# Immune Interferon

## Properties and Clinical Applications

## Pharmacology and Toxicology: Basic and Clinical Aspects

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# *Preface*

Immune interferon (interferon gamma, IFNγ) was discovered 20 years ago. The scientific interest in this cytokine has been steadily growing, as demonstrated by the ever-increasing number of publications reaching a number over 25,000 during this period (Figure 1).

These studies revealed more properties of IFNγ and opened new avenues for its clinical use. Apart from being a natural antiviral agent, IFNγ also turned out to be an important cytokine which plays a pivotal role in the processes of formation and modulation of the immune response. However, in spite of the thousands of scientific papers and a number of reviews concerning the structure, biological activities, and clinical effects of IFNγ, no monograph that summarizes and critically analyzes the results obtained on this subject has been published. This is the goal of the present book.

We have attempted to present data on the different properties of IFNγ and on its role in different pathological processes. The data include experiments involving *in vitro* cell cultures as well as experiments on animals. Clinical trials are also presented regardless of whether the effect of IFNγ was favorable or not. The book is aimed at medical doctors, pharmacists, biochemists and molecular biologists, medical students, and biologists and also businessmen interested in high biotechnology.

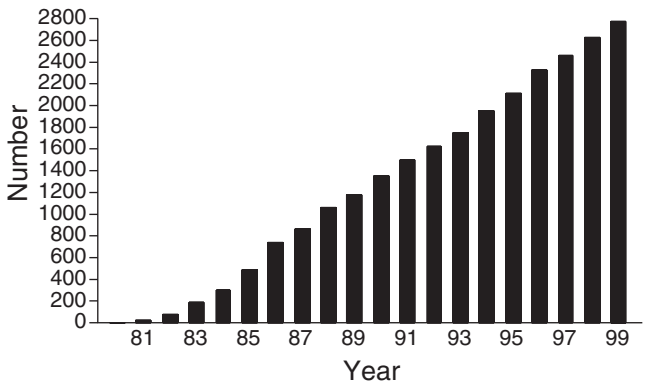


***Figure 1*** Number of publications concerning IFNγ for the corresponding year.

The various diseases discussed here are briefly characterized in relation to IFNγ. Interferons α and β as well as some other cytokines are often mentioned, but only as far as their effect as compared to that of IFNγ or their combination with it is discussed.

Reviews on the properties and application of IFNγ are published every year. Some of them are listed below arranged by year of publication:

Billiau 1981; De Grado et al 1982; Epstein 1982; Gray and Goeddel 1982; Adolf 1985; Bottomley and Toy 1985; Dianzani 1985; Fisher and Grant 1985; Giovanni and Rossi 1985; Joklik 1985; Vilcek et al. 1985b; Kirchner 1986; Revel and Cheboth 1986; Branca 1987; Pestka and Langer 1987; Stanton et al. 1987; Gastl and Huber 1988; Langer and Pestka 1988; Mechti et al. 1988; Schiller and Borden 1988; Borecky 1989; Foon 1989; Romeo et al. 1989; Strander 1989; Gresser 1990; Young and Hardy 1990; Baron et al. 1991; Sen and Langyel 1992; Zhang et al. 1992; Aulitzky et al. 1993; Farrar and Schreiber 1993; Gunther and Otto 1993; Degre 1996; Tannenberger and Hrelia 1996; Johnson et al. 1998.

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# *The Authors*

**Roumen Gueorguiev Tsanev, M.D., Ph.D., D.Sc.,** is the founder of the Institute of Molecular Biology, whose Director he was until 1993. He graduated from the Medical Faculty of the University of Sofia in 1947 and obtained training in Biochemistry and Molecular Biology in Budapest (1954), in Moscow (1957), and in Paris-Saclay (1963).

Dr. Tsanev started his scientific work as a Research Fellow in the Institute of Biology in 1947. In 1954 he founded the Biochemical Research Laboratory that was later transformed into the Institute of Biochemistry (1972) and then into the Institute of Molecular Biology (1978). He became a Professor in 1964. Dr. Tsanev has been an Overseas Fellow of Churchill College in Cambridge (1969 to 1970), was elected as a full member of the Bulgarian Academy of Sciences (1979), and a member of the London–based Academia Europaea (1989). He is also a member of several international scientific organizations: ICRO (International Cell Research Organization at UNESCO), ESGCP (European Study Group for Cell Proliferation), and a reviewer for ICRETT (International Cancer Research Technology Transfer program of IUAC). He has been a member of the advisory boards of the *Journal of Theoretical Biology* (1970 to 1980), *European Journal of Biochemistry* (1972 to 1977), and *Cell Differentiation* (1978 to 1983). He has received a state award for mathematical modeling in biology (1969) and for achievements in biochemistry (1978). At present, Dr. Tsanev continues his active research in the Institute of Molecular Biology where he is also a member of the Scientific Counsel of the Institute.

