Hayman, PK Vogt: The defectiveness of avian myelocytomatosis virus MC29: isolation of long term non-producer cultures and analysis of virus specific polypeptide synthesis. Virology 82:431-448, 1977.

12. Witte, ON, N Rosenberg, M Paskind, A Shields, D Baltimore: Identification of an Abelson murine leukemia virus-encoded protein present in transformed fibroblast and lymphoid cells. Proc. Nat. Acad. Sci. U.S.A. 75:2488-2492, 1978.

13. Stehelin, D, HE Varmus, JM Bishop, PK Vogt: DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal DNA. Nature 260: 170-173, 1976.

14. Risser, R, E Stockert, LJ Old: Abelson virus: a viral tumor antigen that is also a differentiation antigen of BALB/c mice. Proc. Nat. Acad. Sci. U.S.A. 75: 3918-3922, 1978.

15. Elder, JH, JW Gautsch, FC Jensen, RA Lerner, JW Hartley, WP Rowe: Biochemical evidence that MCF murine leukemia viruses are envelope (*env*) gene recombinants. Proc. Nat. Acad. Sci. U.S.A. 74: 4676-4680, 1977.

EVIDENCE FOR THE PRESENCE OF RETROVIRUS MARKERS IN MAN

L. THIRY

Three simian viruses which may be related to human retrovirus strains.

Virologists in search of retroviruses in man must bear in mind that these viruses may be either endogenous with xenotropic properties, or exogenous with ecotropic characteristics. A simian virus of the first type is the Baboon endogenous virus (BeV), the complete genome of which is present in the DNA of all tissues of monkeys taxonomically related to baboons (1). However, the virion is expressed only during pregnancy and then only in the placenta (2). Even in this tissue, the cells are probably not highly permissive for BeV replication, for continuous production of the virus has been obtained only by cocultivation of baboon placentas with cells from other animal species, among which the human rhabdomyosarcoma cell line A204. Using cell hybrids formed from the fusion of mouse cells with a human cell line, (VA) infected with BeV, it was possible to demonstrate that the BeV genome had been integrated in human chromosome 6 (3). However, ^{125}I labelled RNA from BeV hybridized poorly with the DNA of normal human organs (4). Two agents will be cited as examples of exogenous viruses with ecotropic properties, Mason-Pfizer virus (MPMV) and Simian Sarcoma virus (SiSV). The characteristics of MPMV have been reviewed recently (5). This virus was originally isolated from a breast carcinoma of a rhesus monkey. Although it can also be found in placentas and lactating glands of pregnant monkeys it is transmitted horizontally, not vertically. The genome of MPMV is not present in the DNA of normal tissues in the natural hosts. It multiplies freely in cells from monkeys and humans, but not in cells from the many other mammals tested thus far. Although MPMV possesses the capacity to transform monkey and human cells *in vitro*, attempts to induce tumours in various strains of monkeys have been unsuccessful. In contrast, the Simian Sarcoma virus, first isolated from a fibrosarcoma of a woolly monkey (6) can induce tumours in newborn marmosets (7). This virus is replication-defective, but in the presence of a helper virus (Simian Sarcoma-associated virus) it multiplies to high titers in human and other mammalian cells.

Retrovirus footprints in human cancerous and normal tissues.

The complicated results obtained with leukaemic cells have been reviewed recently (8). Attempts to isolate infectious virions must take into account the possibility that tumour cells may contain defective or xenotropic viruses. The multiplication of defective virus can only be obtained in the laboratory by deliberate addition of helper viruses. To detect xenotropic viruses, a wide range of animal cells must be tested empirically. Alternately, one may attempt to produce pseudotypes by cultivating the tumour cells in the presence of a virus which replicates in human cells. The latter virus can provide adequate envelopes for the putative viral genomes originating from the tumour tissues. However, these laboratory

manipulations may introduce other complications. The added laboratory strains may yield recombinants with the patient's viral genes. Sometimes, introduction of viral genes in the experimental protocol may be accidental. This might have occurred when HL-23 virus was isolated after cultivation of cells from a case of acute myeloid leukemia in medium conditioned by a unique human embryo cell strain (9). As discussed below, the possibility that a virus was present in the conditioning medium was made more likely by accumulating evidence that retro viruses are preferentially induced during embryogenesis and differentiation. Actually, the HL-23 isolate was found to contain two viruses, one closely related to SiSV and the other to BeV (10). The obvious tactic was to look for markers of BeV and SiSV in the tissues of leukaemic patients. DNA from several patients with leukaemia hybridized 70 % of the hybridizable RNA from BeV, and Tm of the hybrids was high, while only 23 % of the BeV RNA hybridized to DNA of normal tissues, with a lower Tm (11). To our knowledge, the latter high figure for BeV nucleotide sequences in normal human tissues has not been confirmed by others. SiSV information was not found in the form of proviral DNA in leukaemic or other human tissues. However , cytoplasmic particles hybridizable to SiSV probes were detected in leukaemic cells from 2 patients, but such RNA sequences were also found in normal lymphocytes stimulated in vitro with PHA. The presence of retroviruses can also be demonstrated with the use of the XC or KC plaque assays. SiSV viruses form syncytia on the XC cells, while MPMV and BeV produce syncytia on the KC cells. Bone marrow cells from a child with acute lymphoblastic leukaemia formed plaques on XC cells after stimulation with PHA (8, 12). Negative results were obtained with similarly treated cells from 7 adults with other types of leukaemia and 4 normal adult donors. The samples were not seeded on KC cells, so there is no evidence for or against the presence of a viral component biologically related to MPMV or to BeV.

Several authors have attempted to demonstrate the presence of viral genes by cocultivating the tumour cells with putative permissive cells. In these experiments, attempts are made to insure close initial contact between the two types. We suggest that efficiency of the method might be increased, if the cells were fused with polyethylene glycol. To our knowledge, no such attempt has been reported in the literature. If one looks for infectious virions in the supernatants of tumour cell cultures, these supernatants should be seeded on putative permissive cells pretreated with polybrene to increase the chances of virus penetration. If whole genomes of retroviruses were present in cancer cells, but without production of enveloped virions, infectious DNA should be demonstrable by transfection experiments. By this technique, no positive results were obtained from the leukemic cells of 12 patients, nor did the extracted DNA recombine with or rescue endogenous human virus or BeV (13). The possibility must be considered that only some of the viral genes are integrated

into the host genome. It may be possible to detect RNA transcripts of such genes with the newly developed in situ hybridization technique.

Attempts to detect viral coded proteins have led to conflicting results. Extracts from peripheral blood leukocytes of 5 patients with acute leukemia competed with labelled p30 related to SiSV in a radioimmunoassay (14). In similar assays, a p30 related to those of known retroviruses was detected in both normal and malignant human tissues (15). However, proteolytic enzymes present in tissue extracts can break down the labelled polypeptides. The results of such experiments would then mimic the displacement of the labelled polypeptides by viral polypeptides thought to be present in the cell extracts. Non-specific inhibitors which interfered with radioimmunoassays were found in several human tissue specimens (16).

Antigens related to SiSV (17) or to BeV (18) were detected in cultures of sarcoma cells treated with specific antisera in an indirect immunofluorescence test. Finally, an indirect demonstration of the presence of a retrovirus antigen on the surface of leukocytes from patients with chronic myelogenous leukaemia was obtained by demonstrating that immunoglobulins eluted from the leukaemia cells specifically neutralized the reverse transcriptase of feline leukaemia virus (19).

In regard to the problem of human breast carcinoma, we feel that it is premature to discuss the evidence for markers of mouse mammary tumour virus and/or of MMPV in this type of tumour.

Antibodies to retrovirus antigens in healthy individuals.

Here again we are confronted with apparently discrepant results, even though similar radioimmunoprecipitation procedures were used. By this method, antibodies to SiSV virions were found in most human sera, while the results were negative with purified gp 70 of the same virus (20). Part but not all of the reactivity with SiSV was removed after adsorption of the human sera with calf serum. This fits with the findings that serum components from culture medium become associated with budding retrovirus particles, and suggests that some humans have antibodies directed to calf proteins. Negative results with gp70 may be related to fragility of the molecule, and to the possibility that the chloramine T treatment during radioiodination procedure denatured the reactive determinant. Positive reactions with SiSV virions were confirmed by another group (21), who demonstrated antibodies to BeV particles in about half the adult sera tested. Lack of reactivity of human sera with internal viral proteins was apparent from data showing that human sera did not react with p30 of a virus related to SiSV (22) nor with p25 of MPMV (23).

A role of retroviruses in human reproduction?

a. Reverse transcriptase in spermatozoids.

A DNA polymerase with the properties of reverse transcriptase was isolated from the nuclei of human spermatozoids (25), and showed an immunological relationship to

BeV reverse transcriptase. Immunoglobulins from partners of infertile marriages inhibited both sperm and BeV reverse transcriptase. The authors postulated that the sperm polymerase plays a positive role during fertilization and/or early embryogenesis.

b. Retroviruses during differentiation and embryogenesis.

Soon after the discovery of reverse transcriptase, it was suggested that this enzyme could play a physiological role if present in normal cells. For instance, at some stages of embryogenesis, a reverse transcriptase might use ribosomal RNA as templates to reproduce DNA copies and lead to gene amplification. This hypothesis has not been substantiated by experimental results. However, the suggestion that retroviruses might play a part in normal cell differentiation was strongly supported by the apparent linkage between regulation of type C RNA virus production and cell differentiation in mouse myeloid leukaemic cells (26). Virus production was an early step in the induction of differentiation, indicating that it was a causative factor rather than an effect. Also, the induction of murine B lymphocyte differentiation into immunoglobulin secreting cells was accompanied by budding and release of retrovirus particles (27). However, attempts to demonstrate virus induction in human lymphocytes have failed so far.

A diploid cell culture, HEL-12, was obtained from the lungs of an 8-week-old human embryo and was frozen after a primary growth cycle (28). Reinitiation of cell growth from the frozen stock yielded virus expression after several subcultures and a lag period of 80-120 days. Expression of viral antigens, reverse transcriptase and infectious virus was cyclic. The virus grew on human and other mammalian cells, and was composed of a heterogeneous population with a SiSV-like and a BeV-related component.

We have studied 6 short-term cultures of 6-10 weeks old human embryos, 2 of which were obtained by curettage because of non-progressive pregnancies in women with repeated spontaneous abortions, and 4 were aspiration products from women with social problems (unpublished results). Semi-confluent cultures of heterogeneous cell populations were obtained within 4-7 days. At that point, cells were treated with 30 ug/ml of 5-iododeoxyuridine, in order to increase the chances of retrovirus expression, and then submitted to three tests. Culture media were concentrated and assayed for reverse transcriptase activity in the presence of Mg^{++} or Mn^{++}. A portion of the cells was seeded on KC and on XC cells to test for viral dependent syncytial cell formation. The remaining cells of the foetal culture were labelled with ^{51}Cr and treated with anti-BeV and anti-MPMV sera in the presence of complement. Indications of the presence of a BeV-related virus were obtained in 1 of the 2 cases of non-progressive pregnancy, and 3 of the 4 aspirates from the apparently healthy cases. Positive evidence included : syncytium formation on KC cells, ^{51}Cr release from the foetal cells exposed to anti-BeV serum, and weak but significant Mn^{++} dependent reverse transcriptase activity. Attempts to cultivate the virus(es)

in the human cell line A 204 are in progress.

c. Retrovirus markers in placentas.

Some of the described results tend to support the idea that retroviruses play a part in early implantation of the embryo and/or in its early growth. There is also the possibility that the expression of retroviruses is induced as a consequence of pregnancy. Induction of retroviruses was reported in mice with an allogenic graft. A similar situation might occur in pregnant women bearing a histoincompatible foetal graft. The first description of C-type virus particles in human placentas (29) has been confirmed by several groups, and reverse transcriptase activity has been detected in more than 80 % of full term human placentas (30). These results, however, do not provide unequivocal evidence that retroviruses are preferentially expressed in placental tissues since other organs have not been subjected to comparable studies.

d. Immune responses to retrovirus antigens in pregnant women.

Indications that retrovirus antigens are transiently expressed during pregnancy came from the findings that lymphocytes from pregnant women recognized simian retrovirus antigens and reacted in lymphoblastogenesis assays in vitro, while these reactions were negative in most women with similar numbers of pregnancies, but studied at least 10 months after the last delivery (31). The incidence of positive responses was greater in multiparous than in primiparous pregnant women. In other work, 6 women followed during pregnancy developed cell-mediated reactivity against retrovirus infected cells, as assayed by lymphocytotoxicity.Responsiveness peaked during the second and third trimesters of pregnancy and corresponded with elevated levels of antibodies to the same retrovirus (32).

e. Specificity of retrovirus markers related to human reproduction.

At this point, it is necessary to comment on the retrovirus antigens detected by the various investigators. Reverse transcriptase from human sperm was specifically neutralized by an antiserum to the BeV enzyme. In contrast, reverse transcriptase from full term placentas was not susceptible to BeV antibodies, and the in vitro enzyme activity required Mg^{++} instead of Mn^{++}, an indication that this enzyme was associated with a D-type virus. Apparently, susceptibility of the placental enzyme to anti MPMV antibodies was not tested. In pregnant women, transient lymphocytotoxicity was directed towards cells infected with BeV, and the antibodies precipitated BeV antigens. These tests were not performed with MPMV antigens. Lymphoblastogenesis was induced in vitro by either BeV or MPMV infected cells. Although these two viruses are very different from each other, they share one antigenic determinant . However, in the lymphoblastogenesis assays, women who reacted to BeV antigens did not always respond to MPMV. No reactions against SiSV antigens were detected either by lymphoblasto-

genesis or by lymphocytotoxicity assays.

f. Immune reactions to retrovirus antigens in cord blood.

Our unpublished studies show that :

1. In pregnancies where maternal lymphocytes were significantly stimulated in vitro by either BeV or MPMV antigens, lymphocytes from the cord blood reacted to the same viral antigen as those of the mother. There were some additional cases where positive results were obtained with the infant's blood, but the mothers lymphocytes did not respond. The latter cases occurred mostly in first pregnancies. It was also noted that the indexes of lymphocyte stimulation were higher in cord rather than in maternal blood in first pregnancies. These results indicated that the retrovirus antigens which sensitized the lymphocytes were located at a site available for recognition by both foetus and mother, and that the degree of sensitization of the mothers increased with the number of pregnancies without a corresponding increase in successive foetuses.

2. Neutralizing antibodies to BeV assayed by inhibition of syncytium formation on KC cells were present in 19 of 35 (54 %) of the maternal sera but only in 8 of 35 (23%) of the cord blood sera. In addition, cord sera showed lower neutralizing activity than the maternal sera as determined by the amount of syncytium forming units neutralized by sera at final dilution 1 : 10.

3. Pairs of maternal and cord sera contained complement dependent cytotoxic antibodies specific for ^{51}Cr labelled dog thymus cells producing BeV, or HeLa and rhesus foreskin cells (940C3) producing MPMV, or both. These antibodies were also found more frequently in maternal than cord serum, and the activity of maternal sera was also greater, as detected by the percentage of ^{51}Cr released by sera at final dilutions of 1 : 20 and 1 : 200.

Two possible explanations suggested themselves. The maternal antibodies could have been of the IgM class which cannot cross the placental barrier. Alternatively, maternal IgG antibodies could have been partly adsorbed while filtering through the barrier which apparently can express retrovirus antigens. The placenta has been shown to function as an immunoadsorbent for antibodies directed against paternal HLA antigens. Experiments indicated that both hypotheses were correct. Thirty five maternal sera with cytotoxic antibodies to BeV infected cells were adsorbed 2 times with a suspension of Staphylococcus Cowan A. Thirteen samples retained activity after this adsorption, indicating the presence of IgM or IgG_3 molecules, which do not bind to protein A of the staphylococcus. To distinguish between IgM and IgG_3, the sera were ultracentrifuged in sucrose gradients. Most of cytotoxicity activity against BeV infected cells was found in the 19S region in 13 of the 35 maternal sera. This would explain the lower titers in the related cord sera in these cases. The other 22 mothers, whose antibodies were of the G_1, G_2 or G_4 immunoglobulin subclasses which can filter through the placenta, passed

only a limited amount of anti-BeV antibody to the foetus. This would support the idea that retrovirus antigens expressed by the placenta acted as immunoadsorbents.

We then eluted immunoglobulins from the washed tissue of 17 placentas by treatment at pH2. After concentration, the immunoglobulins preparations were assayed for toxic or blocking activity on dog cells, infected or not with BeV. One preparation was toxic for dog cells in the presence of complement, one was specifically toxic for BeV infected dog cells. Among the 15 non cytotoxic immunoglobulin preparations, 6 (40%) blocked the cytotoxic activity of anti-BeV serum on BeV producing dog cells. It has been shown that antibodies eluted from the placentas show specificities for the trophoblasts. The localized expression of retrovirus antigens in placentas, during pregnancy, may at least partly determine the specificity of the antitrophoblast antibodies. Blocking antibodies to retrovirus antigens may help to protect the foetal tissues against maternal immune response.

g. Retroviruses in pre-eclampsia.

The tests described above were then applied to 28 women with symptoms of pre-eclampsia.

As compared to healthy pregnant women, this group of patients had a higher frequency both of lymphoblastogenic responses to BeV or MPMV antigens (33), and IgM maternal cytotoxic antibodies to retrovirus infected cells (our unpublished results). To determine whether these features are due to an increased expression of retrovirus antigens, or to hyperimmune responses, attempts are under way to detect, localize and quantitate retrovirus antigens which may be present on syncytiotrophoblasts or on the underlying basement membrane. It also appeared that passage of retrovirus antibodies from the mother to the foetus was greater in pre-eclampsia. This might have been due to injury of the choriodecidual junction.

h. Retroviruses, chronic nephritis and kidney graft.

Because retrovirus expression had been described in lymphocytes of mice bearing skin allografts, we attempted to isolate a virus from patients with kidney grafts by cocultivating their leukocytes with SIRC cells (34). In one case, a virus population with dual properties was obtained, those of MPMV, plus those of another virus which could not be typed serologically but which formed plaques on XC cells. It could not be determined if the factors which made the isolation possible were related to the nephritis for which the patient was treated, to activation by the kidney graft or to immunosuppressive treatments. In an attempt to distinguish among these possibilities, sera were obtained from 44 patients prior to renal transplantation, and from 27 of these patients after grafting (35). Neutralization of MPMV syncytium forming units was found in the pre-operative sera of 12 of 24 (50 %) patients with chronic glomerulonephritis but only in 2 of 20 (10 %) of the patients with other renal diseases. The difference between the two groups of patients did not seem to be due to

differences of treatment. In particular, there was no correlation between the number of transfusions received and the incidence of MPMV antibodies. However, seroconversions were observed after renal transplantation and immunosuppressive treatments. Of 19 patients without MPMV antibodies before graft, 10 (53 %) acquired these antibodies within 2-10 months after the graft. BeV antibodies were found in 6 % of the patients with chronic glomerulonephritis and in 9 % of those with other diseases. Seroconversions were in frequent after treatment.

Indications that chronic glomerulonephritis in humans is associated with the expression of retrovirus antigens were also obtained by two other groups.

Postmortem studies of three cases of systemic lupus erythematosus showed that immunoglobulins eluted from the kidneys reacted specifically with the p30 of RD114 virus, which is related to BeV (36). These results fitted well with the report of antigens related to HeL-12 virus in the kidneys and other tissues of a patient who died from SLE (37). As already stated, HeL-12 virus populations appeared to be made of BeV and SiSV components, and the p30 of BeV shares some antigenic determinants with MPMV. Because viral gp proteins were not studied by either of the two groups just cited the possibility has not been ruled out that chronic glomerulonephritis is associated with viruses containing a p30 related to MPMV and BeV, and enveloped by glycoproteins with MPMV specificities. This could explain why neutralizing antibodies to MPMV, rather than to BeV, were found in association with some cases of chronic glomerulonephritis.

I thank doctor RC Hard for helping in the preparation of this manuscript.

REFERENCES

1. Benveniste, R E , M M Lieber, D M Livingston, C J Sherr; Infectious C-type virus isolated from a baboon placenta. Nature 248 : 17-20, 1974.

2. Kalter S S , R J Helmke, M Panigel, R L Heberling, P J Felsburg, L R Axebrod : Observations of apparent C-type particles in baboon (Papio cynocephalus) placentas. Science 179 : 1332-1333, 1973.

3. Lemons R S , W G Nash, S J O'Brien, R E Benveniste, C J Sherr : A gene(BeVi) on human chromosome 6 is an integration site for Baboon type C DNA provirus in human cells. Cell 14 : 995-1005, 1978.

4. Donehower L , F Wong-Stoal, D Gillespie : Divergence of Baboon endogenous type C virogenes in primates : genomic viral RNA in molecular hybridization experiments. J. Virology : 932-941, 1977.

5. Fine D , G Schochetman : Type D primate retroviruses : a review. Cancer Res. 38 : 3123-3139, 1978.

6. Theilen G H , W Gould, M Fowler, D Dungworth : C-type virus in tumor tissue of a woolly monkey (Lagothrix spp.) with fibrosarcoma. J. Natl. Cancer Inst. 47 : 881-889.

7. Wolfe L G , F Deinhardt, G H Theilen, H Rabin, T Kawakami, L K Bustad : Induction of tumors in marmoset monkeys by simian sarcoma virus, type 1 (Lagothrix) : preliminary report. J. Natl. Cancer Inst. 47 : 1115-1120, 1971.

8. Nooter K : Studies on the role of RNA tumour viruses in human leukaemia. In : Publication of the Radiobiological Institute of the organization for Health Research TNO, Rijswijk, The Netherlands, 1979.

9. Gallagher, R E , R C Gallo : Type C RNA tumor virus isolated from cultured human acute myelogenous leukemia cells. Science 187 : 350-353, 1975.

10. Okabe H , R V Gilden, M Hatanaka, J R Stephenson, R E Gallagher, R C Gallo, S.R. Tronick, S.A. Aaronson. Immunological and biological characterization of type C viruses isolated from cultured AML cells. Nature 260 : 264-266, 1976.

11. Wong-Staal F , D Gillespie, R C Gallo : Proviral sequences of baboon endogenous type C RNA virus in DNA of human leukaemic tissues. Nature 262 : 190-195.

12. Nooter K , A M Aarssen, P Bentvelzen, F G de Groot, F G Van Pelt : Isolation of infectious C-type oncornavirus from human leukaemic bone marrow cells. Nature 256 : 595-597, 1975.

13. Nicolson M O , R V Gilden, H Charman, N Rice, R Heberling, R M Mc Allister : Search for infective mammalian type-C virus related genes in the DNA of human sarcomas and leukemias : Int. J. Cancer 21 : 700-706 (1978).

14. Sherr C J , G J Todaro : Primate type C virus p30 antigen in cells from humans with acute leukemia. Science 187 : 855-857, 1975.

15. Strand M , J T August : Type C RNA virus gene expression in human tissue. J. Virol. 14 : 1584-1596, 1974.

16. Stephenson J R , S A Aaronson : Search for antigens and antibodies cross-reactive with type C viruses of the woolly monkey and gibbon ape in animal models and in humans. Proc. Nat. Acad. Sci. USA 73 : 1725-1729, 1976.

17. Zurcher C , J Brinkhof, P Bentvelzen, J De Man : C-type virus antigens detected by immunofluorescence in human bone tumour cultures. Nature 254 : 457:459,1975.

18. Smith H S , J L Riggs, E L Springer : Expression of antigenic cross-reactivity to RD114 p30 protein in a human fibrosarcoma cell line. Proc. Nat. Acad. Sci.USA 74 : 744-748, 1977.

19. Jacquemin P C , C Saxinger, R Gallo : Surface antibodies of human myelogenous leukaemia leukocytes reactive with specific type-C viral reverse transcriptase. Nature 276 : 230-236, 1978.

20. Snyder H W , T Pincus, E Fleissner : Specificities of human immunoglobulins reactive with antigens in preparations of several mammalian type-C viruses. Virology 75 : 60-73, 1976.

21. Kurth R , N M Teich, R Weiss, R T D Oliver : Natural human antibodies reactive with primate type-C viral antigens. Proc. Nat. Acad. Sci. USA 74 : 1237-1241,1977.

22. Stephenson J R , S A Aaronson : Search for antigens and antibodies crossreactive with type C viruses of the woolly monkey and gibbon ape in animal models and in humans. Proc. Nat. Acad. Sci. USA 73 : 1725-1729, 1976.

23. Charman H P , R Rahman, M H White, N Kim, R Gilden : Radioimmunoassay for the major structural protein of Mason-Pfizer monkey virus : attempts to detect the presence of antigen or antibody in humans. Int. J. Cancer 19 : 498-504, 1977.

24. Devare S G , L O Arthur, D L Fine, J R Stephenson : Type D oncornaviruses : immunologic cross-reactivity between major structural proteins of New and Old world primate virus isolates. J. Virol. 25 : 797-805, 1978.

25. Witkin S S , P J Higgins, A Bendich : Inhibition of reverse transcriptase and human sperm DNA polymerase by anti-sperm antibodies. Clin. Exp. Immunol. 33 : 244-251, 1978.

26. Liebermann D , L Sachs : Co-regulation of type C RNA virus production and cell differentiation in myeloid leukemic cells. Cell 15 : 823-835, 1978.

27. Moroni C , G Schumann : Mitogen induction of murine C-type viruses IV. Effects of lipoprotein E. Coli, pokeweed mitogen and dextran sulphate. J. Gen. Virol. 38 : 497:503, 1978.

28. Panem S , E V Prochownik, W M Knish, W H Kirsten : Cell generation and type C virus expression in the human embryonic cell strain HeL-12. J. Gen. Virol. 35 : 487:495, 1977.

29. Kalter S S , R J Helmke, R L Heberling, M Panigel, A K Fowler, J E Strickland, A Hellman : C-type particles in normal placentas. J. Natl. Cancer Inst. 51 : 225-233, 1974.

30. Nelson J, J A Leong, J A Levy : Normal human placentas contain RNA-directed DNA polymerase activity like that in viruses. Proc. Natl. Acad. Sci. USA. 75 : 6263-6267, 1978.

31. Thiry L , S Sprecher-Goldberger, M Bossens, F Neuray: Cell-mediated immune response to simian oncornavirus antigens in pregnant women. J. Natl. Cancer Inst. 60 : 527-532, 1978.

32. Hirsch M S , A P Kelly, D S Chapin, T C Fuller, P H Black, R Kurth : Immunity to antigens associated with primate C-type oncoviruses in pregnant women. Science 199 : 1337-1340, 1978.

33. Thiry L , S Sprecher-Goldberger, M Bossens, F Neuray: Immune response to primate oncornaviruses in pre-eclampsia. Lancet 1 : 1268, 1978.

34. Thiry L , S Sprecher-Goldberger, P Vandenbussche, M Bossens, P Vereerstraeten, O Hestermans-Medard : Mason-Pfizer like virus in kidney grafted patients. Med. Microbiol. Immunol. 165 : 255-269, 1979.

35. Thiry L , S Sprecher-Goldberger, M Bossens, J Cogniaux-Le Clerc, P Vereerstraeten : Neutralization of Mason-Pfizer virus by sera from patients treated for renal diseases. J. Gen. Virol. 41 : 587-597, 1979.

36. Mellors R C , J W Mellors : Type C RNA virus-specific antibody in human systemic lupus erythematosus demonstrated by enzymoimmunoassay. Proc. Natl. Acad. Sci.USA 75 : 2463-2467, 1978.

37. Ordonez N G , S Panem, A Aronson, H Dalton, A I Katz, B H Spargo, W H Kirsten : Viral immune complexes in Systemic Lupus Erythematosus. C-type viral complex deposition at extrarenal sites. Virchows Arch. B. Cell Path. 25 : 355-366, 1977.

DISCUSSIONS OF PAPERS BY R.A. WEISS AND L. THIRY

Dr Lachmann asked whether the 'sarc' gene was still recognised. It was, and coded for a protein of 60,000 daltons, was concerned with transforming cells in culture and with sarcomagenesis, acting as a protein kinase. Sites of mutation in the 'sarc' gene had been precisely located. The 'sarc' gene presumably had some normal function but probably gave rise to oncogenesis when presented in an unscheduled form.

Following this, the role of the oncornaviruses (oncoviruses) in nature was scrutinised. Their natural role was, presumably, in immunology and development. In red jungle fowl, 'en' and 'gag' genes were expressed in embryos; but in grey jungle fowl, these were not detected and the animals remained healthy. It seemed, therefore, that other, as yet undiscovered, oncornaviruses might be present in the grey jungle fowls. Todaro had suggested that, in some strains of mice, up to 7% of the coding sequences represent exogenous retroviruses. These viruses form glycoproteins in plasma and are found in semen. There have been reports of a gene which suppresses oncornavirus production but little more was known. Such genes might act at many stages and there were many species of oncornavirus in mice.

Dr Weiss concluded this discussion by stressing that the role of the oncornaviruses in nature was a chicken and egg argument all the time.

VISNA. THE BIOLOGY OF THE AGENT AND THE DISEASE

G. PÉTURSSON, J. R. MARTIN, G. GEORGSSON, N. NATHANSON and AND
P. A. PÁLSSON

1. INTRODUCTION

In the years 1937-1952 a disease of the central nervous system of sheep was observed in the southwestern part of Iceland. This disease was called visna which means wasting or withering. Visna usually presented as a paralysis of the hind legs, and the course was rather slow but usually progressive, ending in death (1,2).

Visna was associated with a progressive pneumonia called maedi (3), and studies in Iceland proved both syndromes to be caused by closely related viruses (4,5). Investigations of these diseases and other sheep diseases, such ar rida, the Icelandic form of scrapie, led Sigurdsson to formulate his concepts of a special category of infectious diseases, the slow infections (6,7). A particular feature of the pathological picture in natural visna, rather conspicuous demyelinated areas in the central nervous system, suggested that visna might provide insights into the demyelinating disorders of man, such as multiple sclerosis.

Maedi and visna have been eradicated from Iceland by campaign of slaughtering and restocking, which is a rather unique example of eradication of a slow virus disease (3). Work on experimental visna produced by intracerebral virus injection continues in several laboratories in the world, since many aspects of this slow infection continue to pose intriguing questions such as: how does this non-oncogenic retrovirus persist and how does it produce lesions of the central nervous system (8)?

This paper will not attempt to deal with all details

of the published work on visna. Previous reviews will be useful as sources of further information (3,9,10,11,12,13, 14,15).

2. STRUCTURE OF VISNA VIRUS

2.1. Ultrastructure of virions

Electron microscopy of visna-infected tissue culture cells shows that virions are formed by budding from the cytoplasmic membrane (16,17,18). Crescent-shaped budding structures form a particle with a relatively electron - lucent center. This structure appears to condense and to form a smaller (80-120 nm) particle with a central dense nucleoid (30-40 nm), which is believed to represent the fully formed infectious virion. At times an internal membrane can be discerned between the central nucleoid and the outer membrane (19). By negative contrast the visna virions can be seen to be covered by knobs about 10 nm in length (20,21).

The morphology of visna virions is typical for C-type RNA tumor viruses except that the dense nucleocapsid material seems to be closer to the viral membrane during the budding process. Inside the cytoplasm of infected cells structures resembling multilayered budding crescents may sometimes be seen (22). It is not known if they represent incomplete or abortive forms of virus.

2.2. Physicochemical properties

Visna virus is readily inactivated by ethyl ether, chloroform, formaldehyde, ethanol and phenol as well as by trypsin treatment. It is stable at -50°C for months and is not markedly inactivated by several cycles of freezing and rethawing. In medium containing 1% sheep serum 90%

of infectivity is lost after 4 months at 4°C, 9 days at 20°C, 24-30 hours at 37°C and 10-15 minutes at 50°C. The infectivity is relatively stable at pH values between 5.1 and 10 (23).

One of the earliest indications of similarities to RNA tumor viruses was the finding of Thormar that visna virus was relatively resistant to inactivation with ultraviolet irradiation (23). This resistance was similar to that exhibited by avian oncornaviruses. Further similarities to this group of viruses are the isopycnic density of visna virus, 1.15-1.16 g/ml in sucrose, the sedimentation coefficient about 600 S and the isoelectric point, 3.8 (11).

2.3. Nucleic acid

The major nucleic acid component of visna virions is a 60-70 S species of single-stranded RNA cosedimenting with the major RNA component of Rous sarcoma virus and comigrating with this molecule in polyacrylamide gel electrophoresis. A minor RNA component (4-7 S) is also present in purified virus preparations (24,25,26,27).

The behaviour of the 70 S RNA of visna with alterations of ionic strength speaks for considerable secondary structure of the molecule. It can be dissociated by heat into two 36 S subunits (28).

The molecular weight of the 70 S RNA of visna has been estimated to be about 10^7 daltons from sedimentation data and from direct measurements of length (9.3 µm) by electron microscopy of the RNA released from virions and prepared by the Kleinschmidt procedure (29). Shorter pieces of linear RNA (3.2 µm) seem to correspond to the 36 S subunits with a molecular weight of 3×10^6 daltons. Recent studies on retroviruses have led to the conclusion that the genome consists of two 30-40 S subunits with molecular weight of about 3×10^6 daltons each (30). It seems likely that the visna genome will be shown to

contain two subunits also.

The base composition of the 70 S RNA of visna has been determined as C,A,G,U : 28,23,23,26, (27). Long parts of the molecule (100-200 nucleotides) consist of polyadenylic acid (Poly A) and these Poly A sequences have been located at the 3´ end of the molecule just as in Rous sarcoma virus (31,32,33).

Thus, the 60-70 S RNA seems to be formed from 2 subunits of about 10.000 nucleotides each. Some contradictory evidence has been published as to whether these subunits contain identical nucleotide sequences (polyploid model). Transfection with visna proviral DNA apparently required more than one subunit since the kinetics were two-hit (34). Chemical analysis of nucleotides following partial digestion with T1 RNase indicates a genetic complexity of about 10.000 nucleotides, supporting the polyploid model (33,35). It has recently been shown (33) that 19 large RNase T1-resistant oligonucleotides are arranged in the same linear order within all subunits. There are therefore no large redundant segments in the visna virus genome. The methods used ruled out circular permutations, but relatively short terminal redundancies could not be excluded. Thus, it appears that the 36 S subunits are largely identical and that the visna genome is therefore polyploid (probably diploid).

2.4. Virus proteins

The structural proteins of visna virus have been analyzed by gel electrophoresis and by chromatography. The number and distribution of visna polypeptides are quite similar to those of avian tumor viruses except for certain differences in the glycopeptides. A major virus component (40% of the virion mass) of 25.000 daltons (p25, sometimes referred to as p30) seems to be a constituent of the virion core and the glycoprotein (gp135) is thought to be

associated with the surface knobs. These two proteins form the two major precipitin lines in gel immunodiffusion tests with sera from naturally and experimentally infected and hyperimmunized sheep.

The number of polypeptides has been reported as 10-25 (36,37,38,39). Since the combined molecular weight of the polypeptides requires a larger coding capacity than provided by a genome of 10.000 nucleotides (36 S RNA subunits) it has been proposed that the virion polypeptides are not all primary gene products but may result from overlapping proteolytic cleavage of a precursor protein molecule (11). Of course the complete absence of host proteins is difficult to ascertain even with purified virus preparations. Much remains to be done in analyzing and characterizing the protein components of visna and in determining their structural and functional significance.

The envelope of visna virus has been reported to contain neuraminic acid. Visna virus will inhibit hemagglutination by influenza virions and this hemagglutination inhibition activity can be abolished by treating visna virions with neuraminidase. Such treatment did not influence attachment to sheep cells, infectivity nor cell-fusing activity of visna virus (40).

2.5. Reverse transcriptase

Shortly after the discovery of RNA-directed DNA polymerase or reverse transcriptase in avian and murine oncornaviruses a similar enzyme was reported in visna virus (41,42,43).

The polymerase of visna catalyses both the formation of single stranded DNA from an RNA template and subsequent synthesis of double stranded DNA. Several years earlier Thormar (44) reported the early phases of visna virus replication to be sensitive to inhibition of DNA synthesis by 5-bromodeoxyuridine and that actinomycin D decreased virus yield. Lin and others have reported

three different polypeptides with polymerase activity associated with visna virus (45). Their polymerase I was reported to have a molecular weight of 125.000 daltons and to be similar to reverse transcriptase purified from oncornaviruses. Whether or how these three enzymes may be related is not clear.

3. REPLICATION OF VISNA VIRUS

3.1. Virus-cell interactions in vitro

Visna virus differs from many retroviruses in being cytopathic in cultured cells (46). Infected cultures show both rounded individual cells and multinucleated syncytia. This leads to destruction of the cell monolayers and appearance of progeny virus in high titers in the cell culture medium (10^7-10^8 TCD_{50} per ml).

High input multiplicity visna virus causes direct cell-fusion from without as early as 30-60 minutes after inoculation. This effect does not depend on virus multiplication since virus inactivated by ultraviolet light will produce it. Cells that do not support virus multiplication such as BHK21-F cells may be susceptible to virus-induced cell fusion (47).

Adsorption of virus to cells is completed in about 2 hours and virus nucleocapsids enter the cell after fusion of the virus membrane to the cytoplasmic membrane of the cell. The latent period before newly formed infectious virus begins to appear is about 16-24 hours (48). This is followed by an exponential increase of infectious virus for 20-30 hours after which virus production levels off.

After the virus has entered the cell the viral RNA is transcribed into DNA (49). Compounds that inhibit DNA synthesis, especially 5-bromodeoxyuridine, inhibit virus synthesis but only at early stages, within 8 hours after infection (44). By transfection experiments (34), it

has been proven that all the necessary genetic information for the synthesis of complete visna virions is transcribed from RNA to DNA in the infected cell. It was shown by Haase and Varmus that at least some of the DNA provirus is covalently integrated into host cell DNA (49).

Synthesis of viral DNA is thought to take place only in the nucleus. Synthesis of viral RNA takes place in the nucleus with subsequent transport into the cytoplasm (50). By immunofluorescent staining, viral antigens were first detected in the perinuclear cytoplasm but as budding of virus proceeds staining for antigens becomes brighter at the cell surface (51,52).

No visna-virus specific sequences have been detected by hydridization techniques in uninfected sheep cells (49,50). It seems clear that visna is an exogenous virus of sheep that spreads mainly horizontally from one animal to another as does bovine leukosis virus of cattle (53). This is of course consistent with the epizootic behaviour of visna-maedi disease in Iceland and its successful eradication.

Although visna virus is usually grown in cultivated sheep choroid plexus cells, sheep cell cultures from other organs of sheep seem to be quite permissive for virus replication. Cells from certain animal species, especially bovine cells can support virus multiplication (54,55). Cells from most other species tested have either failed to support virus multiplication or produced only minimal titers.

3.2. Possible oncogenic potential of visna virus

Of particular interest is the report (56) that mouse cell lines were transformed by visna virus. No free infectious virus was found in these transformed cell lines, but visna could be rescued from them by cocultivation with permissive sheep cells. The transformed mouse cells

produced tumors when inoculated into X-irradiated mice. Tumor-specific transplantation antigens were found but no crossreaction between various visna-induced tumors (57). Other workers found the visna genome to be associated with mouse cells for up to 20 cell passages but only in a form that could be detected by cocultivation with susceptible sheep cells. They could not find any evidence for cell transformation in these cells nor in hamster cells that produced small amounts of virus for up to 100 days in vitro (58).

MacIntyre observed morphological changes in visna-infected human astrocytoma cells and in sheep cells infected with a strain of virus reisolated from the human cells (59,60). The morphological alterations were reminicent of transformation observed in vitro with oncogenic viruses, accompanied by continued virus production but could not be confirmed as malignant by inoculation into transplantation compatible sheep (61). In this context it should be mentioned that no virus-induced tumors have ever been observed in visna-infected sheep in Iceland, some of which have been followed for a number of years.

4. CLINICAL MANIFESTATIONS OF VISNA

Following intracerebral injection of visna virus, there is a clinically silent period of variable length from a few months up to 8 or 9 years (62). The factors determining the length of this preclinical period are completely unknown at present. When the clinical signs appear they consist of progressive paralysis, especially of the hind quarters. The rate with which weakness increases is different from one case to another and sometimes the disease seems to be stationary for a while. Even if the animals are unable to rise, they remain apparently alert and can still survive for weeks if helped to feed and water. The intervals from onset to death (or prostration

with sacrifice) is usually 1-12 months.

The distribution of incubation periods in two experimental series is shown in figure 1. This figure indicates that the proportion of animals which develop signs can vary, perhaps due to the strain of virus injected. Clearly, some sheep never develop signs even though they are persistently infected as judged by repeated virus isolations and serum antibody response.

Although sheep exhibit no outward signs of disease during the preclinical period, examination of the spinal fluid reveals pleocytosis starting 1-2 weeks after intracerebral injection of virus (1,63,64,65). Most of the cells are macrophages or lymphocytes and a few plasma cells are seen (65,66). Polymorphonuclear leucocytes are practically absent. The number of cells in the spinal fluid increases and reaches a maximum about 1 month after infection. Thereafter it gradually declines but often remains somewhat elevated for a long time (figure 2) with later peaks often appearing towards the end when clinical signs start. No clearcut changes are observed in the peripheral blood.

5. PATHOLOGY

In the early papers of Sigurdsson and his coworkers the pathological features of natural and experimentally transmitted visna were described as inflammatory changes accompanied by demyelination (1,2,67). Later studies by Georgsson and others have primarily focused on characterization of early pathological changes and they have in general confirmed and extended the results of earlier workers (12,63,68). Pathological changes are already quite prominent in many brains as early as 2-4 weeks after intracerebral injection of virus. They consist of inflammatory changes concentrated around the ventricular system and the central canal of the spinal cord, and

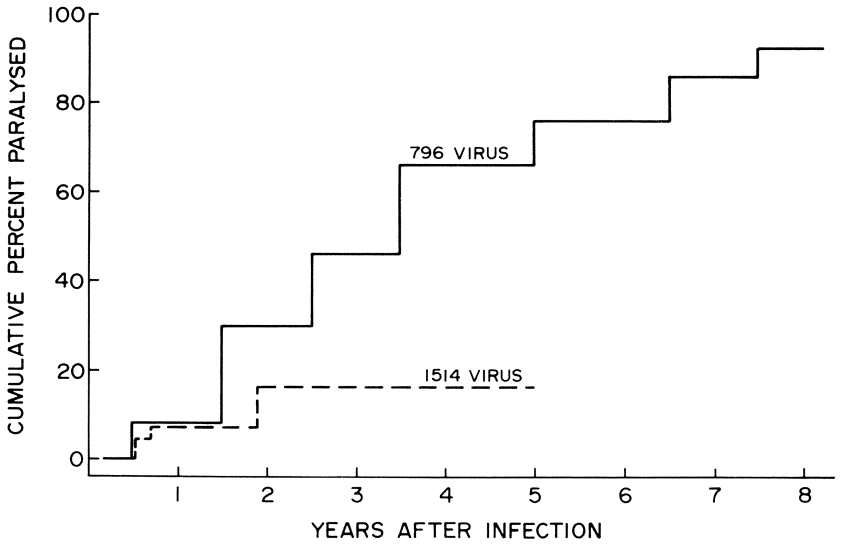


Figure 1. Cumulative incubation periods in two groups of Icelandic sheep injected intracerebrally with 10^6 TCD_{50} of strains 796 (24 sheep) and 1514 (20 sheep) of visna virus. After Pálsson and Gudnadóttir (62), and Pétursson et al (63) and unpublished.

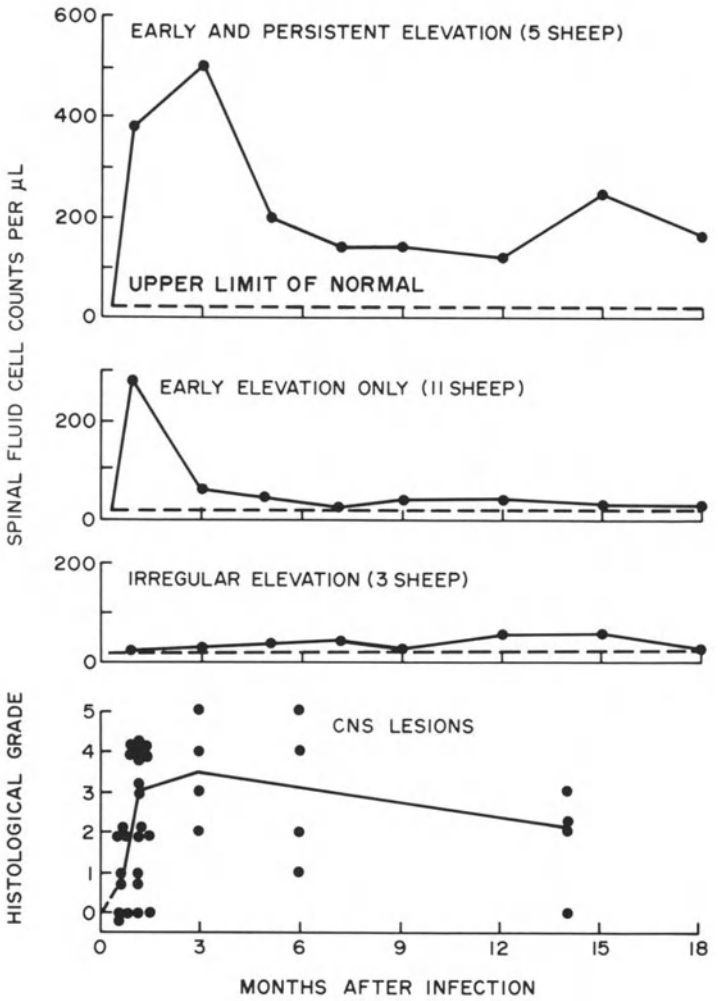


Figure 2. Sequential pathological changes in the CNS of sheep after intracerebral inoculation of 10^6 TCD_{50} of strain 1514 of visna virus. Lower panel: the severity of CNS lesions graded on a scale of 0-6 in 36 sheep. Upper panels: CSF cell counts on a group of 19 sheep which have been sorted into 3 groups to show characteristic patterns. After Pétursson et al (63) and Nathanson et al (65).

extending into adjacent white and grey matter. Inflammation of the meninges and the choroid plexus is also a common feature. Perivascular localization of inflammatory cells is usually observed in visna and glial nodules are also seen. The cell types involved in the inflammatory reaction are mainly mononuclear, and polymorphonuclear leucocytes are not seen. Thus the inflammatory components is similar to that observed in the viral encephalitides.

In some places the inflammatory changes are quite severe with destruction of tissue and liquefaction necrosis and sometimes foci of coagulative necrosis. Inflammation of the choroid plexus can at times be particularly intense with massive proliferation of lymphoid tissue with active germinal centers.

In the early inflammatory lesions evidence of primary demyelination (destruction of myelin with preservation of axons) has not been observed. Present investigations on sheep that have been sacrificed at later stages after infection indicate, however, that primary demyelination may be seen in animals with more advanced lesions (66).

In a series of sheep sacrificed two weeks to 13 months after infection there was little evidence for progression of lesions with time, except during the first month as shown in figure 2. There was, however, a definite correlation between the severity of histopathological lesions and the frequency of virus recovery (63).

Usually the number of cells in the spinal fluid gives a rather good indication of the intensity of inflammatory changes in the central nervous system.

The age of the animals does not seem to have a decisive influence on the character or severity of lesions according to comparative studies on the effect of visna virus on newborn lambs (69) or foetal sheep (70).

6. VIRUS-CELL INTERACTIONS IN VIVO

Visna virus can be isolated from the tissues of infected sheep beginning 1-2 weeks after infection and at any time after that (63). Titers of free infectious virus are usually minimal (table 1) and virus isolation often requires the use of tissue explants. The success of isolation depends to some extent on the number of samples tested and the isolation frequency increases with the number of methods applied. Often one and occasionally two blind passages are required for virus isolation (table 2).

The best source of virus is the central nervous system especially the choroid plexus but lymphoid organs, spleen, mesenteric and mediastinal lymph nodes and lungs are also frequently positive. In the blood the virus is strictly cell-associated and has never been isolated from cell-free plasma. The frequency of successful attempts at virus isolation from buffy coat cells varies considerably (figure 3). Visna has been repeatedly isolated from efferent lymph (71); since this represents a population consisting almost exclusively of lymphocytes, this suggests that the circulating lymphocyte may carry the viral genome. In the spinal fluid some free virus can be found, at least during the first three months after infection, but after that we have found it to be mainly cell-associated there as in the blood (63).

As a result of the low titer of fully formed infectious virus, typical visna virions have with one exception (66) not been detected in tissues from infected sheep by electron microscopy (68). Using immunofluorescence virus antigens have been very difficult to demonstrate in tissue sections. On the other hand Haase and coworkers have demonstrated proviral DNA by in situ hybridization in as much as 18% choroid plexus cells in vivo (72). They reported, however, that only 0.025% of these same cells stained for the p25 antigen by immunofluorescence. By cloning these choroid plexus cells, 14% of them were

found to produce virus in vitro. Thus the evidence speaks for a severe restriction of the production of visna virus in vivo by cells that contain the virus genome, whereas these same cells become readily permissive and produce infectious virus when grown in tissue culture. The restriction in vivo seems to be at the level of transcription (73).

7. IMMUNE RESPONSE TO VISNA VIRUS INFECTION

7.1. Interferon

It has been reported that the replication of visna virus in permissive sheep choroid plexus cell cultures was completely unaffected by high concentrations of sheep interferon induced by polyriboinosinic-polyribocytidylic acid in fetal lambs or in sheep choroid plexus cultures (74,75). This interferon inhibited the growth of other viruses. The resistence of visna to interferon is in contrast to findings with both avian and murine RNA tumor viruses, whose growth is blocked by interferon at a late stage of the replication cycle (76). Cell cultures persistently infected with visna virus are susceptible to infection with vesicular stomatitis and vaccinia viruses indicating that visna virus is a poor inducer of interferon (14,74). It is therefore unlikely that the restriction in vivo of visna virus is mediated through interferon.

Table 1

Virus titers of homogenates (before and after low speed centrifugation) from tissues of visna infected fetal sheep*. After Georgsson et al (70).

Titer per 0.01 g	Whole Homogenate	Supernate of Homogenate
Negative	0	11
1	24	12
10	6	8
≥100	4	5
≥1000	2	0
Totals	36	36

*Limited to virus-positive specimens.

Table 2

Efficiency of three methods of isolation of visna virus from tissues of infected sheep*. After Pétursson et al (63).

Method	Frequency of Isolation Number	Percent	Proportion Requiring Blind passage
Homogenization	68/96	71%	53%
Explantation	42/96	44%	84%
Explantation and Cocultivation	47/96	49%	83%

*Limited to virus-positive specimens. Blind passage: cultures were followed for two weeks and those not showing cytopathic effect were scraped off glass and inoculated into a new set of cultures.

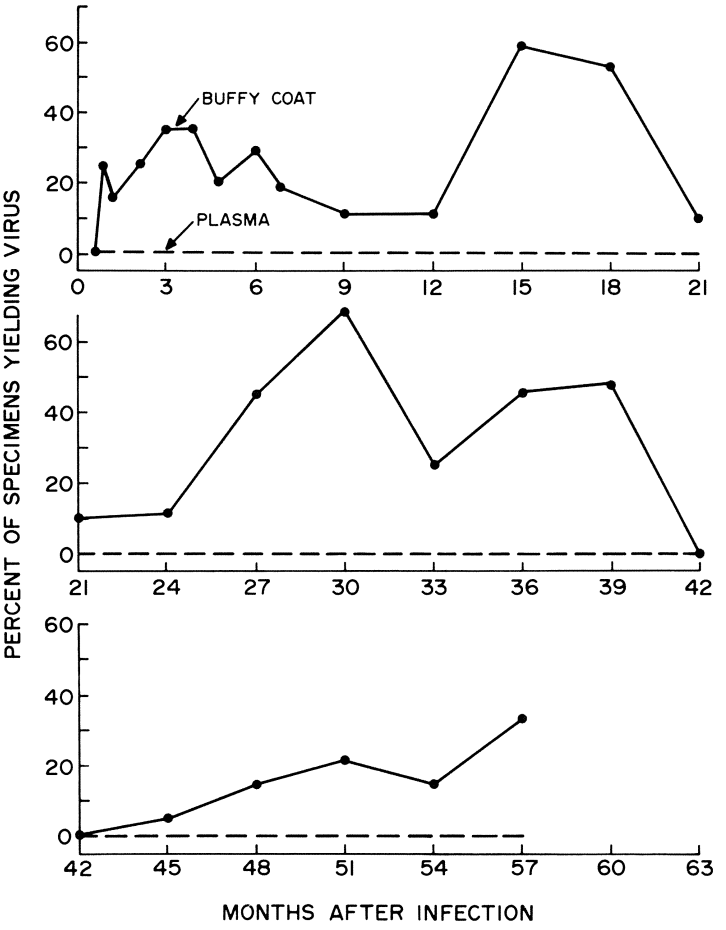


Figure 3. Sequential frequency of isolations of visna virus from the buffy coat and plasma of 19 sheep inoculated intracerebrally with 10^6 TCD_{50} of strain 1514 of visna virus and tested over 57 months. Unpublished.