Dr. Tsanev has published 205 scientific papers, mostly in international journals and books and has presented a number of invited lectures at international and national meetings. His major scientific interests are the biochemistry of nucleic acids, the molecular organization of chromatin in relation to cell proliferation and differentiation, and the regulatory role of genetic networks, including that of cytokines.

**Ivan Gueorguiev Ivanov, M.S., Ph.D., D.Sc.,** is a Professor of Biochemistry and Molecular Biology. He is a head of the Department of Gene Regulations and Deputy Director of the Institute of Molecular Biology at the Bulgarian Academy of Sciences and also teaches biochemistry and molecular biology at the University of Chemical Technology in Sofia. He graduated from the University of Sofia (M.S. in organic chemistry in 1967) and obtained training

in biochemistry (Ph.D. from the Institute of Biochemistry in Sofia in 1975) and molecular biology (D.Sc. from the Institute of Molecular Biology in Sofia in 1989). Dr. Ivanov has obtained postgraduate training at many institutions, such as the Institute of Biochemistry in Szeged, Hungary (1978), the Institute of Biochemistry in Prague, Czech Republic (1980), the Institute of Molecular Biology in Moscow (1982), the Chemistry Department at the University of New Brunswick in Fredericton, Canada (1984), and the Medical Nobel Institute at the Karolinska Institute in Stockholm, Sweden (1986). He has also been a visiting professor at the University of Toronto (Virology Group) in Toronto, Canada (1990 to 1994). Dr. Ivanov has published in refereed journals more than 100 papers on gene synthesis and regulation of gene expression. He is author or co-author of five books (published in Bulgarian) and 25 patents in the field of recombinant DNA technology. He is a member of several scientific societies, councils, and boards in Bulgaria and abroad.

# *Abbreviations*

aa — amino acid residues
aFGF — acid fibroblast growth factor
AFP — alfa fetoprotein
ALL — acute lymphatic leukemia
AML — acute myelogenic leukemia
APC — antigen-presenting cells
Ara-C — cytosine arabinoside
AZT — azidothymidine
bFGF — basic fibroblast growth factor
bp — base pair
cdc — cyclin-dependent kinase
CLL — chronic lymphatic leukemia
CML — chronic myelogenic leukemia
CNS — central nervous system
ConA — concavalin A
CSA — cyclosporine A
CSF-G — granulocyte colony-stimulating factor
CSF-GM — granulocyte-macrophage colony-stimulating factor
CSF-M — macrophage colony-stimulating factor
dsRNA — double-stranded RNA
DTIC — dacarbazine
EGF — epidermal growth factor
G-CSF — see CSF-G
GM-CSF — see CSF-GM
GRO — growth-regulated oncogene
GTP — guanosine triphosphate
GVHD — graft-versus-host-disease
3-HAA — trihydroxyanthranylic acid
HCC — hepatocellular cancer
HIV — human immunodeficiency virus
HLA — human lymphocyte antigen
HPV — human papilloma virus
HS — Herpes simplex
HZ — Herpes zoster
ICAM — intercellular adhesion molecule
ICSBP — interferon consensus sequence binding protein