7.2. Virus antibodies in serum

Antibodies directed against virus antigens are induced following experimental infection of sheep with visna virus and can be demonstrated in serum by various techniques: complement fixation (77), virus neutralization (46), immunofluorescence (52), immunodiffusion (78), passive hemagglutination (79) and as shown recently by the ELISA technique (80).

Most studies have been done on complement-fixing and neutralizing antibodies and they have been characterized to some extent. Complement-fixing antibodies usually appear about 3-4 weeks after infection but neutralizing antibodies not until 2-3 months following infection (figure 4). The titers are fairly high and tend to remain elevated for years (63,81). Precipitating antibodies against the glycoprotein appear at 1-6 months in all infected sheep, while anti-p25 precipitins are slower to appear (3-24 months) and are only seen in a proportion of infected sheep. The complement-fixing and neutralizing antibodies appear both to belong to the IgG1 immunoglobulin class but can be separated on the basis of differences in electrical charge (8,83). Minimal antibody activity in the IgM class has been reported (84) but as yet we have been unable to confirm this.

The complement-fixing antibodies seem to be relatively nonspecific, not distinguishing between various strains of visna or even between visna and maedi strains. Undoubtedly, this reflects the fact that the crude antigen used in complement-fixing tests contains p25 which has group-specific reactivity. The neutralizing antibodies, however, are strain specific. Thus some strains of visna may show little or no crossreaction in neutralization. The kinetics and optimal conditions for the neutralization process have been described by Thormar (85).

The target antigens for the antibodies are not as yet well defined. Neutralizing antibodies are expected to

react with antigens on the virion surface, probably glycoproteins. In immunodiffusion two major lines can be detected. One of them represents the p25 core protein and the other apparently is the major glycoprotein of the viral envelope.

Antibodies to visna virus can be produced in other animals than sheep. Thus, after intensive immunization rabbits will produce complement-fixing, passive hemagglutinating, immunofluorescent and even neutralizing antibodies (79). Nonspecific inhibitiors have been found in low titers in human and in high titers in bovine sera. They are apparently not immunoglobulins (54,86).

7.3. Virus antibodies in the central nervous system

Neutralizing antibodies to visna virus appear in the cerebrospinal fluid in some infected sheep. They have been shown to be produced locally in the central nervous system (63,64,65). In a few sheep with long-term visna infection oligoclonal bands in the gammaglobulin region have been demonstrated by agar gel electrophoresis (65). Some increase in the protein content of the cerebrospinal fluid is often observed (1,15), especially of globulins but not of albumin (64,87).

The presence of plasma cells in the inflammatory infiltrates and sometimes in the cerebrospinal fluid in visna infected sheep is consistent with these findings (66). It is thought that B cell clones generated outside the central nervous system migrate across the blood-brain barrier and produce virus-specific antibodies locally in the central nervous system. Whether this migration is a chance phenomenon or a directed process is not known but the presence of visna virus antigen in the central nervous system probably plays a role. The appearance of neutralizing antibodies in the cerebrospinal fluid may well explain the disappearance of free infectious virus

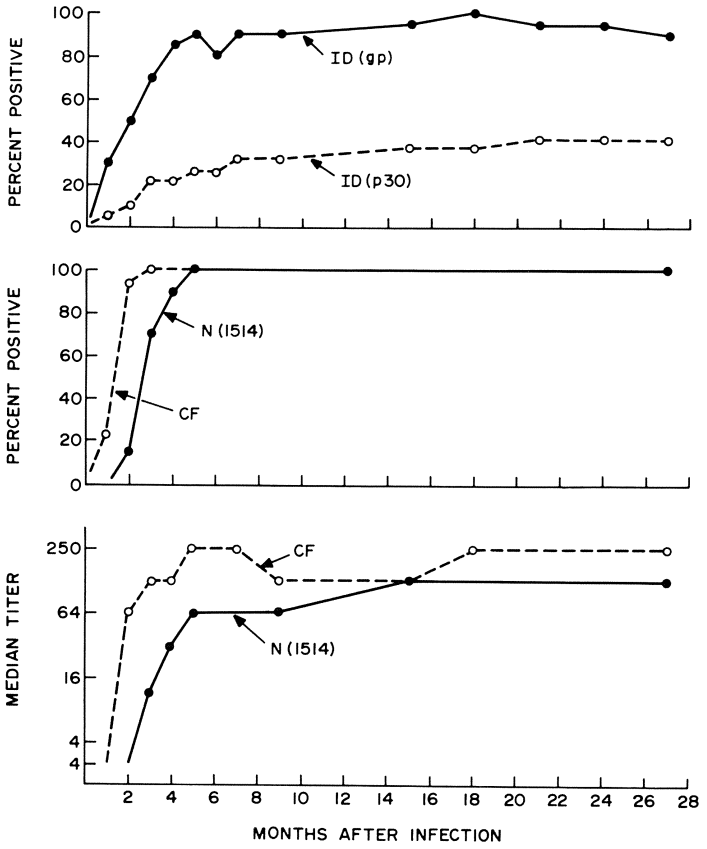


Figure 4. Sequential comparison of serum antibody responses of 19 sheep following intracerebral inoculation of strain 1514 of visna virus. ID: immunodiffusion test to demonstrate precipitating antibody against glycoprotein (gp) and p30 antigens; N: neutralization test; CF: complement-fixation test. After Pétursson et al (63) and Nathanson and Gorham (82).

from this fluid about 3-4 months after infection (63). Further studies on the antibody response in the central nervous system are underway and may throw some light on possible modulating effects of virus antibodies on the course of the infection and on the pathogenesis of lesions.

7.4. Antigenic drift

The phenomenon of antigenic drift of visna virus occurring during the long course of infection in individual animals was first proposed by Gudnadóttir (9). The appearance of new antigenic variants that are poorly neutralized by early antibodies has also been described by Narayan and coworkers (88,89). Narayan has also reported new antigenic variants to arise in tissue culture in the presence of neutralizing antisera and proposed that the appearance of virus variants under antibody pressure may be a mechanism of persistence in vivo (90).

7.5. Cell-mediated immune response

Our knowledge of cell mediated immunity to visna virus is still very scant but some evidence for an early blast transformation response of circulating lymphocytes to visna antigens has been found in sheep hyperimmunized with visna virus (15,71). In intracerebrally infected sheep the response was transitory, the peak occurred between 1 and 2 weeks after infection both in cells of the cerebrospinal fluid and in the peripheral blood and could no longer be detected after six weeks (91).

8. PATHOGENESIS

8.1. Persistence of virus

From the available data it is clear that visna virus is rarely if ever completely eliminated from infected sheep in spite of a fairly good serological response to the virus and a cell-mediated virus specific response at least in the early phases of infection. Several mechanisms have been proposed to explain this failure of the host to clear the infection.

The most plausible explanation is that the viral genome persists as a provirus in at least some cells in vivo. If the genetic information of the virus is not expressed, there will be no target for viral antibodies or a cell - mediated immune response. In this way the virus can escape immune defenses.

The reported insensitivity of visna virus to interferon and the poor interferon-inducing ability of the virus may also favor persistence.

The mechanism of antigenic drift may allow new antigenic variants to escape from strain-specific neutralizing antibody and thus permit new waves of infection. A similar phenomenon has been described with another retrovirus, equine infectious anemia (91). A systematic study of a group of long-term visna-infected sheep with careful sequential comparison of virus isolates has been initiated to determine the importance of antigenic variation in virus persistence.

A defective immune response to visna virus can not be excluded as a factor contributing to persistence. Neutralizing antibodies are rather late in appearance and this may help to establish infection. If our findings of the absence of an antibody response in the IgM class can be confirmed this may contribute to persistence. The inability of the specific immune response to control multiplication in vivo is evidenced by the observation that

immunosuppression of infected sheep does not lead to increased virus titers in the tissues at early stages of infection (93).

8.2. Slowness of clinical evolution

A major factor in the slowness of onset of clinical signs is the limited permissiveness of sheep cells in vivo. As already mentioned (63) there is a strong correlation between the severity of pathological lesions and the amount of virus that can be recovered from the tissues (table 5). The restricted expression of the viral genome may also limit the production of targets for attack by the immune mechanisms of the host.

So far there is no evidence of the production of defective interfering virus particles in visna although such particles have been shown to modify the course of other virus infections in vivo and in vitro (94).

The late onset of clinical signs might be explained by a new element in the pathological picture, such as the development of demyelination. Whatever the mechanism of tissue destruction it seems obvious that the localization of lesions must be of crucial importance in producing the typical paralysis of the hind legs.

8.3. Immunopathogenesis of visna lesions

Several proposals to explain the production of visna lesions in the central nervous system have been made. In view of the cytopathic effect (cytolysis and syncytia) of visna on sheep cells in culture a similar direct effect of virus in vivo has been proposed (47). Multinucleated syncytia have, however, not been observed in the central nervous system in visna (68) as in measles encephalitis.

Indirect effects of visna infection on cells of the

Table 3

Immunosuppression of visna lesions in sheep inoculated intracerebrally with 10^6 TCD_{50} of strain 1514 of visna virus, treated with anti-thymocyte serum and cyclophosphamide, and sacrificed one month after infection. After Nathanson et al (93).

Treatment	Visna Lesion Grade in Individual Sheep	Virus Isolations from CNS
Suppressed	0,0,0,0,0,0,0,2	22/37 59%
Infected Control	1,2,2,3,3,4,4,4	27/39 69%

Table 4

Immunopotentiation of visna, in sheep infected by intracerebral inoculation of 10^6 TCD_{50} of strain 1514 of visna virus, immunized at 3 and 5 weeks with purified virus in CFA, and killed at 8 weeks. Unpublished.

Treatment	Visna Lesion Grade in Individual Sheep	Mean Grade	Percent Positive
Immunized	1,2,2,3,4,4,4,4	3.0*	100%**
Infected Control	0,0,0,0,1,2,3,5	1.4*	50%**

*Not significant **$p < 0.01$

Table 5

Influence of intracerebral dose on severity of early CNS lesions in sheep inoculated with 1514 strain of visna virus and sacrificed one month later. Unpublished.

Dose (TCD_{50})	Visna Lesion Grade in Individual Sheep	Median Grade	CNS Isolations
$10^{8.5}$	2,3,3,3,3,3,4,4	3.0	22/48 46%
$10^{5.3}$	0,0,1,1,1,1,2,4	1.0	14/48 29%

central nervous system have been suggested. The hypothesis that virus-specific antibodies might react with visna antigens on the surface of oligodendrocytes and thus lead to cell lysis and myelin damage (81) has neither been confirmed nor disproven by direct experimental evidence. Because it is difficult to find virus particles by electron microscopy of lesions (68) or to demonstrate virus antigens by immunofluorescence in tissue sections, we do not know which cells of the central nervous system carry viral information or support occasional virus replication.

We have provided evidence that the lesions of visna, at least in the early stages of the disease, are immunologically mediated (93). In table 3 it is shown that an effective immunosuppressive regimen using antithymocyte serum and cyclophosphamide strikingly suppressed the development of lesions in visna infected sheep. The results of an unpublished experiment where we tried to increase the severity of lesions by immunopotentiation by injecting visna infected sheep with high doses of virus in Freund´s adjuvant were consistent with the notion that visna lesions are immunologically produced (table 4).

These results raise the question, whether the target antigens for this immunological attack are viral proteins or antigens of host tissue origin such as myelin components. To explore this question we compared experimental allergic encephalitis of sheep to visna by testing for complement-fixing antibodies to myelin basic protein and a galactocerebroside antigen in both disorders. In sheep with experimental allergic encephalitis, antibodies to both kinds of antigen were produced and a cell-mediated response to basic protein was demonstrated. No such responses were found in early stages of visna (95). Therefore it seems more likely that virus-specified antigens provide the target for an immunological attack by the host, a view which is strengthened by the close correlation of severity

of lesions with the amount of virus in the tissues (63). In recent unpublished experiments we have furthermore shown that the severity of early lesions in visna can be increased by using a large virus inoculum for infection (table 5). As mentioned above, autoimmune reactions to myelin or other host antigens could develop at later stages of the disease and this possibility is currently under investigation.

At the moment we view visna as a persistent retrovirus infection of the central nervous system in which some cells carry the provirus. Apparently more and more of these provirus-carrying cells are activated, producing virus-specified antigens that are attacked by an immune mechanism, probably a cell-mediated response, with production of inflammatory lesions. The possibility of an autoimmune production of myelin damage at later stages is under study.

9. VISNA-LIKE DISEASE OF GOATS

For some time visna of sheep seemed to be a unique example of a slow infection caused by a retrovirus and characterized by inflammatory changes and myelin destruction in the central nervous system. During the last few years reports have appeared describing a disease in goats which exhibits many similarities to the clinical picture and pathological changes of visna. An additional feature of the goat disease, inflammatory changes of the joints, has been described both in the U.S.A. and Germany (96,97,98).

From the published observations it seems possible that the disease syndromes described by German and American workers are similar although certain differences in pathology have been stressed (99). The goat disease seems to run a more acute course than visna, at least in many instances. A retrovirus has recently been found in affected goats by several workers (100,101,102). This

agent shows some antigenic relationship to visna virus although it appears to be a distinct virus (103). The opportunity for comparative studies may provide important new insights into slow infections caused by retroviruses.

ACKNOWLEDGEMENT

Supported in part by USPHS grant NS 11451.

REFERENCES

1. Sigurdsson, B, PA Pálsson, H Grímsson: Visna, a demyelinating transmissible disease of sheep. J Neuropathol Exp Neurol 16: 389-403, 1957.
2. Sigurdsson, B, PA Pálsson: Visna of sheep. A slow, demyelinating infection. Br J Exp Pathol 39: 519-528, 1958.
3. Pálsson, PA: Maedi and visna in sheep. In: Slow virus diseases of animals and man, Kimberlin, RH, (ed), Amsterdam, North-Holland Publ Co, 1976, p17-43.
4. Sigurdardóttir, B, H Thormar: Isolation of a viral agent from the lungs of sheep affected with maedi. J Infect Dis 114: 55-60, 1964.
5. Thormar, H, H Helgadóttir: A comparison of visna and maedi viruses II. Serological relationship. Res Vet Sci 6: 456-465, 1965.
6. Sigurdsson, B: Observations on three slow infections of sheep. Br Vet J 110: 255-270, 1954.
7. Sigurdsson, B: Atypically slow infectious diseases. In: Livre Jubilaire du Dr. Ludo van Bogaert, Brussels, Acta Medica Belgica, 1962, p738-753.
8. Nathanson, N, H Panitch, G Pétursson: Pathogenesis of visna: review and speculation. In: Slow virus diseases of animals and man, Kimberlin, RH, (ed), Amsterdam, North-Holland Publ Co, 1976, p115-131.
9. Gudnadóttir, M: Visna-maedi in sheep. Prog Med Virol 18: 336-349, 1974.
10. Thormar, H, FH Lin, RS Trowbridge: Visna and maedi viruses in tissue culture. Prog Med Virol 18: 323-335, 1974.
11. Haase, AT: The slow infection caused by visna virus. Curr Top Microbiol Immunol 72: 101-156, 1975.
12. Georgsson, G, N Nathanson, PA Pálsson, G Pétursson: The pathology of visna and maedi in sheep. In: Slow virus diseases of animals and man, Kimberlin, RH, (ed), Amsterdam, North-Holland Publ Co, 1976 p61-96.
13. Harter, DH: The detailed structure of visna-maedi virus. In: Slow virus diseases of animals and man, Kimberlin, RH, (ed), Amsterdam, North-Holland Publ Co, 1976, p45-60.
14. Thormar, H: Visna-maedi virus infection in cell cultures and in laboratory animals. In: Slow virus diseases of animals and man, Kimberlin, RH, (ed), Amsterdam, North-Holland Publ Co, 1976, p97-114.

15. Pétursson, G, N Nathanson, PA Pálsson, J Martin, G Georgsson: Immunopathogenesis of visna. A slow virus disease of the central nervous system. Acta Neurol Scand, (Suppl 67), 57: 205-219, 1978.
16. Thormar, H: An electron microscope study of tissue cultures infected with visna virus. Virology 14: 463-475, 1961.
17. Coward, JE, DH Harter, C Morgan: Electron microscopic observations of visna virus-infected cell cultures. Virology 40: 1030-1038, 1970.
18. Dubois-Dalcq, M, TS Reese, O Narayan: Membrane changes associated with assembly of visna virus. Virology 74: 520-530, 1976.
19. Pautrat, G, P de Micco, J Tamalet: Observation en microscopie électronique du cycle infectieux du virus visna sur cellules de plexus choroides de mouton á des températures inférieures á 37°C. C R Acad Sci [D] (Paris) 283: 211-214, 1976.
20. Thormar, H, JG Cruickshank: The structure of visna virus studied by the negative staining technique. Virology 25: 145-148, 1965.
21. Pautrat, G, J Tamalet, C Chippaux-Hyppolite, M Brahic: Etude de la structure du virus visna en microscopie électronique. C R Acad Sci [D] (Paris) 273: 653-655, 1971.
22. Coward, JE, DH Harter, KC Hsu, C Morgan: Ferritin-conjugated antibody labeling of visna virus. Virology 50: 925-930, 1972.
23. Thormar, H: A comparison of visna and maedi viruses. I. Physical, chemical and biological properties. Res Vet Sci 6: 117-129, 1965.
24. Brahic, M, J Tamalet, C Chippaux-Hyppolite: Virus Visna: Isolement d´une molécule d´acide ribo-nucléique de haut poids moléculaire. C R Acad Sci [D] (Paris) 272: 2115-2118, 1971.
25. Brahic, M, J Tamalet, P Filippi, L Delbecchi: The high molecular weight RNA of visna virus. Biochimie 55: 885-891, 1973.
26. Harter, DH, J Schlom, S Spiegelman: Characterization of visna virus nucleic acid. Biochim Biophys Acta 240: 435-441, 1971.
27. Lin, FH, H Thormar: Characterization of ribonucleic acid from visna virus. J Virol 7: 582-587, 1971.
28. Haase, AT, AC Garapin, AJ Faras, JM Taylor, JM Bishop: A comparison of the high molecular weight RNA´s of visna virus and Rous sarcoma virus. Virology 57: 259-270, 1974.
29. Friedmann, A, JE Coward, DH Harter, JS Lipset, C Morgan: Electron microscopic studies of visna virus ribonucleic acid. J Gen Virol 25: 93-104, 1974.
30. Bishop, JM: Retroviruses. Annu Rev Biochem 47: 35-88, 1978.
31. Gillespie, D, K Takemoto, M Robert, RC Gallo: Polyadenylic acid in visna virus RNA. Science 179: 1328-1330, 1973.

32. Vigne, R, M Brahic, P Filippi, J Tamalet: Complexity and polyadenylic acid content of visna virus 60-70 S RNA. J Virol 21: 386-395, 1977.
33. Vigne, R, P Filippi, M Brahic, J Tamalet: Absence of circularly permuted and largely redundant sequences in the genome of visna virus. J Virol 28: 543-550, 1978.
34. Haase, A, BL Traynor, PE Ventura, DW Alling: Infectivity of visna virus DNA. Virology 70: 65-79, 1976.
35. Beemon, KL, AJ Faras, AT Haase, PH Duesberg, JE Maisel: Genomic complexities of murine leukemia and sarcoma, reticuloendotheliosis, and visna viruses. J Virol 17: 525-537, 1976.
36. Mountcastle, W, D Harter, P Choppin: The proteins of visna virus. Virology 47: 542-545, 1972.
37. Haase, AT, JR Baringer: The structural polypeptides of RNA slow viruses. Virology 57: 238-250, 1974.
38. Lin, FH, H Thormar: Substructures and polypeptides of visna virus. J Virol 14: 782-790, 1974.
39. Lin, FH: Polyacrylamide gel electrophoresis of visna virus polypeptides isolated by agarose gel chromatography. J Virol 25: 207-214, 1978.
40. August, MJ, DH Harter, RW Compans: Characterization of visna virus envelope neuraminic acid. J Virol 22: 832-834, 1977.
41. Lin, FH, H Thormar: Ribonucleic-acid-dependent deoxyribonucleic acid polymerase in visna virus. J Virol 6: 702-704, 1970.
42. Stone, LB, E Scholnick, KK Takemoto, SA Aaronson: Visna virus: a slow virus with an RNA dependent DNA polymerase. Nature 229: 257-258, 1971.
43. Schlom, J, DH Harter, A Burny, S Spiegelman: DNA polymerase activities in virions of visna virus, a causative agent of a "slow" neurological disease. Proc Natl Acad Sci USA 68: 182-186, 1971.
44. Thormar, H: Effect of 5-bromodeoxyuridine and actinomycin D on the growth of visna virus in cell cultures. Virology 26: 36-43, 1965.
45. Lin, FH, M Genovese, H Thormar: Multiple activities of DNA polymerase from visna virus. Prep Biochem 3: 525-539, 1973.
46. Sigurdsson, B, H Thormar, PA Pálsson: Cultivation of visna virus in tissue culture. Arch Ges Virusforsch 10: 368-381, 1960.
47. Harter, DH, PW Choppin: Cell-fusing activity of visna virus particles. Virology 31: 279-288, 1967.
48. Thormar, H: The growth cycle of visna virus in monolayer cultures of sheep cells. Virology 19: 273-278, 1963.
49. Haase, AT, HE Varmus: Demonstration of a DNA provirus in the lytic growth of visna virus. Nature [New Biol] 245: 237-239, 1973.
50. Brahic, M, P Filippi, R Vigne, AT Haase: Visna virus RNA synthesis. J Virol 24: 74-81, 1977.

51. Harter, DH, KC Hsu, HM Rose: Immunofluorescence and cytochemical studies of visna virus in cell culture. J Virol 1: 1265-1270, 1967.
52. Thormar H: Visna and maedi virus antigen in infected cell cultures studied by the fluorescent antibody technique. Acta Pathol Microbiol Scand 75: 296-302, 1969.
53. Burny, A, F Bex, H Chantrenne, Y Cleuter, D Dekegel, J Ghysdael, R Kettmann, M Leclercq, J Leunen, M Mammerickx, D Portetelle: Bovine leukemia virus involvement in enzootic bovine leukosis. Adv Cancer Res 28: 251-311, 1978.
54. Thormar, H, B Sigurdardóttir: Growth of visna virus in primary tissue cultures from various animal species. Acta Pathol Microbiol Scand 55: 180-186, 1962.
55. Harter, DH, KC Hsu, HM Rose: Multiplication of visna in bovine and porcine cell lines. Proc Soc Exp Biol Med 129: 295-300, 1968.
56. Takemoto KK, LB Stone: Transformation of murine cells by two "slow viruses", visna virus and progressive pneumonia virus. J Virol 7: 770-775, 1971.
57. Law, LW, KK Takemoto: Specific transplantation antigens of murine neoplasms induced by visna and progressive pneumonia viruses. J Natl Cancer Inst 50: 1076-1079, 1973.
58. Brown, HR, H Thormar: Persistence of visna virus in murine and hamster cell cultures without the appearance of cell transformation. Microbios 13: 51-60, 1975.
59. Macintyre, EH, CJ Wintersgill, H Thormar: Morphological transformation of human astrocytes by visna virus with complete virus production. Nature [New Biol] 237: 111-113, 1972.
60. Macintyre, EH, CJ Wintersgill, AE Vatter: Visna virus infection of sheep and human cells in vitro - an ultrastructural study. J Cell Sci 13: 173-191, 1973.
61. Macintyre, EH, G Pétursson, PA Pálsson: Unpublished data.
62. Pálsson, PA, M Gudnadóttir: Unpublished data.
63. Pétursson, G, N Nathanson, G Georgsson, H Panitch, PA Pálsson: Pathogenesis of visna. I Sequential virologic, serologic and pathologic studies. Lab Invest 35: 402-412, 1976.
64. Griffin, DE, O Narayan, JF Bukowski, RJ Adams, SR Cohen: The cerebrospinal fluid in visna, a slow viral disease of sheep. Ann Neurol 4: 212-218, 1978.
65. Nathanson, N, G Pétursson, G Georgsson, PA Pálsson, JR Martin, A Miller: Pathogenesis of visna. IV Spinal fluid studies. J Neuropathol Exp Neurol in press 1979.
66. Georgsson, G, JR Martin, PA Pálsson, N Nathanson, E Benediktsdóttir, G Pétursson: An ultrastructural

study of the cerebrospinal fluid in visna. Submitted for publication.
67. Sigurdsson, B, PA Pálsson, L van Bogaert: Pathology of visna. Transmissible demyelinating disease in sheep in Iceland. Acta Neuropathol 1: 343-362, 1962.
68. Georgsson, G, PA Pálsson, H Panitch, N Nathanson, G Pétursson: The ultrastructure of early visna lesions. Acta Neuropathol (Berl) 37: 127-135, 1977.
69. Pálsson, PA, G Georgsson, G Pétursson, N Nathanson: Experimental visna in Icelandic lambs. Acta Vet Scand 18: 122-128, 1977.
70. Georgsson, G, G Pétursson, A Miller, N Nathanson, PA Pálsson: Experimental visna in foetal Icelandic sheep. J Comp Pathol 88: 597-605, 1978.
71. Martin, JR, G Georgsson, N Nathanson, PA Pálsson, G Pétursson: Unpublished data.
72. Haase, AT, L Stowring, O Narayan, D Griffin, D Price: Slow persistent infection caused by visna virus: role of host restriction. Science 195: 175-177, 1977.
73. Haase, AT, M Brahic, D Carroll, J Scott, L Stowring, B Traynor, P Ventura, O Narayan: Visna: an animal model for studies of virus persistence. In: Persistent viruses, Stevens, JG, GJ Todaro, CF Fox (eds), UCLA symposia on molecular and cellular biology, Vol XI, New York, Academic Press, 1978 p643-654.
74. Trowbridge, RS: Long-term visna virus infection of sheep choroid plexus cells: initiation and preliminary characterization of the carrier cultures. Infect Immun 11: 862-868, 1975.
75. Carroll, D, P Ventura, A Haase, CR Rinaldo, Jr, JC Overall, Jr, LA Glasgow: Resistance of visna virus to interferon. J Infect Dis 138: 614-617, 1978.
76. Pitha, PM, WP Rowe, MN Oxman: Effect of interferon on exogenous, endogenous and chronic murine leukemia virus infection. Virology 70: 324-338, 1976.
77. Gudnadóttir, M, K Kristinsdóttir: Complement-fixing antibodies in sera of sheep affected with visna and maedi. J Immunol 98: 663-667, 1967.
78. Terpstra, C, GF de Boer: Precipitating antibodies against maedi-visna virus in experimentally infected sheep. Arch Ges Virusforsch 43: 53-62, 1973.
79. Karl, SC, H Thormar: Antibodies produced by rabbits immunized with visna virus. Infect Immun 4: 715-719, 1971.
80. Houwers, D: Personal communication.
81. Gudnadóttir, M, PA Pálsson: Host-virus interaction in visna infected sheep. J Immunol 95: 1116-1120, 1965.
82. Nathanson, N, JR Gorham: Unpublished, 1978.

83. Pétursson, G, B Símonarson, B Magnadóttir: Characterization of antibodies against visna virus. Proc 12th Nordic Veterinary Cong, Reykjavík, 1974, p252.
84. Mehta, PD, H Thormar: Neutralizing activity in isolated serum antibody fractions from visna-infected sheep. Infect Immun 10: 678-680, 1974.
85. Thormar, H: Neutralization of visna virus by antisera from sheep. J Immunol 90: 185-192, 1963.
86. Thormar, H, H von Magnus: Neutralization of visna virus by human sera. Acta Pathol Microbiol Scand 57: 261-267, 1963.
87. Sigurdsson, B, D Karcher, M van Sande, A Lowenthal: Electrophoresis of serum and CSF proteins in sheep neurological diseases. In: "Protides of the biological fluids", Amsterdam,Elsevier Pub Co, 1961, p110-111.
88. Narayan, O, DE Griffin, J Chase: Antigenic shift of visna virus in persistently infected sheep. Science 197: 376-378, 1977.
89. Narayan, O, D Griffin, AM Silverstein: Slow virus infection: replication and mechanisms of persistence of visna virus in sheep. J Infect Dis 135: 800-806, 1977.
90. Narayan, O, DE Griffin, JE Clements: Antigenic mutation of visna in sheep. J Gen Virol 41: 343-352, 1978.
91. Griffin, DE, O Narayan, RJ Adams: Early immune responses in visna, a slow viral disease of sheep J Infect Dis 138: 340-350, 1978.
92. Kono, Y, K Kobayashi, Y Fukunaga: Arc Ges Virus-forsch 41: 1-10, 1973.
93. Nathanson, N, H Panitch, PA Pálsson, G Pétursson, G Georgsson: Pathogenesis of visna. II Effect of immunosuppression upon early central nervous system lesions. Lab Invest 35: 444-451, 1976.
94. Huang, AS: Defective interfering viruses. Annu Rev Microbiol 27: 101-117, 1973.
95. Panitch, H, G Pétursson, G Georgsson, PA Pálsson, N Nathanson: Pathogenesis of visna. III Immune responses to central nervous system antigens in experimental allergic encephalomyelitis and visna. Lab Invest 35: 452-460, 1976.
96. Stavrou, D, N Deutschländer, E Dahme: Granulomatous encephalomyelitis in goats. J Comp Pathol 79: 393-396, 1969.
97. Dahme, E, D Stavrou, N Deutschländer, W Arnold, E Kaiser: Klinik und Pathologie einer übertragbaren granulomatösen Meningoencephalomyelitis (g MEM) bei der Hausziege. Acta Neuropathol (Berl) 23: 59-76, 1973.
98. Cork, LC, WJ Hadlow, TB Crawford, JR Gorham, RC Piper: Infectious leukoencephalomyelitis of young goats. J Infect Dis 129: 134-141, 1974.
99. Cork, LC, WJ Hadlow, JR Gorham, RC Piper, TB Crawford: Pathology of viral leukoencephalo-

myelitis of goats. Acta Neuropathol (Berl) 29: 281-292, 1974.
100. Weinhold, E: Visna-Virus-ähnliche Partikel in der Kultur von Plexus chorioideus-Zellen einer Ziege mit Visna-Symptomen. Zentralbl Veterinaermed [B] 21: 32-36, 1974.
101. Cork, LC: Personal communication, 1978.
102. Crawford, TB: Personal communication, 1978.
103. Cork, LC, O Narayan: Personal communication, 1978.

EPIZOOTIOLOGY OF MAEDI/VISNA IN SHEEP

G. F. DE BOER AND D. J. HOUWERS

'Maedi' (progressive interstitial pneumonia) and 'visna' (meningo-leucoencephalitis) are slowly progressive non febrile contagious diseases of sheep. In the late thirties both conditions (maedi=dyspnoea and visna=wasting) were observed for the first time in Iceland and were considered to be two different diseases. In the same period jaagsiekte or sheep pulmonary adenomatosis (another lung condition of sheep) and rida (scrapie) became also apparent in the Icelandic sheep population.

Sigurdsson and his coworkers were studying these diseases and it became evident that these conditions were characterised by silent but relentlessly progressive lesions which developed over a long period of time. It was thought that these slowly progressive diseases could neither be grouped as acute nor chronic diseases. In acute infections the disease runs a rather regular course, the causative agent enters the body where it multiplies and spreads rapidly so that clinical signs appear after an incubation period of a few days. The hosts defences are mobilised and unless the patient dies the infecting agent is eliminated and convalescence begins. Chronic infections on the other hand are not only much more protracted in their course, they are also much less regular and unpredictable. In order to characterise the group of four sheep diseases, Sigurdsson coined the term 'peculiarly slow progressive infectious diseases' (annarlega haeggengir smitsjúkdómar), often spoken of as 'slow virus diseases' (1).

In 1957 a virus was isolated in tissue culture from the brains of five experimentally transmitted cases of visna. It was subsequently demonstrated that this isolate was not only neutralised by sera from sheep with a disorder of the central nervous system but also by serum samples from sheep affected with maedi (2). When new maedi outbreaks occurred

D. A. J. Tyrrell (ed.), Aspects of Slow and Persistent Virus Infections, 198-220.

in Iceland, the techniques which had been successful for the isolation of visna virus were applied to lungs of maedi sheep and maedi virus became available for experimental studies (3).
In 1964, in the Netherlands virus was recovered from lungs of sheep with 'zwoegerziekte', an interstitial pneumonia resembling maedi (4, 5) and serologically similar viruses were isolated from brain material of sheep with neurological disorders and histopathological lesions of the central nervous system resembling visna (6). Various properties of these isolates were found to be in accordance with data reported for maedi and visna viruses (4, 7, 8). Experimental infections with virus recovered from the lungs of sheep suffering from zwoegerziekte caused progressive interstitial pneumonia (maedi) and meningo-leucoencephalitis (visna). Hence the name maedi/visna virus was proposed for the agent which causes both disease entities (9). One name for the causative agent of the disease, which has been described under various local names, seems well justified since molecular hybridisation studies (10) have shown homologous nucleotide sequences in strains of maedi and visna virus. The name maedi/visna virus gives credit to the Icelandic investigators who were the first to recognise the aetiology of both diseases.

CLINICAL SYMPTOMS OF MAEDI AND VISNA

The first symptoms of maedi are usually observed in sheep older than 3 to 4 years. Illthrift is one of the first signs that may be observed by an experienced sheep farmer. The affected animal shows a greyish discoloration of the fleece and the abdomen is thin. The suspected animals lag behind and show signs of respiratory distress when the flock is moved to another pasture. After some weeks, sometimes months, the increased rate of respiration becomes also manifest without a preceding physical effort. Despite a good appetite affected animals lose weight and finally become cachectic. In the terminal phase of disease the animals lie

down most of the time. The entire clinical stage may last for some months and sometimes for more than a year. The fatal course of the disease is appreciably accelerated when the sheep is kept under conditions of stress e.g. during fostering of lambs. Following intrapulmonary inoculation of 16 sheep of the Texel breed with a zwoegerziekte virus strain of maedi/visna virus, symptoms were first observed after 26 to 31 months in four sheep, whereas the longest interval amounted to 58 months (4). Contact transmission studies have shown that the duration and degree of exposure greatly influenced the time of onset of disease, which again varied from 2 to 5 years (11).

Visna is only observed in sheep over two years of age. The first sign noted is that the sheep lag behind, incoordination becomes apparent when the flock is moved from one pasture to another. Meningitis is an early event of visna, as judged by pleocytosis of cerebrospinal fluid which is present shortly after experimental infection. Despite a persisting appetite the animal looses weight. Gradually the paresis of the limbs progresses and walking becomes difficult. Mostly the hind limbs are affected. Tremors of the head and facial muscles and blindness are occasionally seen. The paresis slowly progresses to paralysis, prostration and death (12).

PATHOLOGY AND PATHOGENESIS

At necropsy overt changes of maedi are confined to the thoracic cavity, lungs and associated lymph nodes. The lungs are enlarged, weighing in advanced cases two to three times as much as normal lungs. The shape is not much altered but the affected tissue is of firm consistency. The normal pinkish-red colour of a healthy sheep lung is replaced by a characteristic greyish colour. The most advanced lesions are usually found in the diaphragmatic lobes and occur less frequently in the cardiac and apical lobes. In about 10% of the cases histological examination is desirable for the diagnosis (5, 13, 14). Often secondary bacterial infections

complicate the lesions. The microscopic lesions develop progressively and consist of a thickening of the interalveolar septa caused by proliferation of alveolar septal cells and infiltration with mononuclear cells, mainly lymphocytes, monocytes and macrophages. Another early feature is hyperplasia of peribronchial, perivascular and lymphoid tissue. As the disease advances the cell infiltrates are replaced by fibroblasts and argentophilic fibres which turn into collagen fibres. Fibrosis with strands of collagen fibres may develop in areas with very pronounced thickening of the interalveolar septa. The process is accompanied by proliferation of alveolar epithelium and smooth muscular tissue around the terminal bronchioles. Vascular alterations which involve the smaller and medium sized arteries are also common in advanced stages of maedi. Very little is known about the pathogenesis of maedi. Infectious virus is mainly recovered from tissue and organs containing lymphoid cells and in the viremic stage infectivity is associated with lymphocytes (4).

Macroscopic lesions of visna are only seen after several weeks of overt clinical symptoms. These consist of emaciation and muscular atrophy. Histopathological lesions of visna are confined to the central nervous system, where meningeal and subependymal infiltrations consisting of lymphocytes, monocytes and some plasma cells are found. Often the infiltrations are small, but in severe cases large areas with intensive inflammation sometimes accompanied by necrosis are observed. Around these lesions extensive perivascular cuffs of lymphocytes, monocytes and a few plasma cells are found (7, 13, 14). The pathogenesis of visna seems to be immunologically mediated. By applying immunosuppression to sheep when experimentally infected, Nathanson *et al.* (15) could demonstrate suppression of the central nervous system inflammatory response, without apparent effect on virus replication.

Maedi/visna virus infections of sheep are characterised by persistence of virus and antibody (16, 17, 18). Only some of the animals that pass through a stage of viremia and develop

antibodies come down with the disease. Persistent subclinical infections lasting up to 5½ years have been observed following intrapulmonary inoculation (19). At autopsy, no macroscopic or microscopic lesions were detected in some experimentally infected animals, despite the isolation of virus from the blood at irregular intervals during the experiment. The conclusion can therefore be drawn that maedi/visna virus infections do not invariably lead to clinical disease and histopathological lesions. The concept of an inevitably fatal course appears to be valid only when clinical signs have been observed. The long incubation period and the slow development of clinical disease (slow virus disease) is thought to be due to a restriction of virus replication, which should only occur in vivo (20). The observed ability of the virus to undergo antigenic modulation (antigenic shift) under the pressure of neutralising antibody was suggested as a second mechanism for the persistence of maedi/visna virus (21).

SEROLOGY

The persistent infection of maedi/visna virus causes an immune response of the host which is usually followed by formation of specific antibodies (16, 17). In an epizootiological study, however, a number of sheep developed histological lung lesions in the absence of detectable amounts of neutralising, complement fixing or precipitating antibody which was tested at intervals for several years (11).

For epizootiological studies two techniques for detection of antibody, the agar gel precipitation test (AGPT) and the complement fixation test (CFT) proved to be most useful. When field samples were examined with both tests, more positives were detected than with each of the tests separately (16, 22, Table 1). AGPT antigen is prepared by freeze-thawing sonication and ether treatment of infected sheep choroid plexus cell cultures. Antigen used in the CFT is prepared of

cell culture supernatant. The test is performed in a microtiter system. AGPT antigen preparations contain a glycoprotein (gp) and a protein fraction of MW 23,000 (p23). In general, serum samples from naturally infected animals show a gp precipitation line and some samples show a p23 precipitation line as well. In our hands some of the samples, which showed no gp line and were scored negative, later showed clear p23 lines in block titrations. So, the outcome of the test is greatly influenced by the actual concentrations of the fractions in the antigen preparation and the concentrations of the respective antibodies in the serum samples. Therefore, the sensitivity of the AGPT can be improved by testing serum dilutions against dilutions of antigen. The incongruity between results with AGPT and CFT of different laboratories and of different techniques in the same laboratory (23) are probably also due to differences in composition of antigen preparations. Variations in gels and testing systems have probably less impact.

Recently, in our laboratory an indirect enzyme-linked immunosorbent assay (ELISA) has been developed for detection of antibodies to maedi/visna virus. The wells of polystyrene microtiter plates are coated with a maedi/visna virus preparation, which was concentrated and purified by differential centrifugation. After coating the plates are rinsed. The test serum samples are diluted in the wells and the plates are incubated. After rinsing, rabbit anti-sheep IgG coupled to horseradish peroxidase is added. Subsequently the plates are incubated, rinsed and the enzyme substrate 5-aminosalicylic acid is added. The tests are read by eye or OD measurement after one hour.

To compare the sensitivity of the three serological techniques, 494 sheep serum samples from the field were tested (Table 1). All samples which were positive in AGPT or CFT were also scored in the ELISA. In addition 57 serum samples were found positive in the ELISA only.

Table 1. Comparison of Agar Gel Precipitation and Complement Fixation tests with an Enzyme-Linked Immunosorbent Assay

	AGPT	CFT	AGPT+CFT	ELISA
No positive samples/ No sheep sera tested	88/494	73/494	103/494	160/494
Percentages positive	17.8	14.7	20.8	30.2

In control tests, serum samples from maedi-free flocks, from sheep suffering from adenomatosis, from gnotobiotic lambs and from lambs hyperimmunised against different viruses were tested and scored negative. The ELISA for maedi/visna virus antibody is sensitive, specific, can be evaluated objectively, needs relatively little antigen and is suitable for screening of large numbers of sera.

DIFFERENTIAL DIAGNOSIS

Lung adenomatosis (jaagsiekte) has been confused with maedi for a long time. Clinically the two diseases are difficult to differentiate. In maedi-affected flocks an occasional weak and dry cough may be heard. Since the interstitial pneumonia is not accompanied by excretion of fluid or mucus in bronchi and trachea, coughing in our opinion is mostly due to lungworm infestations. Jaagsiekte is associated with an increased bronchial secretion, which may result in nasal discharge. Farmers in Iceland called this disease 'vota-maedi' (=wet maedi). The reported incubation period of lung adenomatosis is shorter than that for progressive interstitial pneumonia (24). At autopsy adenomatous lungs are generally not evenly enlarged. In early stages the solitary bacon like tumours are embedded in tissue of normal consistency.
Histopathologically, the two conditions can be differentiated. Maedi starts off with a proliferative reaction of inter-

stitial tissue and lymphoid tissue, whereas the early alterations in jaagsiekte occur in epithelium of bronchioles and alveoli. The solitary adenomatous nodules surrounded by normal parenchyma are a dominant feature in the early stage. The presence of layers of cuboidal or low-cylindric cells lining alveoli or smaller bronchi and the projecting papillary ingrowths are specific for advanced cases of adenomatosis. The alveolar spaces and small bronchioles are often filled with exudate containing large numbers of leucocytes. Metastasis in mediastinal lymph nodes and even in other parts of the body has been reported for jaagsiekte (25, 26), but have never been observed in maedi.

HOST RANGE

Infections with maedi/visna virus seem to be restricted to sheep and goats. Low titres of neutralising activity against maedi/visna virus were observed in serum samples of bovine and human origin (27), but these do not reside in the gamma-globulin fraction of the blood and probably represent non-specific inhibitors. Numerous attempts to infect small laboratory animals with maedi/visna virus have failed (28). Although virus multiplication was shown in tissue cultures of bovine and recently also in astrocyte cultures of human origin (29), there is no indication that maedi/visna virus possesses infectivity for these species in vivo. Sheep farming always involved close human contact. Affected sheep were often slaughtered on the farm without any precautions and meat from diseased sheep was always, and still is, considered suitable for human consumption and marketed accordingly. Fortunately, despite the extensive human exposure, there is no evidence that maedi/visna infections spread to man.

GEOGRAPHIC DISTRIBUTION AND INCIDENCE

Maedi or maedi-like pneumonias, although not all verified by virus isolation, have been reported in sheep in several

European countries, in various parts of the USA, East- and South-Africa and India. The lung condition has been described under various names such as zwoegerziekte, progressive pneumonia, Montana sheep disease, disease of Graaff-Reinet and 'la bouhite' (4, 14).
In 1939, Gíslason observed several diseased Icelandic sheep showing an interstitial pneumonia. Epidemiological observations indicated that all maedi outbreaks could be traced back to a few sheep of the Karakul breed which were imported from Germany in 1933. The disease spread gradually through large parts of the country and by 1945, when the epizootic was at its peak, about 60% of the sheep farming districts were affected. At this time the number of winterfed sheep had declined from 700.000 to 450.000. In individual flocks the annual losses could reach 20-30% (14). The disease in combination with visna, jaagsiekte and scrapie (rida) became an economic disaster for the Icelandic sheep industry. It was therefore decided to slaughter all sheep on every farm within affected districts of the country and to repopulate these farms later with sheep from unaffected areas. This heroic eradication programme took almost ten years to accomplish (1944-1954), but with success. In a few districts in the western and north-western part of the country, 4 to 7 years after the original flocks were removed, maedi was observed again and the control programme had to be continued. The last recurrence of maedi in Iceland was in 1965. The disease had disappeared before laboratory techniques for epidemiological studies became available (7).

Other countries were not so fortunate, but only a few studies were performed on the incidence of maedi/visna virus infections. In 1971 a serological survey was performed with a random sample of sheep sera from various areas in the Netherlands. About 3000 serum samples were collected from larger flocks and per flock about 10% of the adult sheep were bled. Serum samples were tested for precipitating and complement fixing antibodies with AGPT and CFT. Antibody was detected in about 28% of the sheep sera tested. The majority of the seropositive sheep were detected with the AGPT and an additional 2% by the use of CFT. In most flocks at least one

animal with antibodies was present. The data suggested a widespread maedi/visna virus infection in the Dutch sheep population (22).

In the last ten years an increase of maedi pneumonias has been observed in sheep of Denmark, Norway, Sweden and Germany (30, 31). Especially in Denmark and Norway maedi/visna virus infections seem to be increasing,but exact information on the incidence is still lacking. Many infections in these four countries were connected with import or trade of sheep of the Texel breed or their crosses with native breeds. However, in serological studies little attention was given to indigenous breeds. In Germany for example, antibodies to maedi/visna virus were detected in 50% of serum samples from a flock of Merino sheep in which clinical symptoms of maedi had never been observed (32).

Maedi/visna (ovine progressive pneumonia) was found to be prevalent in the major sheep-producing areas of the USA. Recently Cutlip *et al.* (33) collected about 1400 serum samples from cull sheep of 5 to 10 years of age at slaughter plants. Precipitating antibodies to maedi/visna virus were present in more than 40% of samples from midwestern and northwestern states. Virus was recovered from the lungs of 20 to 46% of such sheep from the same areas. In addition, in Idaho, relatively high incidences of precipitating antibody were observed in another serological survey of range sheep (34). The presence of precipitating antibody was largely determined by age, the percentage of positive sheep increasing from 16 in yearlings to 83 in ewes older than 7 years.

Originally, in the late thirties only sporadic cases of visna occurred in Iceland, but annual losses of some farms could reach 10% and in a few flocks the visna mortality exceeded that caused by maedi. After the end of the eradication campaign visna had disappeared from the field. Later, the disease was observed in a few other countries and usually in association with maedi (24). It is noteworthy that in Germany visna was seen by prevalence in sheep of the Merino breed (35).

Maedi/visna in goats seems to be a rare event. In both Germany and the Netherlands only one flock was observed to be affected. The German flock suffered from disorders of the central nervous system (36, 37) and the Dutch goats from interstitial pneumonia. From both outbreaks viruses were recovered in tissue culture which were similar to maedi/visna virus. Since the goats in the Netherlands were kept in close contact with a zwoegerziekte-affected sheep flock, it is likely that they had been infected by horizontal exposure. In India, however, maedi/visna infections of goats seem to be more common. Both interstitial pneumonia (maedi) and leucoencephalitis resembling visna have been reported and virus was isolated from lungs (38).

SUSCEPTIBILITY OF DIFFERENT BREEDS OF SHEEP

So far no clearcut data have been obtained for differences in susceptibility to maedi/visna virus infection between various breeds of sheep. The heavy losses in Iceland caused by maedi and visna could be suggestive for a genetically determined higher susceptibility of the Icelandic sheep, but Icelandic sheep are maintained under more severe stress than in other countries. In addition, the relatively high mortality of both conditions may be due to the introduction of virus in an immunologically virgin population. In our experimental studies with a zwoegerziekte strain of maedi/visna virus (9), lambs of Icelandic and Texel breeds were employed. Clinical symptoms of maedi and visna became manifest earlier in the Icelandic sheep, but these were infected with the small lungworm (Muellerius capillaris) and the sheep of the Texel breed were specific pathogen free. Lungworms may have had an enhancing effect on the course of the disease.
In a few countries certain breeds are predominantly infected. In the Netherlands this is no surprise, since 99% of sheep in this country are of the Texel breed. In Hungary, however, the disease has only been described in Merino sheep (39). In the Idaho survey significant differences were noted between the percentages of serologically positive sera from

sheep of various breeds within three large flocks (34). Rambouillets demonstrated a significantly lower incidence of precipitating antibodies than five other breeds while such antibodies were more frequently present in Finn crosses than in the other five.
Very little is known about differences of resistance or susceptibility of strains or blood lines within the same breed. During the epizootic in Iceland the impression was obtained that certain strains within the Icelandic breed were more resistant than others and crosses between native ewes and Border Leicester rams appeared to be particularly resistant (24). Our epizootiological observations indicated a positive correlation between serologically positive ewes and positive offspring within the Texel breed. This results probably from a horizontal (lactogenic) transmission of maedi/visna virus, but may also be influenced by genetically determined differences in susceptibility to infection.

EPIZOOTIOLOGY

In 1933, in Iceland, the Karakul sheep were kept in quarantine for two months and thereafter, showing no symptoms of disease, were sent to 14 farms in different districts of the country. In retrospect, at least two of the rams carried the infection of maedi and gave rise to two epizootics in different parts of the country. It soon became clear that maedi was a contagious disease with an incubation period of at least 2 to 3 years. The disease was later successfully transmitted by direct contact between healthy and diseased sheep, by contaminating drinking water with faeces from sheep affected with maedi and by injecting maedi lung material intrapulmonarily and intravenously (24). Sheep farming practices in Iceland are conductive to the spread of maedi/visna virus. All sheep are closely housed on the farms during winter and the size of the flock is kept as large as the food supply permits. During the summer months sheep from different farms roam freely in the hills, but here the communicability of maedi seems to be low, even in

its clinical stage (24). Stress and the degree of direct contact seem to be prerequisites for the development of clinical disease. In the Netherlands, severe losses (up to 15% mortality) are observed in a few larger flocks. In addition, in the individual animal, parasitic infestation (lung worms) may enhance the development of clinical pneumonia.

To study the transmission of maedi under natural conditions, we performed a field trial with four flocks of about 40 sheep which were separated from the parent flock at different times after birth (11). A total of 146 pregnant ewes of the Texel breed was purchased from 11 farms in the provinces of Zeeland and Noord-Holland (Isle of Texel) in the Netherlands. The age of the ewes ranged from 2 to 6 years. The farms were selected on the basis of a 10 to 20% loss of adult sheep due to zwoegerziekte in the preceeding years. The sheep were brought together shortly before the lambing season on an evacuated farm. Over a period of two months a total of 220 lambs were born from 135 ewes. Lambing was supervised day and night, and the lambs were assigned by lot into four groups.

The lambs of group No 1 were delivered into sterile towels and immediately transferred to a climatic chamber on the institute's farm. Lambs of group No 2 were left with their mothers for 9 to 11 hours, were allowed to take up colostrum from the dams, and were thereafter transported to a second climatic chamber. Both groups were reared artificially for a period of 2 to 3 months and thereafter transferred to separate paddocks of about two hectares each. Group No 3 was weaned at the age of 6 weeks and was then transferred to a third farm. Lambs of group No 4 were allowed the normal contact with the ewes of the parent flock for a period of one year. Two years after initiating the trial, the total numbers of sheep present in groups Nos 1, 2, 3 and 4 were respectively 50, 40, 38 and 38. These flocks were kept under observation for respectively 8, 6, 7 and 4 years. Serum samples were collected from the sheep of the four flocks twice a year. Results of serological

testing with AGPT, CFT and neutralisation tests of one sample per year are presented in Table 2.
During the 8 years of observation, no specific antibody to maedi/visna was detected in serum samples from sheep of flock No 1. No symptoms of maedi were observed and plasma clot cultures of various organs of these animals yielded no virus either. In flock No 2 a low percentage of sheep with antibody was detected with the AGPT and CFT, whilst the neutralisation tests remained negative during an observation period of 6 years. Signs of maedi were only observed in one five year-old animal. It showed respiratory distress and was the first sheep of this flock from which virus was recovered. Thereafter another four yielded virus. At autopsy lungs of two sheep of this flock weighed over 1000 g. Histological lesions indicative of maedi were observed in the lungs of three sheep. In contrast to flocks No 1 and No 2, antibodies to maedi/visna virus were frequently present in serum samples of flocks No 3 and No 4. The highest score was observed in flock No 4, in which the number of positive sera was about three times higher than in flock No 3 (Table 2). Most sheep of flocks No 3 and No 4 which died or had to be euthanized, suffered from maedi. Flock No 4 was slaughtered in the fourth year because the majority showed emaciation and laboured breathing. Histopathologic lesions of maedi were observed in 55% of the sheep and virus was recovered from 10 of 35 sheep tested. In flock No 3, these figures were somewhat lower and symptoms of maedi were mainly seen in the fifth and sixth year of the trial. The total of maedi/visna virus infected sheep detected at post mortem by virological, serological and histological examination in flocks No 1 to No 4 amounted to 0/50(0%), 11/40(28%), 28/37(76%) and 30/37(81%) respectively.
The trial provided evidence for horizontal transmission of maedi/visna virus. The results obtained with flock No 2 demonstrate that lambs may become infected within 10 hours after birth. A prolonged contact exposure, however, results in a more severe infection, which is reflected by the total number of serologically, virologically and histopathologic-

Table 2. Percentage of serologically positive sheep in the four flocks which were contact-exposed to the parent flock for different periods.

Flock No	Exposure to parent flock	Serological test	Age of sheep (years)							
			1	2	3	4	5	6	7	8
1	none	AGPT	0	0	0	0	0	0	0	0
		CFT	0	0	0	0	0	0	0	0
		NT	0	0		0		0		
2	10 hours	AGPT	5	7	3	6	9	11		
		CFT	0	3	3	3	3	7		
		NT	0	0		0	0	0		
3	6 weeks	AGPT	23	28	33	55	47			
		CFT		3	26	18	13			
		NT	25	14	16	0	0			
4	1 year	AGPT	67	67	55	64				
		CFT	62	65	56	26				
		NT	63	60	41					

ally positive and of clinically affected sheep observed in the three flocks.

In an earlier study (4), we reported the recovery of maedi/visna virus from milk of ewes (of flock No 4), one to five months after lambing. The infection of lambs of flock No 2 therefore was presumably via ingestion of colostrum, although fostering by the dam may have contributed. Lambs of flock No 3 were fostered for a period of 6 weeks. The rate of infection in this flock rose to 76% as compared with 28% in flock No 2. Flock No 4 was weaned from the parent flock at 5 to 6 months of age and again contact-exposed between 7 and 12 months. In winter, they were housed every night in a small barn together with the parent flock. The differences in the degree of contact exposure between the two flocks are well expressed in the time of onset of disease and differences in severity of clinical signs. In addition to ingestion of colostrum and milk, the lambs of flocks No 3 and No 4 may have been exposed to maedi/visna virus via the respiratory route or via ingestion of other materials such as faeces.

Recent observations in the field indicate that practically all serologically positive sheep are born from infected ewes. This is highly suggestive of the importance of lactogenic transmission in the epizootiology of maedi/visna, but one has to keep in mind that genetic predisposition may give a similar picture. The horizontal virus spread may probably be much faster when the infection is introduced for the first time in a sheep flock. We recently observed 90% seropositive sheep in a flock of imported Scottish Halfbreds, which had been in contact with maedi/visna infected sheep for 1½ year.

Sigurdsson *et al.* (40) obtained negative results in their experiments designed to test the role of sheep keds (Melophagus ovinus) in the transmission of disease. However, their experiment had to be terminated after 15 months and so the sheep ked remains a possible candidate for transmitting maedi/visna virus. We found no evidence for the transmission of maedi/visna virus via the small lungworm (Muel-

lerius capillaris, 11).
In our abovementioned field trial, no evidence was obtained for vertical transmission of maedi/visna virus. In addition, plasma clot cultures of various tissues of a total of 30 fetuses from maedi-affected ewes were invariably negative for virus (11). Therefore, since vertical transmission of maedi/visna virus does not occur, or at least is of no importance for the epizootiology of the disease, methods for controlling the disease appear feasible.

CONTROL OF MAEDI/VISNA

The economic losses caused by maedi/visna virus can be calculated in terms of earning capacity. No therapeutic methods are available and in the field sheep are usually culled from affected flocks before the age of 5 years. By this procedure mortality rates are kept low, but the earning capacity is not optimal. Control programmes should aim at elimination of the infection from the flock. At present the following procedures seem feasible:

1. The results obtained with flock No 1 of our field trial suggest that in a relatively short time a maedi-free flock can be obtained by artificial rearing of lambs which are separated from their dams immediately after birth. This procedure is based on the assumption that there is no intra-uterine transmission. It requires a strict hygienic regime which is difficult to perform under the usual farming conditions. The use of bovine colostrum could be considered to reduce neonatal infections. It seems to be the method of choice for experimental flocks. Recently by this approach maedi/visna virus was eliminated from a flock of the North Dakota State University (41).
2. In 1972, we initiated an attempt to develop a control programme on the basis of serological testing of a flock composed of 34 five year-old sheep and 41 of their two year-old progeny (flock No 3 of our field trial). The sheep were examined and sampled every six months and

the sera tested by AGPT and CFT. All clinically suspected and all seropositive sheep were eliminated. No sheep were introduced from outside, the ewes being served by rams born in the preceeding year. Except for the closed management, the flock has been kept under average Dutch farming conditions. The results of the serological tests are presented in Table 3.

Table 3. Decline of number of seropositive sheep (AGPT + CFT) in a maedi infected flock by slaughter of serologically positive and clinically diseased animals at six month intervals.

	Sheep born in 1967	Sheep born in 1971 and later	Total percentage positives
1972	17/34	0/43	22.1
1973	9/17	5/41	24.1
1974	1/ 7	3/36	9.3
1975		0/52	0
1976		0/49	0
1977		0/84	0
1978		0/104	0

No further seropositive sheep were detected later than three years after initiating this experiment. All 24 sheep which have been slaughtered since then, were examined virologically and by histological techniques. None of these sheep yielded virus and no histological lung lesions were observed. We tentatively conclude that maedi has been eradicated from this flock. As mentioned earlier a number of virus infected sheep do not react serologically and, following the above procedure, will only be detected after clinical disease has developed. The infection may thus linger in the flock for several years without being traced. In order to define the limits of usefulness of the above method the experiments have been extended to a few other farms in the country. The procedure of these field trials is as described above, but

in addition to slaughter of seropositive ewes, their offspring in also being eliminated since we observed a correlation in serological results between seropositive ewes and their progeny. A regular culling of seropositive sheep is certainly more economical than slaughter of all sheep of infected farms, followed by restocking with maedi/visna-free sheep. This procedure was successfully applied in Iceland (24), but was only feasible as a desperate attempt to save the sheep industry in this country.

REFERENCES

1. Sigurdsson, B: Rida, a chronic encephalitis of sheep with general remarks on infections which develop slowly and some of their special characeristics. Brit Vet J 110: 341-354, 1954.
2. Sigurdsson, B, H Thormar, PA Pálsson: Cultivation of visna virus in tissue culture. Arch ges Virusforsch 10: 368-381, 1960.
3. Sigurdardóttir, B, H Thormar: Isolation of a viral agent from the lungs of sheep affected with maedi. J Infect Dis 114: 55-60, 1964.
4. De Boer, GF: Zwoegerziekte. A persistent virus infection in sheep. Thesis, University of Utrecht: 1-211, 1970.
5. Ressang, AA, GF de Boer, GC de Wijn: The lungs in zwoegerziekte. Path Vet 5: 353-369, 1968.
6. Ressang, AA, FC Stam, GF de Boer: A meningo-leucoencephalomyelitis resembling visna in Dutch zwoeger sheep. Path Vet 3: 401-411, 1966.
7. Thormar, H, H Helgadóttir: A comparison of visna and maedi viruses II. Serological relationship. Res Vet Sci 6: 456-465, 1965.
8. Thormar, H: A study of maedi virus. Proc Int Conf on Lung Tumours. Perugia, Italy. Severi L (ed): 393-402, 1965.
9. De Boer GF: Zwoegerziekte virus, the causative agent for progressive interstitial pneumonia (maedi) and meningo-leucoencephalitis (visna) in sheep. Res Vet Sci 18: 15-25, 1975.
10. Harter, DH, R Axel, A Burny, S Gulati, J Schlom, S Spiegelman: The relationship of visna, maedi and RNA tumour viruses as studied by molecular hybridization. Virology 52: 287-291, 1973.
11. De Boer GF, C Terpstra, J Hendriks, DJ Houwers: Epizootiological studies of maedi/visna of sheep. Res Vet Sci, in press.
12. Sigurdsson, B, PA Pálsson, H Grímsson: Visna a demyelinating transmissible disease of sheep. J Neuropathol Exp Neurol 16: 389-403, 1957.

13. Georgsson, G, N Nathanson, PA Pálsson, G Pétursson: The pathology of visna and maedi in sheep. In: Slow virus diseases of animals and man, Kimberlin RH (ed) Amsterdam-Oxford, North Holland Publishing Company, 1976, p61-96.
14. Pálsson, PA: Maedi/visna, a slow virus disease. Bulletin OIE, in press.
15. Nathanson, N, H Panitch, PA Pálsson, G Pétursson, G Georgsson: Pathogenesis of visna. II. Effect of immunosuppression upon early central nervous system lesions. Lab Invest 35: 444-451, 1976.
16. De Boer, GF: Antibody formation in zwoegerziekte, a slow infection in sheep. J Immunol 104: 414-422, 1970.
17. Gudnadóttir, M, PA Pálsson: Host-virus interaction in visna infected sheep. J Immunol 95: 1116-1120, 1965.
18. Gudnadóttir, M, K Kristinsdóttir: Complementfixing antibodies in sera of sheep affected with visna and maedi. J Immunol 98: 663-667, 1967.
19. Terpstra, C,GF de Boer: Precipitating antibodies against maedi/visna virus in experimentally infected sheep. Arch ges Virusforsch 43: 53-62, 1973.
20. Haase, AT, O Stowring, D Narayan, DE Griffin, D Price: Slow persistent infection caused by visna virus: Role of host restriction. Science 195: 175-177, 1977.
21. Narayan, O, DE Griffin, J Chase: Antigenic shift of visna virus in persistently infected sheep. Science 197: 376-379, 1977.
22. De Boer,GF, C Terpstra: The incidence of maedi/visna virus infections in the Netherlands. Tijdschr Diergeneesk 99: 655-658, 1974.
23. Bruns, M, B Frenzel, F Weiland, OC Straub: Vergleich verschiedener serologischer Methoden zum Nachweis von Antikörpern gegen Maedi/Visna Virus. Zbl Vet Med B 25: 437-443, 1978.
24. Pálsson, PA: Maedi and visna in sheep. In: Slow virus diseases of animals and man, Kimberlin RH (ed), Amsterdam-Oxford, North Holland Publishing Company, 1976, p17-42.

25. Enchev, S: On the malignant (metastasizing) forms of what is known as pulmonary adenomatosis (jaagsiekte) in sheep. CR Acad Bulg Sci 16: 441-444, 1963.
26. Nobel, TA, F Neuman, U Klopfer: Metastases in pulmonary adenomatosis of sheep. Refuah Vet 25: 57-52, 1968.
27. Thormar, H, H von Magnus: Neutralization of visna virus by human sera. Acta Path Microbiol Scand 57: 261-267, 1963.
28. Thormar, H: Visna-maedi infection in cell culture and in laboratory animals. In: Slow virus diseases of animals and man, Kimberlin RH (ed). Amsterdam-Oxford, North Holland Publishing Company, 1976, p97-114.
29. MacIntyre, EH, CJ Wintersgill, AE Vatter: A modification in the response of human astrocytes to visna virus. Am J Vet Res 35: 1161-1163, 1974.
30. Krogsrud, J, H Udnes: Maedi (progressive interstitial pneumonia in sheep). Diagnosis epizootiology, prevention and control programme in Norway. Bulletin OIE, in press.
31. Straub, OC: Ueber die Isolierung von Maedi Visna Virus (MVV) aus einem deutschen Schafbestand. Berl Münch Tierärztl Wschr 83: 357-360, 1970.
32. Bruns, M, OC Straub, F Weiland, S Feiler: Detection of antibodies against glycoprotein of maedi/visna virus released as soluble antigen in cell cultures. In preparation.
33. Cutlip, RC, TA Jackson, GA Laird: Prevalence of ovine progressive pneumonia in a sampling of cull sheep from Western and Midwestern United States. Am J Vet Res 38: 2091-2093, 1977.
34. Gates, NL, LD Winward, JR Gorham, DT Shen: A serologic survey of the prevalence of ovine progressive pneumonia in Idaho range sheep. In preparation.
35. Schaltenbrand, G, OC Straub: Visna/maedi in einer unterfränkischen Merinofleischschafherde. Dtsch Tierärztl Wschr 79: 10-12, 1972.
36. Weinhold, E: Visna-Virus-ähnliche Partikel in der Kultur von Plexus Chorioideus Zellen einer Ziege mit Visna-Symptomen. Zbl Vet Med B 21: 32-36, 1974.

37. Weinhold, E, B Triemer: Visna bei der Ziege. Zbl Vet Med B 25: 525-538, 1978.
38. Dwivedi, JN: Studies on jaagsiekte and maedi (pulmonary adenomatosis complex) in sheep and goats with special reference to their infections vis-à-vis cancerous nature. Mathura, India; U.P. College of Vet Sci Husb: 1-52, 1974.
39. Süveges, T, A Széky: Incidence of maedi (chronic progressive interstitial pneumonia) among sheep in Hungary. Acta Vet Acad Sci Hung 23: 205-217, 1973.
40. Sigurdsson, B, PA Pálsson, A Tryggvadóttir: Transmission experiments with maedi. J Infect Dis 93: 166-175, 1953.
41. Molitor, TW, MR Light, IA Schipper: Elevated concentrations in serum immunoglobulins due to infection by ovine progressive pneumonia virus. Am J Vet Res 40: 69-72, 1979.

DISCUSSIONS OF PAPERS BY G. PÉTURSSON ET AL. AND G. F. DE BOER AND D. J. HOUWERS

The possibility of studying the effect of immunosuppression in long-term experiments with visna was mentioned. Dr Pétursson replied that this was not possible as the animals succumbed rapidly; possibly a modified immunosuppressive regime might be feasible. The presence of antibodies to visna in the cerebrospinal fluid in very high titre was queried; these antibodies were indeed present, sometimes in titres up to four times greater than those observed in the peripheral blood.

Antigenic variation in visna infections was discussed. This was a real phenomenon and variants of virus were recovered at different times from the same animal, the new variant being less readily neutralised by early serum than the early variant. Much more systematic work was necessary in this area.

THE KEY ROLE OF CELL MEMBRANE MODULATION IN THE BIOLOGICAL EFFECTS OF INTERFERON

C. CHANY, M. F. BOURGEADE, M. BERGERET, D. SERGIESCU, A. PAULOIN AND F. CHANY-FOURNIER

Interferon has been regarded for a long time only as an inducible antiviral protein released by virus-infected cells. However, a great variety of antigens of bacterial origin (1), lectins (2), or other substances, can induce interferon in animals or white blood suspensions. This observation greatly complicates the relatively simple original concept on the role of these substances in acute or chronic infections. In the present state of knowledge interferons can be divided into two main cat gories:

- Type I interferon is produced in virus-infected B lymphocytes (3) or other somatic cells. Although leukocyte interferon is different from the fibroblast variant in molecular weight and antigenic structure (4-6), they are all pH resistant and heat-sensitive.

- Type II interferon is released by antigen-sensitized T lymphocytes after restimulation by the same antigen or lectins. In contrast, this variant is heat-resistant, but acid-labile (Table 1).

Table 1

Interferon	Lymphocytes	Other Somatic Cells	Inducer
Type I	B	+	Virus RNA
Type II	T	0	Viral or other antigens (DH)x

xDH means delayed hypersensitivity

Many aspects of type I interferon production and action are well documented (7). Not much is known, however, on the biological role of type II interferon. We will, therefore, focus on presently available knowledge on type I interferon and point out possible common features of the two types.

During acute infection, interferon is produced locally by the infected cells and by both B and T lymphocytes. It diffuses thereafter into the inflammatory area and into the bloodstream (8). It acts on target cells which can be located at distant sites. In this respect interferon resembles other messenger molecules such as lymphokines or hormones. The effect on target cells is a general slow-down of cell metabolism which results in vitro in a decrease of cell replication (9) and is associated with the development of the antiviral state. It is as yet uncertain whether the antiviral activity appears or not in interferon-treated cells in the absence of a challenge virus (10). Studies of interferon action in cell-free systems seem to indicate that dsRNA has to be added to the cell sap to induce or increase a number of metabolic steps which result in the inhibition of viral protein synthesis and the degradation of mRNA (11-13). There is no evidence whatsoever that these inhibitory steps are really selective for virus metabolism. If this is correct, it means that the challenge virus triggers or enhances the antiviral state in interferon-treated cells. Thus in the absence of infection, interferon could modify somewhat differently the biological status of the cell.

The primary site of the biological action of interferon is a cell membrane modulation followed, as first demonstrated by Paucker (9), by a general repressing effect on cell metabolism. In addition, a change in the phenotypic expression occurs. This can be shown when cancer cells are carried for a long period in the presence of interferon. These cells recover contact inhibition and no longer produce colonies in soft agar, even though the cells continue to divide at a lower rate (14, 15) (Fig. 1).

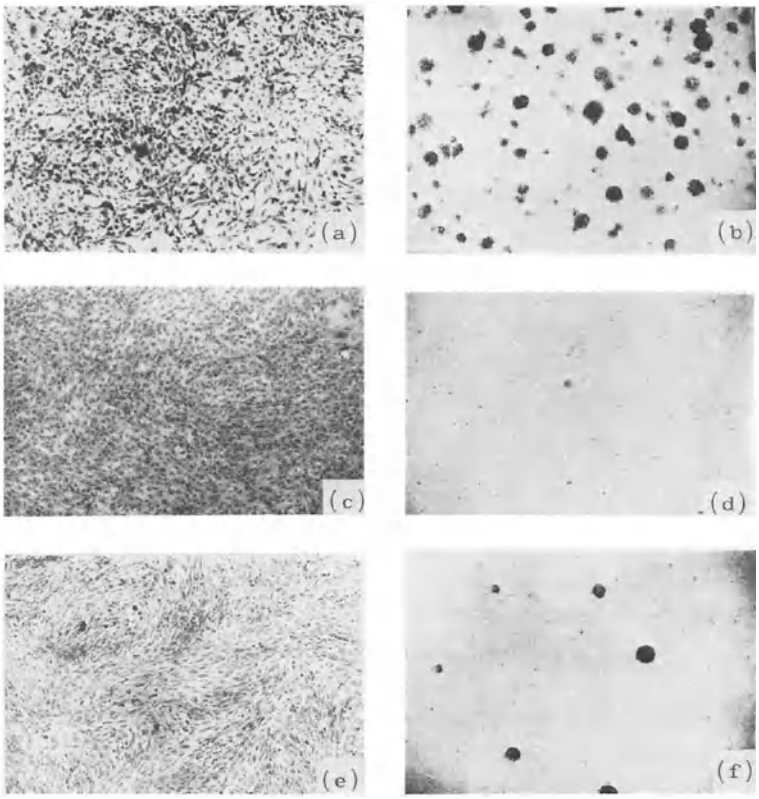


Figure 1[x]

(a) Murine sarcoma virus (MSV)-transformed mouse embryonic fibroblasts (MEF).

(b) Same cells producing colonies in soft agar.

(c) Same cells grown in the presence of interferon for 200 passages (MSV-IF+).

(d) MSV-IF+ cells grown in soft agar. Practically no development of colonies.

(e) Normal MEF used for transformation by MSV.

(f) Reappearance of colonies in MSV-IF+ cells when interferon is omitted from the tissue culture medium for 30 passages.

[x]Reprinted from the J. Gen Virol. (1970), 7, 206-207 with slight modification.

Thus, the mechanism of cell membrane modulation is, in our view, the key problem for a better understanding of the apparently contradictory effects which can be produced by interferon during chronic infection or disease.

ROLE AND MECHANISM OF CELL MEMBRANE MODULATION IN THE DEVELOPMENT AND MAINTENANCE OF THE ANTIVIRAL STATE

Binding of interferon to the cell. It has been shown that in order to act interferon has to bind to the cell membrane (16). Binding is a temperature-independent step and can occur at 4°C, but activation of the antiviral state only appears at 37°C. Further studies in somatic Cercopithecus monkey-mouse (or human-mouse) cells have shown that the presence of only one monkey 29 chromosome (or human 21) in every cell is necessary for the expression of primate interferon sensitivity. Trypsin treatment of one monkey-mouse hybrid clone, followed by the suspension of the cells in a serum-free medium for 3-4 h, abolishes the sensitivity of cells to primate, but not to mouse, interferon (Table 2). This experiment suggests that the primate interferon receptor, more exposed than the murine variant, is temporarily destroyed by the proteolytic enzyme. It can be concluded therefrom that a membrane-bound glycoprotein is responsible for interferon binding and is probably the interferon cell species specific component. It is also likely that in the hybrid cells, the mouse components are responsible for the subsequent cytoplasmic changes which occur in the cell (17).

The existence of another but non-specific binding site has been suggested by Besançon and Ankel (18). Interferon covalently bound to sepharose can induce antiviral activity in sensitive cells after contact in the absence of detectable leakage (19, 20). Such sepharose-bound interferon loses its antiviral properties when gangliosides (in the case of murine interferon GM 2) (21) or lectins (PHA) (22) are added to the beads. The role of gangliosides in interferon action is also substantiated by (a) interferon resistance of cells lacking mature monosialogangliosides (23) and (b) disappearance of interferon sensitivity induced by cho-

Table 2[x]

Cell type	Dispersing agent	Interferon: Human WBC, No serum	Interferon: Human WBC, 2% calf serum	Interferon: Mouse, No serum	Interferon: Mouse, 2% calf serum
Parental monkey	Trypsin	0.07	3.42	N.D.	N.D.
Hybrid clone M	Trypsin	0.49	2.06	2.76	2.86
Parental mouse	Trypsin	N.D.	N.D.	1.13	1.75
Mouse L cells	Trypsin	N.D.	N.D.	2.80	2.97
Parental monkey	EDTA	2.86	3.07	N.D.	N.D.
Hybrid clone M	EDTA	2.23	2.25	N.D.	N.D.

Antiviral effect of primate and mouse interferon in trypsinized or EDTA-dispersed parental and clone cells. Numbers represent $\log_{10}$ inhibition of VSV yield in interferon-treated cells as compared to contact cells subjected to the same experimental conditions but incubated with media containing no interferon. N.D. means not done.

[x]Reprinted from Proc. Nat. Acad. Sci. USA 70 (1973) 559, with some modifications.

lera toxin or hormones which can also bind to gangliosides (24), although the effect is probably indirect.

Cell membrane modulation. As here shown, interferon binds to at least two cell membrane constituents. On the other hand, the now well-established model of membrane structure, devised by Singer and Nicolson (25), predicts the lateral mobility of the glycoproteins and glycolipids which are its structural parts. The sigmoidal shape of the interferon dose-response relationship can be especially well analyzed in somatic primate-mouse hybrid cells which, as has been shown earlier, contain at least two interferon species-specific receptor proteins. Indeed, in the same hybrid cell, the dose-response relationship can be different for mouse or primate interferon. It is therefore likely that this modification is due to the number, exposure, or activity of these receptors. The expected results will be

a remodulation of the cell membrane depending on the concentration of the interferon molecules and on the mobility of the membrane constituents.

Membrane-bound glycoproteins are anchored on cytoplasmic cytoskeletal structures (thin filaments made up of actin, thick filaments formed by myosin in a hammer-like structure, and tubuline). These constituents govern the lateral mobility of the cell membrane-bound glycoprotein. Their integrity could be important for interferon action.

Indeed, data available show that inhibition of the cytoskeletal system by drugs such as cytochalasin B, vinblastine, or colchicine inhibit in parallel the antiviral action of both type I and II interferons (26, 27). In cells infected with Sarcoma virus temperature-sensitive in Sarcoma (src) gene, the shift from non-permissive to permissive temperature results in the loss of the synthesis of microtubules and microfilaments (28, 29). Sarcoma cells containing defective cytoskeletons also respond poorly to interferon. Sodium butyrate, which improves in such cells the synthesis of these components, also enhances interferon sensitivity, while the same drug is inactive in normal cells (30, 31).

The interferon-induced modifications of the cell membrane are not only necessary to establish the antiviral (or other) effects of interferon, but are also necessary for its maintenance. When this interferon-induced distribution of cell membrane constituents is modified either from the inside (by inhibitors of the cytoskeleton) or from the outside by PHA (32), the established antiviral state decays in 6-8 h and the cell recovers sensitivity to viruses. When the cells are retreated with interferon, antiviral activity can be reinduced.

RELATIONSHIP OF CELL MEMBRANE MODULATION TO NON-ANTIVIRAL EFFECTS OF INTERFERON

Effects on the immune system. In addition to its effect on the reduction of cell proliferation and changes in the phenotypic expression of the cells already mentioned, interferon also has a complex effect on the immune system. Phagocytosis of carbon particles by mouse peritoneal macrophages can be increased (33). This can explain the suppression of infection due to organisms such as E. Coli, Listeria monocytogenes, Chlamidia, plasmodia Berghei, etc.

T-lymphocytes sensitized to L 1210 cells incubated in the presence of interferon show enhanced cell mediated cytotoxicity (34). More complex results have been obtained using T cells sensitized to a monkey-mouse hybrid cell after the immunization of syngeneic mice. Interferon treatment of the target cells increases cytotoxicity after primary (low level) sensitization. On the contrary, the same treatment protects target cells against lymphocytes hypersensitized after secondary in vitro treatment with the target cells. It is not difficult to relate these contradictory effects to cell membrane modulation, since interferon-treated target cells show, in addition, a significantly increased spontaneous lysis in the presence of ^{51}CR. Such surface changes could be responsible for the increased expression of H-2 antigens on interferon-treated lymphocytes (35).

Interferon has been shown to act on delayed hypersensitivity in mice. Using the ear-swelling and footpad-swelling tests, interferon decreases or completely inhibits sensitization of mice when injected prior to the antigen or decreases its expression in already sensitized mice (36).

B cell functions can also be affected by interferon. Small amounts of interferon can increase, while large amounts decrease, antibody production in vitro (37). In addition, interferon treatment of T lymphocytes inhibits lymphoblastoid transformation by lectins (38).

Effects of the two interferons during acute and chronic infections. As stated earlier, the problem is relatively simple during primary infection. In the inflammatory area, type I interferon is produced and after hypersensitization of the T cells in a later step, type II interferon can also be secreted. Indeed, it has been shown that sensitized T lymphocytes can produce type II interferon in the presence of macrophages when triggered by Vaccinia (39) or Herpes (40) viruses. Interferon, after induction of the here described cell membrane modification, will induce a number of metabolic steps leading to the blockage of virus infection. An important link between cell membrane and cytoplasmic events is missing. The possible role of cyclic nucleotides is uncertain (41).

In chronic infections the problem is much more complex. Undoubtedly type I and II interferons will similarly protect uninfected cells and limit the extension of the lesions.

In cells already infected by the virus, several possibilities have to be considered. The virus replicates in the cells without producing a lethal injury. For RNA tumor viruses it has been shown that interferon can limit virus replication to some extent, acting much less on viral protein synthesis than on the budding of the particles from the cell membrane. They could become not only reduced in quantity but also altered in quality, resulting in the production of non-infectious particles (42-44).

In other cases, cells can be modified during an incomplete viral cycle. This is true for SSPE where nothing is known at present about the sensitivity of such cells to interferon. Several therapeutic trials using purified human interferon, injected either by the subcutaneous or intrathecal route, fail to show any modification in the course of the disease (unpublished data). Similar results have been observed during the acute phase of rabies. During this infection, patients can be maintained alive for about three weeks and die very often when the circulating antibody rises to a peak level. Interferon inhibits to a certain extent antibody synthesis but is without effect on the clinical

expression of the disease (unpublished data).

However, during chronic hepatitis B, where the infectious virus is expressed, intensive and prolonged interferon treatment has at least in some cases beneficial effects. A few patients have been completely cleared of the virus, with disappearance of all biological manifestations of the disease (45).

Interferon as an aggravating factor in chronic disease. In two experimental diseases interferon seems to be an aggravating factor in the pathogenic course of the disease. During choriomeningitis infection in mice, injection of antibody to mouse interferon protects mice against glomerulonephritis by neutralizing the endogenous interferon induced by the virus. This protection occurs in spite of a significant increase of virus titer in the animals (46).

NZB mice and their NZB/NZW hybrids develop an autoimmune disease comparable to human Systemic Lupus Erythematosus. In NZB mice, the main character of the disease is the appearance of anti-red cell antibodies and hemolytic anaemia. In NZB/NZW hybrids, anti-nuclear factors and anti-DNA antibodies are found associated to immune complex resulting in glomerulonephritis which eventually kills the animal. The administration of interferon inducers (47-49) or prolonged interferon treatment starting at birth (50) increases mortality in the animals when compared to the placebo group. It is of interest that in interferon-treated NZB mice, prior to death, anti-nuclear antibodies can be detected although they are generally only present in the NZB/NZW hybrids. Most of the animals develop a reticulum cell sarcoma which could contribute to their premature death. It is unknown by what mechanism interferon produces such unusual adverse effects. It is likely that modifications of cellular immunity (such as accelerated decrease of repressor T cell functions, already deficient in these animals) could be involved.

In summary, the biological role of interferon is more complex than its antiviral action. It is possible that type II interferon could regulate immune functions especially during delayed hypersensitivity. However, not much is known about the metabolic processes it induces in the cell. It is likely that some steps are common (51) with type I, while other are different (27).

Type I interferon is produced by practically all somatic cells during viral, or some bacterial infections. In both cases its synthesis and different biological effects are submitted to a negative control mechanism. Thus both production and action are transitory phenomena. Cell membrane modulation seems to be the key step for the induction, primary amplification, maintenance, and degradation of most, if not all, biological effects (52).

REFERENCES

1. Youngner, JS, SB Salvin: Production and properties of migration inhibiting factor and interferon in the circulation of mice with delayed hypersensitivity. J Immunol 14: 1914-1922, 1973.
2. Wheelock, EF: Interferon-like virus inhibitor induced in human leukocytes by phytohemagglutinin. Science 149: 310-311, 1965.
3. Yamaguchi, T, K Handa, Y Shimizu, T Abo, K Kumagai: Target cells for interferon production in human leukocytes stimulated by Sendaî virus. J Immunol 118: 1931-1935, 1977.
4. Duc-Goiran, P, B Galliot, C Chany: Studies on virus-induced interferons produced by the human amniotic membrane and white blood cells. Arch Virusforsch 34: 232-243, 1971.
5. Levy-Koenig, RE, MJ Mundy, K Paucker: Immunology of interferons.I. Immune response to protective and non-protective interferons. J Immunol 104: 785-790, 1970.
6. Havell, EA, B Berman, CA Ogburn, K Berg, K Paucker, J Vilcek: Two antigenically distinct species of human interferon. Proc Nat Acad Sci USA 72: 2185-2187, 1975.
7. Baron, S, F.Dianzani (eds): Texas reports on biology and medicine. The interferon system: A current review to 1978, 35, University of Texas Medical Branch, 1977.
8. Baron, S: Mechanism of recovery from viral infection. In Advances in virus research 10, Smith,DA, MA Lauffer (eds), Academic Press Inc., New York, 1963, p.39-60.
9. Paucker, K, K Cantell, W Henle: Quantitative studies on viral interference in suspended L cells. III. Effect of interfering viruses and interferon on the growth rate of cells. Virology 17: 324-334, 1962.
10. Friedman, RM, DH Metz, RM Esteban, DR Tovell, LA Ball, IM Kerr: Mechanism of interferon action: inhibition of viral messenger ribonucleic acid translation in L-cell extracts. J Virol 10: 1184-1198, 1972.
11. Kerr, IM, RE Brown, LA Ball: Increased sensitivity of cell-free protein synthesis to double-stranded RNA after interferon treatment. Nature (London) 250: 57-59, 1974.
12. Farrell, PJ, GC Sen, MF Dubois, L Ratner, E Slattery, P Lengyel: Interferon action: two distinct pathways for inhibition of protein synthesis by double-stranded RNA. Proc Nat Acad Sci USA 75: 5893-5897, 1978.
13. Revel, M: Interferon-induced translational regulation. In Texas reports on biology and medicine, Baron, S, F Dianzani (eds), 35, 1977, p. 212-218.

14. Chany, C, M Vignal: Etude du mécanisme de l'état réfractaire des cellules à la production d'interféron, après inductions répétées. Compt Rend Acad Sci Paris 267: 1798-1800, 1968.
15. Chany, C, M Vignal: Effect of prolonged interferon treatment on mouse embryonic fibroblasts transformed by murine sarcoma virus. J Gen Virol 7: 203-210,1970.
16. Friedman, RM: Interferon binding: the first step in establishment of antiviral activity. Science 156: 1760-1761, 1967.
17. Chany, C, A Grégoire, M Vignal, J Lemaître-Moncuit, P Brown, F Besançon, H Suarez, R Cassingena: Mechanism of interferon uptake in parental and somatic monkey-mouse hybrid cells. Proc Nat Acad Sci USA 70: 557-561, 1973.
18. Besançon F, H Ankel: Binding of interferon to gangliosides. Nature 252: 478-480, 1974.
19. Ankel, H, C Chany, B Galliot, MJ Chevalier, M Robert: Antiviral effect of interferon covalently bound to sepharose. Proc Nat Acad Sci USA 70: 2360-2363,1973.
20. Chany, C, H Ankel, B Galliot, MJ Chevalier, A Grégoire: Mode of action and biological properties of insoluble interferon. Proc Soc Exp Biol Med 147: 293-299, 1974.
21. Besançon, F, H Ankel, S Basu: Specificity and reversibility of interferon ganglioside interaction. Nature 259: 576-578, 1976.
22. Besançon, F, H Ankel: Inhibition of interferon action by plant lectins. Nature 250: 784-786, 1974.
23. Vengris, VE, FH Reynolds, Jr, MD Hollenberg, P Pitha: Interferon action: role of membrane gangliosides. Virology 72: 486-493, 1976.
24. Friedman, RM, LD Kohn: Cholera toxin inhibits interferon action. Biochem Biophys Res Commun 70: 1078-1084, 1976.
25. Singer, SJ, G Nicolson: The fluid mosaic of the structure of cell membranes. Science 175:720-731, 1972.
26. Bourgeade, MF, C Chany: Inhibition of interferon action by cytochalasin B, colchicine and vinblastine. Proc Soc Exp Biol Med 153: 501-504, 1976.
27. Bourgeade, MF, C Chany, TC Merigan: Type I and type II interferons: differential antiviral actions in transformed cells. J Gen Virol, submitted for publication.
28. Wang, E, AR Goldberg: Effects of the src gene product on microfilament and microtubule organization in avian and mammalian cells infected with the same temperature sensitive mutant of Rous Sarcoma Virus. Virology 92: 201-210, 1979.
29. Pollack, R, M Osborn, K Weber: Pattern of organization of actin and myosin in normal and transformed cultured cells. Proc Nat Acad Sci USA 72: 994-998,1975.
30. Bourgeade, MF: Effet du butyrate de sodium sur la sensibilité à l'interféron de cellules normales et transformées par le virus du sarcome murin. Compt Rend Acad Sci Paris 187: 391-394, 1978.

31. Bourgeade, MF, C Chany: Effect of sodium butyrate on the antiviral and anticellular action of interferon in normal and MSV transformed cells. Submitted for publication.
32. Pauloin, A, F Chany-Fournier, L Epstein, C Chany: Dégradation par la phytohémagglutinine de l'état antiviral induit par l'interféron. Compt Rend Acad Sci Paris 284: 1119-1122, 1977.
33. Huang, K: Effect of interferon on phagocytosis, in Texas reports on biology and medicine, Baron, S, F Dianzani (eds), 35, 1977, p. 350-356.
34. Lindhal, P, P Leary, I Gresser: Enhancement by interferon of the specific cytotoxicity of sensitized lymphocytes. Proc Nat Acad Sci USA 69: 721-725, 1972.
35. Bergeret, M, A Grégoire, C Chany: Different effect of interferon on target cells after treatment with lymphocytes exposed to primary or secondary sensitization. Submitted for publication.
36. De Maeyer-Guignard, J, A Cachard, E De Maeyer: Delayed-type hypersensitivity to sheep red blood cells: inhibition of sensitization by interferon. Science 190: 574-576, 1975.
37. Gisler, RM, P Lindhal, I Gresser: Effects of interferon on antibody synthesis in vitro. J Immunol 113: 438-444, 1974.
38. Lindhal-Magnusson, P, P Leary, I Gresser: Interferon inhibits DNA synthesis induced in mouse lymphocyte suspensions by phytohaemagglutinin or by alloqeneic cells. Nature New Biol 237, 120-121, 1972.
39. Epstein, LB, DA Stevens, TC Merigan: Selective increase in lymphocyte interferon response to vaccinia antigen after revaccination. Proc Nat Acad Sci USA 69: 2632-2636, 1972.
40. Rasmussen, LE, GW Jordan, DA Stevens, TC Merigan: Lymphocyte interferon production and transformation after herpes simplex infections in humans. J Immunol 112: 728-735, 1974.
41. Friedman, RM, I Pastan: Interferon and cyclic-3'5'-AMP: potentiation of antiviral action. Biochem Biophys Res Commun 36: 735-740, 1969.
42. Pitha, PM, WP Rowe, MN Oxman: Effect of interferon on exogenous, endogenous, and chronic murine leukemia virus infection. Virology 70: 324-338, 1976.
43. Friedman, RM, JM Ramseur: Inhibition of murine leukemia virus production in chronically infected AKR cells; a novel effect of interferon. Proc Nat Acad Sci USA 71: 3542-3544, 1974.
44. Billiau, A, H Sobis, P De Sommer: Influence of interferon on virus particle formation in different oncornavirus carrier cell lines. Int J Cancer 12: 646-653, 1973.
45. Greenberg, HB, RB Pollard, LI Lutwick, et al: Effect of human leukocyte interferon on hepatitis B virus infection in patients with chronic active hepatitis. New Eng J Med 25: 517-522, 1976.

46. Gresser, I, L Morel-Maroger, P Verroust, Y Rivière, JC Guillon: Anti-interferon globulin inhibits the development of glomerulonephritis in mice infected at birth with lymphocytic choriomeningitis virus. Proc Nat Acad Sci USA 75: 3413-3416, 1978.
47. Steinberg, AD, S Baron, N Talal: The pathogenesis of auto-immunity in New Zealand mice. I. Induction of antinucleic acid antibodies by polyinosinic-polycytidylic acid. Proc Nat Acad Sci USA 63: 1102-1107,1969.
48. Walker, SE: Accelerated mortality in young NZB/NZW mice treated with the interferon inducer, tilorone hydrochloride. Clin Immunol Immunopathol 8: 204-212, 1977.
49. Sergiescu, D, I Cerutti, E Efthymiou, A Kahan, C Chany: Adverse effects of interferon treatment on the life span of NZB mice. Biomedicine, in press.
50. Heremans, H, A Billiau, A Colombatti, J Hilgers, P De Somer: Interferon treatment of NZB mice: accelerated progression of autoimmune disease. Infect Immun 21: 925-930, 1978.
51. Wietzerbin, J, S Stephanos, R Falcoff, E Falcoff: Some properties of interferons induced by stimulants of T and B lymphocytes. In Texas reports on biology and medicine, Baron, S, F Dianzani (eds), 35, 1977, p. 205-211.
52. Chany, C: Membrane-bound interferon specific cell receptor system: role in the establishment and amplification of the antiviral state, Biomedicine 24: 148-157, 1976.

DISCUSSION OF PAPER BY C. CHANY

This discussion opened with a question as to whether conconavelin A was more effective than phytohaemagglutinin in blocking receptors for interferon. Dr Chany replied that there was no evidence for this. There was one report that the receptors could be 'capped' but this could not be accepted. Gangliosides gc2 and gc3 were the most important glycoproteins concerned with the action of interferon; a relevant observation in this context was that transformed cells were less sensitive to interferon than non-transformed cells.

Dr Lachmann asked for further information on interferon types I and II. In reply, Dr Chany outlined the facts as known. Type I is produced by leucocytes and also by other cells (for example, fibroblasts). It is pH resistant glycoprotein. Type II interferon was produced by T lymphocytes, following previous sensitisation by antigens and was a heat-stable, pH-labile glycoprotein. The actions of types I and II interferon differed, type II interferon having a greater anti-cellular and a lesser anti-viral effect than type I interferon. Types I and II interferon were antigenically different and there was not complete cross-neutralisation.

The mechanism of action of type II interferon was not known, although it appeared that the two types shared receptors. An interesting observation was that trisomy-21 cells were more susceptible to both types of interferon than normal diploid cells.

IMMUNE RESPONSES IN THE CEREBROSPINAL FLUID

A. LOWENTHAL AND D. KARCHER

The immune response may be either referred to cellular or to humoral immunity in cerebrospinal fluid (CSF) as in all other biological fluids. The study of cellular immunity in CSF has never been thoroughly undertaken, however, recent work has brought important information in this field, and allowed to identify the T lymphocytes (1).

The study of humoral immunity, on the other hand is much more important and a large literature is devoted to this subject. These researches enabled us to discover with agar electrophoresis the phenomenon of 'restricted heterogeneity' in human CSF (2). Different other electrophoretic methods and quantitative measurements, mainly immunological methods were later utilized and confirmed our results. In addition, culture of CSF cells showed that these cells produce immunoglobulins (3, 4). These points will be discussed in relation to slow viral diseases such as multiple sclerosis (MS), subacute sclerosing panencephalitis (SSPE), visna and distemper. On the other hand, we shall only mention the spongiform encephalopathies where all the examinations gave normal results and experimental allergic encephalomyelitis (EAE) where information on CSF is still incomplete.

MATERIAL AND METHODS.

A. Patients. For patients, and specially those affected with MS, the first need which arises is a clear clinical definition of the disease. A rigorous definition has to be reached before starting to study the biological material and the CSF. A clinical case of multiple sclerosis cannot, in our opinion, be accepted for scien-

tific investigations without a prior CSF electrophoretic examination. Cases with increased cell counts in the CSF, frequently used by some authors, should be considered as exceptional.

Besides MS, SSPE, other acute encephalitis, such as necrotizing encephalitis, and chronic encephalitis, such as trypanosomiasis, filariasis, neurosyphilis as well as acute and chronic, bacterial and viral meningitis, can provide material for investigation. This material may either be of human or of animal origin.

B. Identification of T lymphocytes can be done by methods described by Kam-Hansen (1). For the cell cultures, we will mention in particular Sandberg-Wollheim's work (3,4).

C. Identification and determination of immunoglobulins.

1) electrophoretic methods. The electrophoretic examinations are practically always effected on concentrated CSF. The question arises whether concentration produces artefacts and therefore method applicable to the study of non-concentrated CSF seems indicated. Besides these methods, there are some indirect methods such as colloïdal reactions, formation of double ring precipitations and immunoelectrophoresis. In our opinion, their contribution to the study of the immunological phenomena observed in CSF is lesser than the electrophoretic methods.

2) quantitative determinations of the immunoglobulins, IgG as well as IgM, were often carried out. They allow either a direct interpretation of the results or an interpretation based on the calculation of ratios which allows comparions of the distribution of immunoglobulins and other proteins (albumin e.g.) in CSF and in serum. We mention this approach although we give preference to direct quantitative determination. The restricted heterogeneity of the IgG's as seen in MS or SSPE makes, in our opinion a comparison with the so called homogeneous albumin fractions very difficult.

3) information relative to the immune reaction in CSF can also be obtained by measuring free or bound κ and λ light chains. The results confirm those of protein electrophoresis.

RESULTS

a) research on cellular immunity suggests that there is a decrease of the T cell depressive activity in CSF (1). In 30 % of cases of multiple sclerosis the number of cells is increased. These cells are usually lymphocytes, and in particular T lymphocytes (95 %).

The depressive immune activity of these T cells could be decreased, or perhaps, according to some authors their stimulating activity increased. The study of cellular immunity in the blood of patients suffering from multiple sclerosis has not provided so far any difenite information. The same is true for numerous slow viral diseases, with the exception perhaps of SSPE. In summary, in CSF, we are faced with an undoubted and important phenomenon : the modification of T lymphocytes.

b) studies investigating humoral immunity showed in human CSF, (before it was described in experimental animals) that there may be γ globulin fractionation, later called oligoclonal reaction (fig. 1). We think that the title 'antibody of restricted heterogeneity' used in experimental work in recent years, is more appropriate. It is probable that the detection of this reaction was more easy to achieve in CSF than in serum for purely technical reasons : the fact that in normal CSF there are practically no γ globulins. It has to be underlined that this observation was made first in CSF and thus is a neurological contribution. It was shown later that these γ globulins are mainly IgG's and only recently some papers referred to IgM's. This fractionation of the IgG is not specific for a disease. Once the fractionation

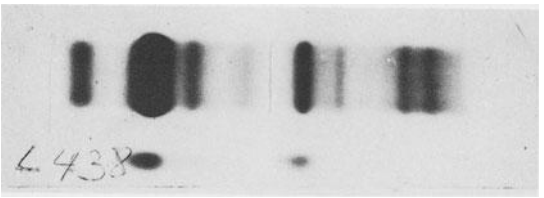


Figure 1 : Cerebrospinal fluid protein electrophoresis with fractionation of the γ globulins.

has settled in, it remains qualitatively the same for years. The quantitative interpretation of the pherograms is not simple; for this reason it is not easy to demonstrate that this fractionation remains quantitatively constant. It persists, even after a complete and protracted clinical recovery from some diseases - for instance necrotizing encephalitis. According to Vandvik, these fractionated immunoglobulins would be Igl's (5).

c) The IgM's (6, 7, 8, 9, 10, 11, 12) are frequently, but not always, increased in the CSF of patients affected with multiple sclerosis. The fractionation of the IgG and an increase of the IgM (11) are very often associated. Increases of the IgM may reach up to 5 times their normal values. There are still technical problems in the interpretation of the IgM determinations. The IgA (7, 8, 12) may also be increased. The concentrations of IgA, IgM and IgG can vary independently.

DISCUSSION

We wish to discuss four different points :

A. What definition can we presently give to the immune reaction, observed in the CSF in slow viral diseases as SSPE and MS ?

B. Should the IgG, present in the CSF in cases of slow viral diseases, be considered as homogeneous, like the IgG in experimental hyperimmunization ? What are the known immune activities of these homogeneous IgGs ?

C. where does the synthesis of these IgG's take place ? In the CSF or in other organs or tissues ?

D. what is the physiopathological significance of this hyperimmune process ? Is it a physiological or a pathological reaction ?

A. In MS we could define the immune reaction, observed in CSF, as characterized by a decrease of the depressive activity of the T lymphocytes, associated with a restricted heterogeneity of the IgG and occasionally an increase of the IgM. Due to the fact that immunoglobulins with restricted heterogeneity are technically easier to show in CSF than in serum, one came to the conclusion, maybe too hastily, that these immunoglobulins are produced locally in the central nervous system.

Moreover, in 80 % of cases of SSPE, in some cases of MS (± 30 %) and in diseases such as the Guillain-Barré syndrome, the fractionation is observed in serum as well as in CSF. This is also the case in acute encephalitis, like necrotizing encephalitis and in some chronic encephalitis, such as trypanosomiasis and filariasis. The restricted heterogeneity of the immunoglobulins could also be shown indirectly in MS serum, (it is observed only sporadically by electrophoretic methods) (fig 2) by precipitating the γ globulins with several antigens (13).

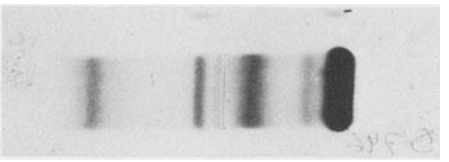


Figure 2 : serum protein electrophoresis in multiple sclerosis with fractionation of the γ globulins.

It seems probable that restricted heterogeneity is a general phenomenon that can be found in CSF and in serum in many neurological (and non neurological) diseases. Restricted heterogeneity in serum is a typical consequence of experimental hyperimmunization.

Until now, no investigation has been undertaken in the field of experimental hyperimmunization, to find out if oligoclonal reactions seen in the serum can also be found in CSF. Yet we can assume that they will probably be seen in CSF and this for two reasons :

1) the same homogeneous γ globulins are observed in serum and in CSF, in cases of myeloma, which are undoubtedly non-neurological diseases and where the homogeneous immunoglobulins have a non-neurological origin,
2) in ataxia telangiectasia, a disease characterized by a deficiency of IgA, a restricted heterogeneity of the IgG appears in serum, after repeated infections or after parenteral treatment with γ globulins (14). Undoubtedly, it is difficult to admit that these IgG are produced in the central nervous system.

Thus it is not excluded, and even highly probable, that a fractionation of immunoglobulins due to extra-neural processes will also be reflected in the CSF. Nor is it excluded that, in diseases where no anomalies in the serum are observed, as is frequently the case in MS, the anomalies in the serum may reamin hidden for technical reasons. We can thus assume that fractionation of the γ globulins can be considered a general phenomenon which probably develops to a maximum in the CSF, in disease states.

As far as we are concerned, we would consequently define the immune reaction observed in diseases such as SSPE and even MS, as a general hyperimmunization with probably an inhibition of the depressive action of the T lymphocytes. The hypothesis of hyperimmunization is confirmed by the fact that the measles antibody levels

in SSPE are extremely high in serum and tissues, as well as by the presence of antibody of restricted heterogeneity. Quantitatively, with regard to the other proteins, the restricted heterogeneity is also more intense in CSF than in serum. In MS we believe that the humoral immune reaction is similar to that in SSPE.

B. Experimental hyperimmunization in animals leads to the formation of partially specific homogeneous immunoglobulins. The number of fractions experimentally obtained varies from case to case. Very often 2 main and 5 secondary fractions are found. In addition to these fractions, CSF and especially serum display a background of heterogeneous immunoglobulins. Similar electropherograms are observed in serum of patients suffering from MS or SSPE. The multiplicity of these fractions raises some questions : are they different antibodies, active against different antigenic sites of single protein, complex proteins, or are each of these fractions active against different viral antigens, or are they immun-complexes? The measles virus consists of numerous proteins, different from one another, and could thus stimulate different antibodies. This could be one explanation for the fractions multiplicity in SSPE; it is still, at present, only an hypothesis of some authors who showed that different viral proteins can precipitate various IgG .

Some comments ought to be made. It has to be noted on the one hand, that precipitation does not involve all the serum fractions, and on the other hand, that the CSF IgG, at least in MS and to a lesser degree in SSPE (13), is immunologically active against numerous and various viral antigens. It is difficult in these experiments to state precisely what is to be referred to the homogeneous fractions and what to the background in the γ globulin region after agar gel electrophoresis. Only precise quantitative determinations could answer this question. The question can also be raised whether hyper-

immunization provokes a specific or a generalized stimulation of the humoral immune response. The increase of antibody titres against the antigen in experimental hyperimmunization and the measles antibody titres in SSPE, argues in favour of specificity. Specificity however cannot be considered to have been demonstrated, as long as quantitative measurements have not been carried out. We shall return to this in connection with SSPE serum.

Hence two questions are raised with regard to the fractions seen in neurological diseases : are they homogeneous and/or are they specific? We have been able to demonstrate in SSPE that the cathodic IgG serum fraction after electrophoresis is homogeneous by the study of the terminal aminoacid sequence of the variable part of the light and the heavy chains (15).

Confirmation of this homogeneity is given by the calculation of the κ and λ light chains'ratios. These ratios reveal a predominance either of the κ or the λ chains in serum and in CSF of SSPE and MS. The answer is yet not as clear as in myelomatosis where homogeneity has been proven beyond doubt.

What are these homogeneous immunoglobulins? Attempts were made to identify them by direct methods or by absorption methods. Fractionation of the immunoglobulins on Sephadex or DEAE columns or by isoelectric focusing and thereafter measurement of their measles antibody activity show very clearly in the case of SSPE that only some fractions, and mainly the most cathodic ones, have a very high measles antibody activity (16). The curve obtained by measuring the measles antibody activity in the various SSPE serum IgG fractions after isoelectric focusing shows two main, very cathodic peaks, and runs parallel with the optical density curve measured for the same proteins after agar gel electrophoresis.

This is only partial evidence that the γ globulins are measles antibodies. In absorbing this immunoglobulins with antigens, in the case of SSPE with measles virus,

it is possible to precipitate them out partially if not totally. Other antigens, different from the measles virus can, in MS, and even in SSPE, partially precipitate out some IgG. Precipitation is qualitatively demonstrable. The quantitative study of this precipitation has, up to the present time, never been done and is very difficult to achieve. Therefore the question of whether all IgGs are really precipitable remains open.

Finally, even if absorption is complete, which fractions are of interest with regards to the pathogenesis of the disease,remains unknown.

Our personal study and those we have mentioned, point to the fact that in SSPE serum, homogeneous immunoglobulins may be found, that great majority of these immunoglobulins are measles antibodies, and these conclusions are similar to those expressed by Vandvik and Norrby. We can add a personal observation : these antibodies show a specific idiotypy (17) characteristic for the patient, rather than for the disease. For the time being, one can add no more. All these results were obtained with serum and CSF of SSPE and MS patients. The studies pertaining to the serum were more successful for obvious reasons.

From the foregoing, the conclusion can be drawn that a hyperimmune state exists in SSPE and probably also in MS. This process leads to the synthesis of homogeneous measles antibodies in SSPE with specific idiotypy. One does not know if this reaction is a physiological or a pathological one.

C. Where does this hyperimmunization reaction occur? Quite a few observations show that this reaction occurs in the central nervous system :

1) the anomalies are more distinct in CSF than in serum; injection of labelled γ globulins made by Frick (18) showed that these γ globulins reached the CSF in cases of MS, but that some of the γ globulins are synthesized elsewhere. Research by Cutler (19).

on the vetricular fluid of patients suffering from SSPE confirms that some of the γ globulins are produced inside the meningeal envelope. The calculation of ratios for the distribution between serum and CSF proteins, such as Schuller (20) and Tourtellotte (21) are using, leads to similar conclusions, demonstrating that the most specific and active immunoglobulins are relatively more concentrated in CSF than in serum;

2) the study of immunoglobulins produced by CSF cell cultures (3, 4) confirms this point. These cells produce immunoglobulins with 'restricted heterogeneity'. These results were obtained by incorporating radioactive aminoacids in immunoglobulins produced by CSF cell cultures and subsequent, electrophoretic examination of these immunoglobulins. One has to remember, that the patients, who were all MS, had all more than 20 cells per mm3, which is exceptional. We have to remark that the oligoclonal reaction is seen in CSF in the absence of cellular reaction, and even in the absence of any neurological semeiology. Secondly we think that Sandberg-Wollheim's results should be confirmed by other methods than electrophoresis. Isolated IgG's easily show as an homogeneous band by electrophoresis.

3) in addition, it was possible to show immunoglobulins in the nervous tissue by immunohistological methods, in particular in certain 'glial' cells, which may be derived from blood.

But the fact that oligoclonal immunoglobulins are present in the serum, argues against the hypothesis of of an unique origin in the central nervous system. Should one admit that a phenomenon, localized in the central nervous system, could generate enough immunoglobulins to modify the composition of the blood and even of some organs in SSPE and MS, during years ? This seems difficult to accept.

In our opinion, we believe that we are confronted

with a general immune response with perhaps a particularly active component in the central nervous system. This could explain why, so often, these diseases end clinically with a neurological component. Local synthesis in the CNS probably occurs. It can be the consequence of a phenomenon occurring in the nervous tissue as well as in the cells present in the CSF. But such a local phenomenon cannot explain the fractionation of serum γ globulins which implicates necessarily a generalized hyperimmune reaction occuring in the nervous system as well as in other tissues. For us in SSPE, and probably in MS, the immune reaction is a generalized one, better seen or better demonstrated in CSF and located in the CNS as well as in other tissues.

D. How have we to interprete this hyperimmune reaction? Is it a continuous and relatively specific stimulation of antibody synthesis by one or more persistent antigens? This is more than evident, but does not explain how these antigens can persist in the presence of their antibodies. Depression of cellular immunity, the action of a depressive factor which would influence the cellular reaction and the antigen/antibody reaction, could be put forward. A depressive T lymphocyte factor affecting cellular immunity was found by several authors and was confirmed by the observations made by Kam-Hansen (1) in the CSF. By precipitating serum IgG with measles virus total absorption is not achieved (22). Therefrom the hypothesis was expressed that a factor present in serum would inhibit this precipitation. This factor could be a thermostable α 2 globulin and can be determined by RIA. We have not observed this factor in CSF. We wonder if we are not in the presence of one and the same factor which would act simultaneously on the cellular and the humoral immunity. Maybe it could be a factor acting on lymphocytes before they differentiate in T or B lymphocytes.

Has the hyperimmune reaction, as described, to be considered as a physiological, a pathological or ultimately a genetic reaction? It was suggested in the experimental section that hyperimmunization may be influenced by genetic factors. The existence of these genetic factors in SSPE or in MS is still to be proven. Or could it be a pathological reaction, the pathology probably being due to anomalies brought about by the inhibiting factor mentioned before? The fact that free κ and λ light chains (23) were found in CSF of those patients, argues in favour of a pathological immune response.

To us, the immune process seen in the CSF and serum in slow viral diseases, such as SSPE, is a hyperimmune reaction leading to the formation of homogeneous specific antibodies. This reaction can develop thanks to the presence of a factor inhibiting the immune mechanism in serum.

The immune reaction due to the existence of the antigen and the presence of an inhibiting factor is a pathological one. This hyperimmune process may extend to antibodies other than the one mentioned above. Although in SSPE the antigens are to be found in the measles virus, for MS we have no knowledge of the identity of the antigen. Autoimmune reactions against myelin proteins or even IgG (22) were mentioned in MS as well as antibodies against many different viral proteins.

We wish to add the following remarks :

1. <u>The results obtained by the determination of IgM.</u> For IgM (6, 7, 8, 9, 10, 11, 12) and even for IgA (8, 12), a local synthesis is suggested. The variations of the IgM levels might follow closely the clinical phenomena of exacerbations and remissions. The results of these determinations should be accepted with reservation. Determination of IgM by immune complex assays could be an answer to the technical problem. Let us remember

that the changes of IgM may not be correlated with those observed for IgG. The changes in the IgM would be more frequent in SSPE CSF than in MS CSF.

2. The immune complex assays (24). Here as well the results are equivocal. It is not excluded that, when the technical problems have been solved, we might come to interesting conclusions.
3. In spontaneous and experimental spongiform encephalopathies, electrophoresis and immune reactions remained normal in CSF and serum.
4. In EAE increase of γ globulins or IgG were reported in CSF. As far as we know, electrophoretic examinations were never carried out.

CONCLUSION.

We may conclude by saying that, in at least one of the diseases, considered as slow viral disease, SSPE, the hypothesis that the immune reaction observed in CSF is due to a generalized hyperimmunization provoked by a persistent antigen, is accepted. This hyperimmunization leads to the production of homogeneous and relatively specific antibodies. It is not excluded that under the influence of a persistent antigen, the humoral system is stimulated in its entirety and that other antibodies are also more actively produced than in normal or basic conditions. We ought to speak here about a generalized relatively specific hyperimmunization. Generalization of the humoral reaction could explain the large number of different IgG fractions observed after electrophoresis and isoelectric focusing. Persistence of the pathological antigen responsible for the hyperimmunization could be explained by the protection of this antigen against the antibodies by a specific inhibitor of the immune reaction. It seems that a genetic factor does not influence the immune reaction. The presence of κ and λ free chains allow to consider the hypothesis that the immune reaction has a pathological character and only is observed in predisposed individuals, for still unknown

reasons.

SUMMARY

By electrophoretic study of the CSF proteins we could, for the first time, demonstrate the restricted heterogeneity of the IgG. This restricted heterogeneity can be explained as a hyperimmunization due to the persistance in patients of an antigen protected against immune reactions by a factor inhibiting the antigen/antibody reaction. The immune phenomenon as a whole, would only be observed in specific individuals. This would explain the rarity of some of these diseases, such as SSPE.

REFERENCES

1. Kam-Hansen, S: Active T cells in blood and CSF in MS and controls. In: Humoral Immunity in Neurological Diseases, Karcher, D, A Lowenthal, D Strosberg (Eds), Plenum Press, London, 1979, (in press).

2. Lowenthal, A: Agar gel electrophoresis in neurology, Elsevier Publishing Co., Amsterdam, 1964.

3. Sandberg-Wollheim, M: Immunoglobulin synthesis in vitro by cerebrospinal fluid cells in patients with multiple sclerosis. Scand J Immunol 3: 717-730, 1974.

4. Sandberg-Wollheim, M: Immunoglobulin synthesis in vitro by cerebrospinal fluid cells in patients with meningoencephalitis of presumed viral origin, Scand J Immunol 4: 617-622, 1975.

5. Vandvik, B, JB Natvig, D Wiger: IgG 1 subclass restriction of oligoclonal IgG from cerebrospinal fluids and brain extracts in patients with multiple sclerosis and subacute encephalitides, Scand J Immunol 5, 427-436, 1976.

6. Ehrenkranz, NJ, ES Zemel, C Bernstein, K Slater: Immunoglobulin M in the cerebrospinal fluid of patients with arbovirus encephalitis and other infections of the central nervous system. Neurology 24: 976-980, 1974.

7. Schuller, E, N Delasnerie, M Hélary, M Lefèvre: Serum and cerebrospinal fluid IgM in 203 neurological patients. Eur Neurol, 17: 77-82, 1978.

8. Nerenberg ST, R Prasad, ME Rothman: Cerebrospinal fluid, IgG, IgA, IgM, IgD, and IgE levels in central nervous system disorders. Neurology 28: 988-990, 1978.

9. Ziola B, T Vuorimaa, A Salmi, M Pancluis, P Halonen, T Arnadottir, G Enders: Measles IgM antibody persistence: Is it a marker for chronic measles virus infections? Acta Neurol Scand 57, suppl 67: 237-238, 1978.

10. Sindic C, C Cambiaso, PL Masson, EC Laterre: Determination of IgM in the cerebrospinal fluid by particle counting immunoassay (PACIA). In: Humoral Immunity in Neurological diseases, Karcher D, A Lowenthal, D Strosberg (Eds), Plenum Press, London, 1979, (in

press).

11. Olsson JE, H Link: Immunoglobulin abnormalities in multiple sclerosis. Arch Neurol 28: 392-399, 1973.

12. Mingioli ES, W Strober, WW Tourtellotte, JN Whitaker, DE McFarlin: Quantitation of IgG, IgA and IgM in the CSF by radioimmunoassay. Neurology 28: 991-995, 1978.

13. Nordal HJ, B Vandvik, E Norrby: Oligoclonal virus antibodies in healthy adults and neurological patients. In: Humoral Immunity in Neurological Diseases, Karcher D, A Lowenthal, D Strosberg (Eds), Plenum Press, London, 1979, (in press).

14. Lowenthal A, K Adriaenssens, B Colfs, D Karcher, R Van Heule: Oligoclonal gammapathy in ataxia telangiectasia. Z Neurol, 202 : 58-63, 1972.

15. Strosberg AD, D Karcher, A Lowenthal: Structural homogeneity of human subacute sclerosing panencephalitis antibodies. J Immunol, 115, 157-160, 1975.

16. Karcher D, G Matthyssens, A Lowenthal: Isolation and characterization of IgG globulins in subacute sclerosing panencephalitis. Immunology, 23: 93-99, 1972.

17. Strosberg AD, D Karcher, A Lowenthal: Structure and idiotype of human homogeneous antibodies. Immunology 4249, Fed. Proc 969, 1975.

18. Frick E, L Scheid-Seydel: Untersuchungen mit I^{131} markiertem γ -Globulin zur Frage der Abstammung der Liquoreiweisskörper. Klin Wschr 36: 857-863, 1958.

19. Cutler RWP, GV Walters, CF Barlow: ^{125}I-labelled protein in experimental brain oedema. Arch Neurol 11, 225-238, 1964.

20. Schuller E: Cerebrospinal fluid immunoglobulins. II. Variations in different pathological situations. La nouvelle presse médicale, 8: 427-432, 1979.

21. Tourtellotte WW: Cerebrospinal fluid in Multiple Sclerosis. In: Handbook of Clinical Neurology, Vinken PJ, GW Bruyn (Eds), North Holland Pub, Amsterdam, 1970, p 324-382.

22. Karcher D, M Noppe, A Lowenthal: A heat stable serum inhibitor of an antigen antibody reaction of subacute sclerosing panencephalitis. J Neurol, 261/1: 51-56, 1977.

23. Bollengier F, A Lowenthal, N Henrotin: Bound and free light chains in SSPE and MS serum and CSF. Z klin Chem, 13: 305-310, 1975.

24. Masson PL: Circulating immune complexes in neurological disorders. In: Humoral Immunity in Neurological Diseases. Karcher D, A Lowenthal, D Strosberg (Eds), Plenum Press, London, 1979, (in press).

DISCUSSIONS OF PAPERS BY A. LOWENTHAL AND D. KARCHER

Dr Kreth commenced discussion of this paper by pointing out that hyper-immunisation in SSPE was probably a pathological process and not a normal one. There was little evidence for a possible failure of measles antigen in the peripheral circulation to form immune complexes. It was therefore not known how measles antibody production in SSPE took place in vivo; certainly, there was some evidence for high antibody levels before the onset of clinical signs. It might be that there was something wrong with the regulation of antibody production and that SSPE patients could not stop or regulate the production of antibody and this production had to go on and on. Such a regulatory error might involve interactions between regulatory and suppressor T cells and might be extremely specific. It could be related to the degree to which the patient had been exposed to the antigen. It was noted that, in SSPE patients, plasma exchange had no effect and the antibody levels rose extremely rapidly in spite of this procedure.

Dr Norrby asked why one should invoke a congenital immunological abnormality when there were clearly large amounts of antigen present. The position differed from MS, where the immune reaction was important. In SSPE, 80% - 90% of the immunoglobulin was directed against measles antigen but, in MS, the proportion was very much lower, most of the immunoglobulin being directed against other antigens.

INFECTIONS IN IMMUNODEFICIENT PATIENTS

A. D. B. WEBSTER

Introduction

Antibodies protect humans against many virus infections. This is shown by the finding that patients with primary hypogammaglobulinaemia, who are given regular gammaglobulin injections, do not suffer from the common virus infections of childhood such as measles, varicella and mumps. However, the mechanisms involved in eliminating established virus infections are not well understood. The traditional view is that cell mediated immunity plays a predominant role, probably though the killing of virus infected cells (cytotoxicity) by lymphocytes and macrophages. Nevertheless, the fact that some patients with primary hypogammaglobulinaemia develop chronic echovirus infection suggests that, in this instance, antibodies may also be important.

The virus infections which occur as complications of hypogammaglobulinaemia should not be confused with the virus infections which cause immunodeficiency. Both foetal rubella (1) and infectious mononucleosis in children (2) may cause hypogammaglobulinaemia but the infection does not persist in an overt form.

This chapter will describe the virus infections which occur in patients with primary defects of cellular or humoral immunity. Virus infections are not a recognised complication of the rare primary defects in neutrophils or the complement system.

PRIMARY ANTIBODY DEFICIENCY

SEX LINKED HYPOGAMMAGLOBULINAEMIA

This rare inherited disease is characterised by recurrent bacterial infections of the upper and lower respiratory tract, meningitis and occasionally septic arthritis in the first two years of life (3). The organisms involved are usually pneumococci and H.influenzae although they are also prone to mycoplasma infections. Virus infections, except for those due to certain enteroviruses, are not a troublesome complication.

The patients have very low levels of serum immunoglobulins and are

unable to make antibody when immunized. A characteristic feature, from the laboratory point of view, is the complete absence of circulating B lymphocytes. However, they do have cells in the bone marrow which are thought to be the precursors of B cells (4). Cellular immunity is intact, the patients showing normal delayed hypersensitivity skin reactions, macrophage inhibition factor production and normal in vitro lymphocyte transformation to mitogens and allogeneic cells. Their circulating T lymphocytes have normal 'natural' non-specific cytotoxicity against various target cells (5). Cytotoxicity against antibody coated cells, such as chicken erythrocytes, is also normal ('K' cell killing) but the killing of antibody coated allogeneic lymphocytes is severely depressed (6). This finding suggests that the latter type of cytotoxicity is mediated by a B lymphocyte.

Echovirus infections

In 1956, Janeway (7) described a condition like dermatomyositis in a child with sex-linked hypogammaglobulinaemia. The main features were the gradual onset of brawny oedema of the extremities with flexion contractures of the knee and elbow joints which produced a characteristic posture. In 1971, we investigated an 11 year old boy (AG) with sex-linked hypogammaglobulinaemia who presented with a two months history of severe headaches. He then developed brawny oedema of the arms with some swelling of the elbow and wrist joints (8). Echovirus 11 was cultured from his cerebral spinal fluid which also contained raised protein and mononuclear cells. A skin and muscle biopsy showed marked perivascular cuffing by mononuclear cells. There was relatively little muscle fibre destruction which explained why the serum creatinine phosphokinase level was normal. Although the oedema in his arms rapidly improved after treatment with human immune plasma, his headaches persisted and he had frequent grand mal convulsions. Central nervous system features were variable and, during good phases, he was able to return to school. He developed hydrocephalus six months after the start of his illness which required an atrial ventricular shunt. Two years later, he died suddenly with respiratory centre failure.

The autopsy on patient AG showed a severe chronic meningitis

with thickening of the leptomeninges of the brain and spinal cord. There were scattered destructive lesions in the brain showing marked astrocytic proliferation, microcalcification and perivascular lymphocytic infiltration. There was depletion of neurones in some parts of the central grey matter, cerebella nuclei and cerebellum.

Wilfert et al (9) reported six similar cases in the U.S.A. Echovirus was isolated from the cerebral spinal fluid of all patients although not all had central nervous system features. A subclinical hepatitis was common. Headaches, deafness and convulsions occurred in some patients and one had iridocyclitis. Although only two of the patients in this series had died at the time of reporting, it is generally believed that the disease is nearly always ultimately fatal.

Table 1 shows the features of the three patients we have managed. Patient WF developed episodes of erythema and swelling of the lower legs two years before he complained of headaches and sensory disturbances. His central nervous system disease progressed despite treatment with immune serum and he died of respiratory centre failure shortly after a bone marrow graft. MR was noticed to have flexion deformities of his elbows and knees at a routine clinical examination. Echovirus 17 was cultured from his cerebral spinal fluid, which also had a high protein and cell count, despite the absence of central nervous features.

Age at presentation of echovirus infection	Echovirus type isolated from CSF	Clinical features	Outcome
24	3	Oedema of lower legs, deafness, headaches, sensory disturbances	Died after 3 year illness of respiratory centre failure. Attempted bone marrow graft.
11	11	Headaches, deafness oedema of arms grand mal convulsions hydrocephalus	Died after $2\frac{1}{2}$ year illness of respiratory centre failure
18	17	Flexion deformities of elbows and knees, deafness	Condition unchanged after 18 months

Footnote: All three patients had severe congenital hypogammaglubulinaemia (two with affected male relatives) with absent circulating B lymphocytes and normal cellular immunity).

Treatment

All three of our patients have been treated with hyperimmune animal serum, patient AG receiving repeated infusions of horse serum while patients MR and WF were given sheep serum. The horse serum was obtained commercially but we raised our own immune sheep serum. This was done by propagating the echovirus from each patient on MRC 5 fibroblasts in an isolated laboratory. The tissue culture fluid was then processed to produce an inactivated vaccine by methods similar to those used for the production of polio vaccine. The sheep were immunized with the vaccine in Freunds complete adjuvant at multiple intradermal sites. Booster injections of vaccine in Freunds incomplete adjuvant were given at monthly intervals until an adequate antibody titre was reached. Most sheep produced a serum antibody titre of > 1:512 (measured by haemagglutination inhibition or neutralization) within about two months.

Two of the patients received about 200 ml of whole immune sheep serum intravenously every 2 - 3 weeks. Despite treatment for one year, the central nervous system features in patient WF progressed until his death. The swelling of his lower legs also persisted. MR received similar therapy for about a year and a repeat muscle biopsy, taken three months after starting serum therapy, was much improved. The flexion deformities of his elbows and knees have persisted. For the last seven months, we have been unable to isolate echovirus from his cerebral spinal fluid although the last two specimens have contained a virus-like agent (see Tyrrell's chapter). The dermatomyositic features in patient AG improved rapidly when he was given human plasma containing anti-echovirus 11 antibody at a titre of 1:80 (haemagglutination inhibition test). However, his central nervous system disease progressed despite treatment with hyperimmune horse serum, maternal peripheral blood white cells taken after she had been immunized with an echovirus 11 vaccine, and various attempts to non-specifically stimulate cellular immunity (i.e. BCG vaccination, transfer factor, Lamprene). Patient AG initially showed evidence of non-specific depression of cellular immunity with poor lymphocyte transformation and absence of delayed hypersensitivity skin reactions. However, these returned to normal with improvement in his general condition and we were able to demonstrate specific immunity against

echovirus 11 using a macrophage inhibition test (8).

Our experience indicates that the dermatomyositic features of this disease can be improved by treatment with hyperimmune animal serum. However, there is general agreement in both the U.S.A. and this country that such therapy does not alter the progression of the central nervous system disease. Since very little antibody is likely to cross the blood brain barrier, a more rational approach would be to give intrathecal hyperimmune globulin. Such therapy has been used successfully in the treatment of neonatal tetanus (10). It is probably dangerous to inject whole animal serum intrathecally as this commonly produces local inflammation when given intravenously. However, purified hyperimmune sheep globulin would probably be safe. Interferon therapy should also be considered but this would have to be given intrathecally as it does not cross the blood brain barrier.

Bone marrow transplantation is the only method currently available for reversing the immunological defect. However, in the absence of a histocompatible sibling, this is not worth consideration. The immunosuppression given to patient WF during his bone marrow transplant from his histocompatible sister did not seem to cause dissemination of the echovirus infection, and his death from respiratory centre failure a few weeks later was probably fortuitous.

Prophylaxis

The gammaglobulin therapy given to these patients usually offers no protection against echovirus infection. This is because the gammaglobulin is prepared one or two years before it is given to the patient and therefore does not contain antibody to the prevalent echovirus serotypes in the community at the time. An alternative approach would be to produce hyperimmune sheep globulin against a wide range of common echovirus serotypes. This could then be given at roughly monthly intervals to susceptible patients. The finding that three out of about 15 patients with either proven or probable sex-linked hypogammaglobulinaemia have developed echovirus infection, shows that this is a common complication and that it would be economically sensible to invest in such a prophylactic regime. Such therapy would only need to be given to those patients with childhood onset hypogammaglobulinaemia with absent circulating B lymphocytes.

Poliovirus

The incidence of paralytic poliomyelitis due to natural infection is probably not raised in patients with primary hypogammaglobulinaemia. However, there does seem to be a raised incidence of vaccine associated poliomyelitis and Wright et al (11) reviewed five cases. The disease usually has a prolonged incubation period of more than two months and at least two cases have survived with only minor muscle weakness. This complication does not occur in patients already receiving gammaglobulin therapy and none of the 28 children in the Medical Research Council series (12), who were given oral polio vaccine, developed paralytic disease. However, two of these patients continued to excrete the vaccine strain in the stools for up to 32 months.

ADULT ONSET 'VARIABLE' HYPOGAMMAGLOBULINAEMIA

Herpes zoster infection occurred in 18% of the patients in our series. This nearly always affected patients who were not receiving gammaglobulin therapy and was self-limiting and localised. Some patients have had two or three attacks involving different dermatomes. This high incidence of Herpes zoster may be explained by the finding that many of these patients have defects in cellular immunity as shown by absent delayed hypersensitivity skin reactions and poor in vitro lymphocyte transformation (13). The common occurrence of Herpes zoster in Hodgkin's disease, and other lymphomas associated with similar defects in cell mediated immunity, supports this view. However, not all the patients with Herpes zoster in our series have demonstrable defects in cellular immunity. Herpes zoster is not a complication of sex-linked hypogammaglobulinaemia so that the antibody deficiency alone cannot be responsible.

Herpes simplex (labialis) is extremely rare in patients with hypogammaglobulinaemia regardless of whether they are receiving gammaglobulin therapy.

SELECTIVE DEFECTS IN CELLULAR IMMUNITY

There is a very rare group of patients with T lymphocyte deficiency. This may be caused by a non-familial foetal abnormality causing

absence of the thymus (14) or to an inherited deficiency of the enzyme, nucleoside phosphorylase (15). There is very little information concerning virus infections in thymic aplasia but patients with nucleoside phosphorylase deficiency are prone to severe, and sometimes fatal, varicella and cytomegalovirus infections. For these reasons, prophylactic gammaglobulin injections should be given to these patients.

SEVERE COMBINED IMMUNODEFICIENCY

This disease is characterised by a susceptibility to bacterial, fungal and viral infections, usually starting within the first few months of life (16). Affected infants fail to thrive and usually die within the first two years unless given a successful bone marrow graft. There is an autosomal and sex-linked recessive form of the disease, one cause for the latter variety being a deficiency of the enzyme, adenosine deaminase (17).

Affected children are prone to a variety of viruses such as adenovirus, cytomegalovirus and measles. They frequently suffer from unexplained diarrhoea which may be caused by viruses although they are rarely isolated. Viral encephalitis is common and viruses such as echovirus, Herpes simplex and measles virus have been isolated from the brains of some children at autopsy. Dayan (18) found histological evidence of viral encephalitis in 9 of 23 brains examined at autopsy from children with severe combined immunodeficiency. Immunization with live viral vaccines is hazardous and generalised fatal vaccinia is a well-known complication. There is also a raised incidence of vaccine-associated paralytic poliomyelitis which is usually fatal (19, 20). Nevertheless, a few patients have received live oral polio vaccine without complication (21).

CONCLUSION

The finding that children with severe defects in T lymphocyte function often die from viruses supports the view that cellular immunity is an important defence mechanism. However, this does not seem to apply to echoviruses where either antibody or B lymphocytes are required to eliminate the infection. The chronicity of the central nervous system infection in patients with hypogamma-

globulinaemia, even in the absence of neurological signs, should encourage a search for viruses in other chronic inflammatory diseases of the central nervous system. These patients also provide a challenge to find a way of treating chronic virus infections of the central nervous system.

REFERENCES

1. Soothill, JF, K Hayes, JA Dudgeon: The immunoglobulins in congenital rubella. Lancet 1: 1385-1388, 1966

2. Purtilo DT, JPS Yang, S Allegra, D De Florio, LM Hutt, M Soltani, G Vawter: Hematopathology and pathogenesis of the X-linked recessive lymphoproliferative syndrome. Am J Med. 62: 225-233, 1977

3. Webster ADB: Immunodeficiency. In: Medical Immunology, Holborow EJ and WG Reeves (eds), Academic Press, 1977, p474-537

4. Pearl ER, LB Vogler, AJ Okos, WM Crist, AR Lawton, MD Cooper: B lymphocyte precursors in human bone marrow: an analysis of normal individuals and patients with antibody-deficiency states. J.Immunol 120: 1169-1175, 1978.

5. Koren HS, DB Amos, RH Buckley: Natural killing in immunodeficient patients. J.Immunol. 120: 796-799, 1978

6. Sanal SO, RH Buckley: Antibody-dependent cellular cytotoxicity in primary immunodeficiency diseases and with normal leukocyte subpopulations: importance of the type of target. J.Clin.Invest. 61: 1-10, 1978

7. Janeway CA, D Gitlin, JM Craig, DS Grice: 'Collagen disease' in patients with congenital agammaglobulinaemia. Trans. Ass. Am. Physicians 69: 93-97, 1956

8. Webster ADB, JH Tripp, AR Hayward, AD Dayan, R Doshi, EH MacIntyre, DAJ Tyrrell: Echovirus encephalitis and myositis in primary immunoglobulin deficiency. Arch.Dis.Child 53: 33-37 1978

9. Wilfert CM, RH Buckley, T Mohanakumar, JF Griffith, SL Katz, JK Whisnant, PA Eggleston, M Moore, E Treadwell, MN Oxman FS Rosen: Persistent and fatal central nervous system echovirus infections in patients with agammaglobulinaemia. N.Engl.J.Med.296: 1485-1489, 1977

10. Sanders RKM, R Joseph, B Martyn, ML Peacock: Intrathecal antitetanus serum (horse) in the treatment of tetanus. Lancet 1: 974-977, 1977

11. Wright PF, MH Hatch, AG Kasselberg, SP Lowry, WB Wadlington, DT Karzon: Vaccine associated poliomyelitis in sex linked agammaglobulinaemia. J.Pediatr.91: 408-412, 1977

12. MacCallum FO: Antibodies in hypogammaglobulinaemia. In: Hypogammaglobulinaemia in the United Kingdom. Medical Research Council SRS 310. Her Majesty's Stationery Office, London. p.72-85, 1971

13. Webster ADB, GL Asherson: Identification and function of T cells in the peripheral blood of patients with hypogammaglobulinaemia. Clin.Exp.Immunol.18: 499-504, 1974

14. Lischner HW, DS Huff: T-cell deficiency in Di George syndrome. In: Immunodeficiency in Man and Animals. Birth Defects: original article series, Vol 11, No. 1. p16-21, 1975

15. Biggar WD, ER Giblett, RL Ozere, SD Grover: A new form of nucleoside phosphorylase deficiency in two brothers with defective T cell function. J.Pediatr. 92: 354-357, 1978

16. Rosen FS: Immunity deficiency in children. Br.J.Clin.Pract. 26: 318-322, 1972

17. Meuwissen HJ, B Pollara, RJ Pickering: Combined immunodeficiency associated with adenosine deaminase deficiency. J.Pediatr. 86: 169-181,1975

18. Dayan A.D: Chronic encephalitis in children with severe immunodeficiency. Acta Neuropathol. 19: 234-241, 1971

19. Davis LE, D Bodian, D Price, J Butler, JH Vickers: Chronic progressive poliomyelitis secondary to vaccination of an immunodeficient child. N.Engl.J.Med.297:241-245,1977

20. Feigin RD, MA Guggenheim, SD Johnsen: Vaccine related paralytic poliomyelitis in an immunodeficient child. J Pediatr. 79: 642-647, 1971

21. Lopez C, WD Biggar, BH Park, RA Good: Nonparalytic poliovirus infections in patients with severe combined immunodeficiency. J Pediatr 84: 497-502, 1974.

DISCUSSIONS OF PAPER BY A.D.B. WEBSTER

Dr ter Meulen opened the discussion, asking why there had been no recoveries from these patients of enteroviruses other than ECHO viruses. In answer, Dr Webster stated that this was not known; certainly, Coxsackie virus infections did occur in these patients and there was a higher incidence of paralysis with vaccine strains of poliovirus. In connection with the myositis observed in these patients, Dr Tyrrell commented on the similarity of some strains of ECHO virus (e.g. 9 and 24) to Coxsackie viruses. Following a query concerning the effects of natural measles virus infection in these patients, it was noted that, in this country at least, they were usually recognised in early infancy and treated with prophylactic gamma-globulin, which contained measles virus antibodies.

Dr Cathala stressed that the disease in these patients was a chronic meningitis and not a neurological disease. In a final exchange, a postulated comparison between the effects of dengue virus infection and the conditions which Dr Webster had described was not upheld.

VIRUS-LIKE AGENTS FROM PATIENTS WITH MENTAL DISEASES AND SOME CHRONIC NEUROLOGICAL CONDITIONS

D. A. J. TYRRELL, R. PARRY, T. J. CROW, E. JOHNSTONE AND N. FERRIER

My colleagues and I have described how a virus-like cytopathic effect (CPE) was detected in cultures inoculated with CSF collected from patients with schizophrenia and some chronic progressive nervous disease, such as Huntington's chorea (1, 2). The main observations were that within a few days of inoculation a focal CPE was seen, particularly in stationary unchanged cultures of the MRC-5 strain of human embryo lung fibroblasts incubated at 33°C. The CPE tended to disappear and could only rarely be passed serially. However the agent causing it was partly characterised by experiments on CSF and it was shown to be particulate by filtration, and also appeared to resist lipid solvents, heat at 56°C and grew in cultures treated with BUDR. It (or they) has been therefore called provisionally a virus-like agent(s) or VLA. Such observations prompt us to ask a series of questions, to which at the moment we have only a few answers, but this paper gathers together our more recent observations and our ideas under the headings of these questions.

IN WHAT CLINICAL CONDITIONS CAN WE DETECT VLA?

We have received carefully collected and stored CSF from a variety of patients. The most nearly "normal" fluids came from patients undergoing spinal anesthesia for conditions such as benign prostatic hyperplasia, and from patients who had myelograms or air studies for conditions such as backache in which no abnormality was found. We also had some patients with general medical conditions such as cardiac arrest, or septicaemia, and with acute nervous system infections such as bacterial aseptic meningitis or unexplained encephalitis. The present catalogue of results is shown in table 1, which indicates that such specimens are usually negative but can be positive in patients with acute neurological syndromes. One patient with hypogammaglobulinaemia had suffered from an echovirus infection which had subsided following intensive specific immunotherapy (3).

Table 1 Results of tests on CSF from certain surgical and medical cases

Clinical features of cases	Proportion tested showing CPE
Lumbar puncture for spinal anaesthesia or for radiological study of backache*	3/22
Various mainly infectious intracranial diseases - stroke, convulsions, subarachnoid haemorrhage or cranial nerve palsy	1/12
Meningism or meningitis	1/7
Encephalitis	0/3
Cardiac arrest, septicaemia, headache, vomiting	0/6
Acute unexplained fluctuating loss of consciousness	3/3
"Hysterical" hemiplegia	1/1
X-linked hypogammaglobulinaemia with CNS involvement	1/1
"Sea-blue" histiocytosis	1/1

* The clinical records on some of these patients are incomplete - some patients did have neurological syndromes.

Table 2 Tests on cases of psychiatric illness, Huntington's chorea and multiple sclerosis

	Proportion tested showing CPE
Schizophrenia	10/14
Affective disorders	
Hypomania	2/3
Depression	3/3
Confusional state	1/1
Severe anxiety state	*0/1
Huntington's chorea	3/4
Multiple sclerosis	4/8

* A VLA was detected by throat swab but the CSF was negative.

However, we have evidence that VLA may be detected in multiple sclerosis, particularly in those with active or progressive disease (table 2) and also in Huntington's chorea. We have been interested to detect VLA in several cases with what might be labelled acute confusional or organic mental states; some patients had mild respiratory or influenza-like infections and signs suggestive of CNS involvement such as meningism and headache - three were drowsy or apparently unconscious. Our original study concentrated on patients with schizophrenia and we now detect VLA in about two-thirds of cases. More recently we have begun to study patients with other forms of mental disease and we have detected VLA in the first small group of cases of affective disorders. Examples of the CPE are shown in fig. 1. The clinical features of the schizophrenic patients have been summarized - those of the other cases will be reported later.

CAN VLA BE CONTINUOUSLY PROPAGATED IN VITRO?

There is some evidence that the CPE is due to a replicating agent. Not only have a few foci spread gradually through a cell sheet, but occasionally we have been able to produce CPE in further cultures by passing the culture fluids to further cultures of MRC-5 or of brain tumour cells up to a total of three passages. It is clear that the CPE is usually reduced by using cultures which have been recently changed or are rolled. In recent months we have had further interesting results. We received tissue from the frontal and temporal cortex of a recently deceased patient with Huntington's chorea. This was inoculated into roller tissue cultures. A cytopathic effect developed in rhesus monkey kidney cells and can now be propagated serially in HeLa cells. It is now necessary to study the agent in detail to determine whether it is related to the disease. We cannot exclude that it might have been derived from the rhesus kidney cells. A similar CPE was seen in cultures inoculated with throat swab material from a patient with schizophrenia and this was at first thought to be due to an adenovirus. This agent likewise will be characterized and using similar techniques we shall continue our efforts to get a continuously propagated agent from at least representative cases of other types of disease. It thus seems that by persistence an occasional VLA can be persuaded to grow serially, but great care

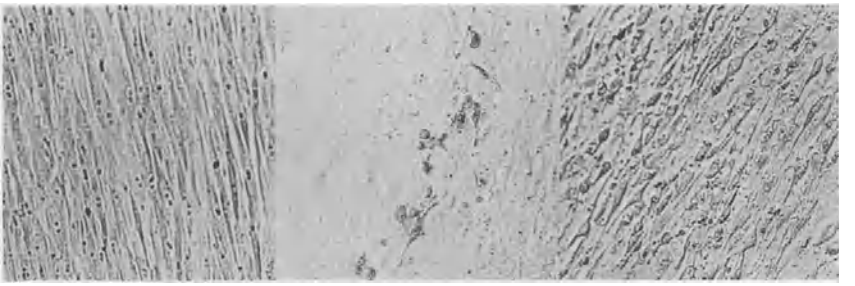


Fig. 1 Cytopathic effect produced in human embryo lung fibroblast cultures (MRC-5) inoculated with CSF. Unstained.

a) Uninoculated culture

b) Culture inoculated 2 days previously with 0.1 ml of CSF from a patient with Huntington's chorea. An early focus.

c) Culture inoculated 4 days previously with 0.1 ml of CSF from a patient with multiple sclerosis. An extensive cytopathic effect.

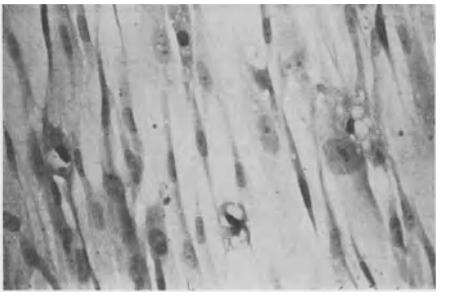


Fig. 2 Fixed and stained MRC-5 cells inoculated with CSF from patient with schizophrenia. Note the vacuolation of the cytoplasm and nuclear changes. Stained haematoxylin and eosin.

will have to be exercised to ensure that what is grown really is a relevant organism and not a passenger virus or laboratory contaminant.

WHAT IS THE VIROLOGICAL SIGNIFICANCE OF THESE OBSERVATIONS?

We have considered the possibility that these results were spurious and due to the use of unsatisfactory cells or to faulty observations. We think these are unlikely explanations for we have observed many hundreds of uninoculated cultures and only rarely seen any changes comparable with the CPE, and in many instances the CPE is so marked, rapid and extensive that untrained observers have readily recognized it. Furthermore many of the observations were made by observers who did not know the origin of the specimen and in each test there have been specimens from a mixture of cases. We have often passed uninoculated cells and have not seen a comparable CPE, suggesting that it is unlikely to be an agent latent in our cultures. Furthermore the strongly positive specimens have been positive in HeLa and monkey kidney cells as well as in MRC-5 cells. Some of our cultures contain mycoplasmas and we also get occasional contamination with fungi, bacteria or protozoa, but the properties of the agent deduced by tests on CSF are apparently incompatible with a contaminating mycoplasma or other organism. After some initial studies we arranged for all the laboratory manipulations of our VLA to be confined to one exhaust ventilated cabinet into which known viruses were never introduced - this was intended to minimize the risk that virus was carried across from other cultures. The fact that the agent cannot be serially passed makes it unlikely that it is due to a laboratory-grown virus, and contamination from positive specimens is unlikely because the titre of these is low and numerous uninoculated control cultures have never shown the effect. Thus the best hypothesis is still that we are seeing the effect of a virus-like agent in the patient's CSF.

We would like to know whether we are dealing with one agent or several. The properties, so far as they go, suggest no obvious differences though one agent from a case of "sea blue" histiocytosis with choreo-athetosis is apparently chloroform labile. The CPE varies in scale and also in the speed with which it develops, but qualitatively it seems to be the same whatever the type of case and

in unstained cultures resembles that due to viruses, for example rhinoviruses. No characteristic inclusions have been seen and the changes seem to be mainly vacuolation of the cytoplasm and coarsening of the nuclear chromatin, followed by pyknosis or karyorrhexis (fig. 2). Eventually one hopes this will be supported by more detailed and specific studies such as immunofluorescence and electron microscopy. Nevertheless we could be dealing with several closely related viruses, for instances a number of serotypes of a single agent, or even quite unrelated agents - we should remember that at an equally early stage of research the reoviruses were grouped with the enteroviruses and only later was echovirus type 10 renamed reovirus type 1.

WHAT IS THE RELATIONSHIP OF VLA TO DISEASE?

We wondered at first whether VLA was abnormal but previously undetected inhabitant of the CSF. Failure to recognize a normal symbiont as such has mislead investigators in the past. It is not ethical to collect CSF from completely normal subjects, but we are impressed that generally we have not found VLA in CSF of patients with diseases unrelated to the CNS, such as mild orthopaedic disabilities, surgical conditions such as benign prostatic hypertrophy and even serious general medical conditions such as septicaemia and cardiac arrest. We could postulate, of course, that VLA is released by damaged nervous tissue, and might be a non-specific indicator of damaged nervous tissue. However, samples of CSF from patients with severe and extensive acute CNS disease, such as meningitis and encephalitis, were uniformly negative.

Thus probably the simplest hypothesis is that VLA is aetiologically associated with some CNS diseases including schizophrenia, affective psychoses, Huntington's chorea and multiple sclerosis. It is regularly found in close association with the diseased tissue and not in unaffected CNS, and it is capable of damaging cultured nervous system cells as well as a variety of others. However the evidence would be much reinforced if we could produce CNS disease by inoculating VLA into the CNS of animals, and experiments of this type have now begun. If they are positive they will fulfil the remaining third clause of Koch's postulates.

We have some evidence that VLA may cause mild and recoverable conditions. For instance there is the small group of adult patients who had rather rapid impairment or loss of consciousness without any explanation apart from signs suggestive of a mild infection, and two of these recovered rapidly and completely. We have also found VLA in the CSF of an elderly man with backache and of a 14 year old girl who appeared to have a respiratory infection with meningism, followed by an attack of sinusitis - again both of these recovered.

IF VLA IS PROVED TO BE IMPORTANT WOULD THIS VITIATE RESULTS WHICH SUGGEST THE IMPORTANCE OF GENETIC AND ENVIRONMENTAL FACTORS IN THESE DISEASES?

In most of the diseases in which we have found VLA there is little or no evidence of case association, let alone case-to-case transmission of an infectious agent. The best hypothesis would appear to be that VLA is widespread in the community. Since we have positive results with two throat swabs and one faeces it is not difficult to imagine how it could be transmitted without involving the CNS at all. Genetic predisposition might then determine whether clinical illness occurs, as in the case of Huntington's chorea. Nevertheless it is completely obscure at the moment how a genetic influence might operate - it could affect the susceptibility of cells to the virus or the ability of the immune system to respond.

On the other hand in schizophrenia probably other factors besides the genetic predisposition would be important - for example the age at which infection occurs could have an important influence. Then the content of a schizophrenic illness would be very much influenced by the social and educational background; for instance these must determine which language hallucinating voices speak, and whether the influences are conceived of as coming from outer space. It must also be clear that the changes in the brain can be described in many ways, in terms of electrical activity, or the types of neurones or receptors affected, e.g. dopaminergic. Nevertheless the changes so described could all be the consequence of a virus invading a series of susceptible cells.

Others have considered the possibility that schizophrenia might be due to a virus infection and found the idea plausible (4, 5)

though at the time in most cases there was no direct evidence to support the idea.

The pathogenesis could still be complicated and be partly mediated by immune processes. It could perhaps have something in common with the long term damage produced in the mouse CNS by Theiler's virus, a picornavirus (6, 7) or in the so called immune polioencephalitis which occurs in old mice, particularly of the C58 strain, and now known to be transmitted by a probable togavirus (8).

CONCLUSION

This can be only a preliminary report, a sketch map of what we think may well be an exciting new land, an area of knowledge in which we can explore the interactions between some new organisms and the central nervous system of man. Clearly much more work is needed so that details may be filled in and many unknown facts discovered.

ACKNOWLEDGEMENTS

We wish to thank again the many individuals referred to in our recent publication, without whose efforts this research would not have been possible.

REFERENCES

1. Tyrrell, DAJ, RP Parry, TJ Crow, E Johnstone, IN Ferrier: Possible virus in schizophrenia and some neurological disorders. Lancet i: 839-841, 1979.
2. Crow, TJ, IN Ferrier, EC Johnstone, JF Macmillan, DGC Owens, RP Parry, DAJ Tyrrell: Characteristics of patients with schizophrenia or neurological disorder and virus-like agent in cerebrospinal fluid. Lancet i: 842-844, 1979.
3. Webster, ADB, JH Tripp, AR Hayward, AD Dayan, R Doshi, EH Macintyre, DAJ Tyrrell: Echovirus encephalitis and myositis in primary immunoglobulin deficiency. Arch. Dis. Childh. 53: 33-37, 1978.
4. Torrey, EF, MR Peterson: Slow and latent viruses in schizophrenia. Lancet ii: 22-24, 1973.
5. Crow, TJ: Viral causes of psychiatric disease. Postgrad. med. J. 54: 763-767, 1978.
6. Theiler M: Spontaneous encephalomyelitis of mice, a new virus disease. J. exp. Med. 65: 705-719, 1937.
7. Lipton HL, MC Dal Canto: Contrasting effects of immunosuppression in Theiler's virus infection of mice. Infect. Immun. 15: 903-909, 1977.
8. Martinez D, B Wolanski, AA Tytell, RG Devlin: Viral aetiology of age-dependent polioencephalomyelitis in C5B mice. Infect. Immun. 23: 133-139, 1979.

DISCUSSION OF PAPER BY D. A. J. TYRRELL ET AL.

Discussion on Dr Tyrrell's paper opened with a question on the use of immunosuppressed animals in future investigations. Dr Tyrrell replied that experiments in a wide range of animals were contemplated and reminded the audience of a disease of the mouse central nervous system which was initially thought to be immunological in origin. Here, only old mice, or mice which had been treated with cyclophosphamide, were susceptible. In due course, it appeared that a togavirus was responsible.

Dr Crow mentioned the use of behavioural alterations (already being used as a model system in the investigation of schizophrenia) in the detection of mild degrees of illness. He suggested the testing of brains from such animals in tissue culture. As these virus-like agents are now capable of recovery from faeces and throat swabs, it now seemed feasible to test for their presence in unaffected normal subjects.

Dr Thiry suggested that these virus-like agents might resemble the adeno-associated viruses. Dr Tyrrell, in reply, drew attention to the limited host range of the virus-like agents. Of 7 clones of cells from one foetus, only one was suitable for the culture of these agents. It was also noted that transformed MRC-5 cells were resistant to infection with these agents. Professor Chany recalled that, in 1960 - 1961, Dr Gresser, working in his laboratory, identified similar agents which could not be successfully passaged.

In answer to further questions, Dr Tyrrell stated that the cytopathic effect developed usually within 2 or 3 days (often 24 hours) and that the stage in the disease at which specimens had been obtained varied from 3 days to more than 20 years. In further discussion, it appeared that there was a significant literature on the development of schizophrenia following virus infections and that, in 1918 - 1919, schizophrenia followed the influenza pandemic. Vilyuisk encephalitis could present as a schizophrenic illness which progressed to a dementia.

In a discussion of technical details, it was apparent that the cytopathic effect only appeared if the conditions were just right; thus immunofluorescence with convalescent sera and similar procedures were not possible as satisfactory cover-slip preparations could not

be made. The agents were sensitive to freezing (-40°C) and to freezing and thawing but resisted overnight storage at 4°C and could be safely kept for longer periods in liquid nitrogen or at -70°C. These virus-like agents were deposited by ultracentrifugation.

Dr Kimberlin, predicating that this might be a scrapie-like agent, suggested that it should not be inoculated into neonatal animals nor should immunosuppression be used. Professor Fraser observed that these experiments reminded him of ones currently under way in Belfast where a slightly different cytopathic effect had been produced in VERO cells inoculated with human marrow specimens.

In conclusion, it was suggested that a study of close relatives of patients with Huntington's chorea should be set up; it was agreed that such a study would be quite feasible.

DISCUSSION ON DR. SANGER'S PAPER*

This interesting paper led to a lengthy discussion, beginning with a consideration of the mode of spread of infection of viroids. As a point of general interest, the necessity for asymptomatic infection to occur was considered to be important in the understanding of persistent infections. In the case of viroids, the mode of spread from one plant to another was imperfectly understood and the vectors were not known. On the analogy of the experimental means of transfer and the natural spread associated with the use of pruning shears, both of which procedures involve damage to the plant, the possibility of transmission by biting insects was seriously considered but there was no data at all on this at present.

Uptake of virus, following such injury, possibly involved receptors and the virus might be taken into the cell by pinocytosis. With the possible exception of TMV, facts concerning these possible routes were not known for either plant viruses or viroids. Spread through the infected plant might occur *via* plasma links between the cells (plasmodesmata).

Discussion then shifted to the replication of viroids. These contain no protein; no translation of RNA into protein can be detected *in vitro*. Their primary structure was that of a +ve strand which cannot produce protein. There was no good evidence of an instructional codon nor of stop signals. It might be that this +ve strand RNA could behave like a -ve strand, with the -ve copy acting as a template. In infected plant tissues, there was no evidence for a viroid-coded protein; one recent report of such a protein lacked force, as similar proteins could also be produced as a general pathological response following fungal or viral or double viral infections. The possibility was considered that protein did not play a role in viroid replication and that the RNA was acting by itself. There was no reverse transcriptase but an RNA-dependent replicase had been demonstrated. The possibility of a loose packaging system, using host proteins, could not be excluded.

The situation of viroids in the cell was also unclear. Infectivity was associated with chromatin. It was possible that viroids might be associated with molecules of cellular origin which could confer stability.

*Though Dr. Sanger's paper was presented at the workshop, it unfortunately does not appear in this volume.

The circularity of viroid RNA was alluded to. This circularity is associated with a high degree of secondary structure and Dr Sanger had found that up to 99.5% of viroids displayed a circular structure, unlike the position in Dr Diener's laboratory, where 80% were linear in structure. It was observed that, whereas circular structures can readily form linear structures by secondary folding, the formation of circular structures from linear structures would require a ligase. During the isolation of viroids, it appears that $Mg^{\cdot}$ and an alkaline pH may lead to artefacts; thus 3mM $Mg^{\cdot}$ at pH 9.0 will lead to a phosphate di-ester breakage and thus circular forms would tend to become linear.

Dr Tyrrell closed the discussion with a reference to the entirely new concepts which consideration of the viroids had induced.

DISCUSSION ON DR. CATHALA'S RECENT WORK

This began with a question on interference between scrapie and CJD; so far, this had not been demonstrated, although it was noted that TME can block scrapie replication. Dr Cathala mentioned that primary transmission of CJD to mice had been successful on two occasions in her hands, although inoculation of guinea-pigs had been unsuccessful.

Further discussion centred on the epidemiology of CJD. Australia (free from scrapie) did not have an appreciably lower prevalence of CJD than other countries; a similar lack of correlation was shown for Iceland, Japan and China (where there are no sheep but CJD is believed to be present). The incidence of CJD in vegetarians was unknown. Dr Cathala speculated that, in the elderly, patients with CJD might be misdiagnosed as Alzheimer's disease; in such a case, the patients would be consigned ot long-stay wards or to an old people's home and the final diagnosis at necropsy would never be known.

DISCUSSION ON THE DIRECTIONS OF FUTURE RESEARCH

As there had previously been much discussion on viroids, this topic was dealt with briefly. Control of the viroid diseases of plants was not possible as there were no effective virucides. The first aim of viroid research would be to improve diagnosis as, at present, bioassay was not reliable and nucleic acid assay was not feasible in field conditions. Screening for viroid disease would follow and, in turn, control by exclusion of plants with viroid disease from propagation. These processes would be carried out by the gardener or agriculturist, thus demonstrating the close relationship in this area between applied and basic research.

In scrapie, the problem of the zero phase, where no marker was available, was important, as was the situation where experimental animals develop severe neurological signs without corresponding neuropathology. There had been too much emphasis on the scrapie agent as such and more work should be done on the basic biochemical defects which resulted in death or malfunction. The nature of the molecular lesion should be studied and neurochemical probes should be more extensively used. Few sequential studies had been carried out and these should be set up to study the earliest changes, particularly those at the post-synaptic side of the synapse. The 35 nm tubular filamentous spherical particles which have been described should be further investigated. Dr Tyrrell suggested that the neurophysiology of the disease should be studied. Scrapie was a steadily progressive disease; why was this so? Does reactivation occur? As scrapie replicates in the spleen, organ culture of that organ might be helpful. Prolines, purine metabolism and the effect of scrapie on the enzymes related to purine metabolism should be investigated.

In SSPE and distemper, Dr Norrby suggested that the serological response should be studied further. Similarly, the molecular aspects of SSPE deserved more attention and more strains of SSPE virus should be obtained in an effort to detect markers on different strains. In vivo changes in the virus should be studied and it was proposed that a spectrum of SSPE virus strains should be assembled, and their relations with the immune defence system investigated. Doubt was expressed whether the study of tissue culture adapted virus strains was entirely

valid. The question was raised whether there were one or more strains of wild measles virus; the answer lay in studying homogeneity of such strains by restriction of enzyme analysis, in a similar way to that used with herpes simplex virus.

Although doubts were expressed, it was suggested that a follow up of normal uncomplicated measles in childhood might be attempted. As SSPE occurs only in 1 in 1 x 10^6 children, selected cases only could be included and attempts might be made to identify a subgroup which might be at risk. The imperfect models of SSPE which exist in animals should be studied further.

Dr Thiry called for a collaborative clinical/virological group to investigate the role of oncornaviruses in man. The topics she suggested were infertility, repeated spontaneous abortions, hydatidiform mole and pre-eclampsia. The investigation of newly diagnosed patients with subacute lupus erythematosus and acute diffuse glomerulonephritis was also suggested. Protocols for sampling were needed and, with technical improvements now available, it should be possible to search for and assess the role of these viruses in man.

As for Maedi/Visna, it was felt that it was possible that some disease in man might resemble this condition and this should be studied further. In vivo variation of visna/maedi clearly needed further attention.

As a member of the general group of topics included in this meeting, interferon and its immunology deserved further research.

The meeting was closed by Dr Tyrrell with thanks to the participants and to the EEC.

INDEX OF SUBJECTS