IDO — indoleamino-2,3,-dioxygenase
IFNγR — IFNγ receptor
IL — interleukin
IPF — idiopathic pulmonary fibrosis
IRF — interferon regulatory factor
IU — international unit
LAK — lymphokine-activated killer (cell)
LFA — leukocyte function antigen
LPS — lipopolysaccharide
M-CSF — see CSF-M
MDP — muramyl dipeptide
MDR — multidrug-resistant
MDS — myelodysplastic syndrome
MethA — methylcholantren sarcoma
MGSA — melanoma growth-stimulating activity
MIG — membrane-bound globulins
mRNA — messenger RNA
MS — multiple sclerosis
MTD — maximal tolerated dose
NK — natural killer (cell)
NO — nitric oxide
nt — nucleotide
PAF — pertorin-activated factor
PCNA — proliferating cell nuclear antigen
PCR — polymerase chain reaction
PDGF — platelet-derived growth factor
PEG — polyethylene glycol
PgE2 — prostaglandin E2
PHA — phytohemagglutinin
PKC — protein kinase C
PMA — phorbol myristate acetate
RA — rheumatoid arthritis
RCC — renal cell carcinoma
SICAM — soluble ICAM
SLE — systemic lupus erythematosus
SMC — smooth muscle cells
SSc — systemic sclerosis
STAT — signal transduction and transcription activator
TAD — transporter for antigen presentation
TCR — T-cell receptor
TGF — transforming growth factor
Th — T helper (cell)
TIL — tumor-infiltrating leukocytes
TNF — tumor necrosis factor
TP — thymidine phosphorylase
VEGF — vascular endothelium growth factor
VSV — vesicular stomatitis virus

# *Contents*

**Part II: Interferon gamma in pathological processes and its clinical application**

# *chapter one*

# *The cytokine family*

Cytokines are an important group of proteins realizing intercellular communications among cells of the same tissue (paracrine) or of different tissues (endocrine). Such communications are essential for the correct synchronized reactions of the cells of different tissues. In the literature different designations are used to denote these factors: lymphokines, monokines, interleukins, and growth factors, which are now unified under the common name *cytokines*. The main cytokines include interleukins 1 to 18 (IL-1 to IL-18); interferons α, β, and γ (IFNα, IFNβ, and IFNγ, respectively); tumor necrosis factors α and β (TNFα and TNFβ); colony-stimulating factors — granulocyte, macrophage, and granulocyte-macrophage (CSF-G, CSF-M, CSF-GM, respectively); basic and acid fibroblast growth factors (aFGF and bFGF); and others.

Normally these proteins are synthesized in minute amounts and are released under the effect of very specific local stimuli. They exert their action on target cells through binding to highly specific receptors on the surface of these cells. Different cytokines have synergistic as well as antagonistic interactions. A typical example is the antagonistic relations of cytokines produced by the helper T lymphocytes, Th1 and Th2 (Reynolds et al. 1987; see Figure 1.1).

The Th1 group of CD4+ T helper cells synthesizes type I cytokines (IL-2, IFNγ, and IL-12) and is responsible for the cellular immunity (see Chapter 3, Section 3.3). The Th2 group of T cells synthesize type II cytokines (IL-4, IL-5, IL-6, IL-10, IL-13) and stimulate humoral immunity. Th1 cells are responsible for the delayed-type hypersensitivity (for review, see Black 1999), a reaction obtained also by stress (Dhabhar et al. 2000). There are also Th0 helper lymphocytes which synthesize both types of cytokines.

The CD4+ helper cells, depending on a number of factors, respond to antigens by developing either a Th1 or a Th2 phenotype (Karp and Chan 1994). One such factor is the balance between IL-12 and IL-4, with the former stimulating the Th1 and the latter the Th2 response (Chen et al. 1996). According to some data, IL-12 and PrE2 determine the level of IFNγ produced by activated CD4+ cells (Hilkens et al. 1996a). PrE2 inhibits cellular immunity by increasing the level of cAMP which in turn activates protein

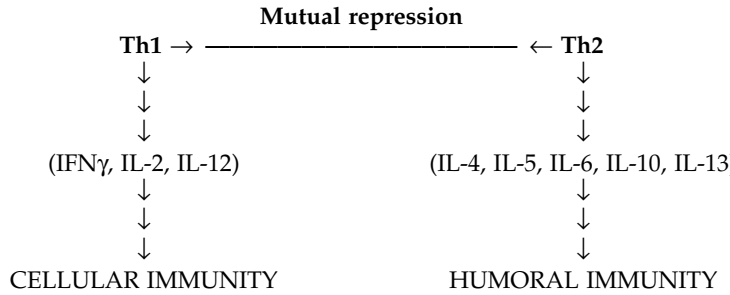


***Figure 1.1*** Relationship between two types of T helper lymphocytes.

kinase A (PKA). This leads to a decreased production of IL-2 and also a decreased concentration of IFNγ (Uotila 1996). However, although there are numerous data showing that the two groups of cytokines have an antagonistic relationship and mutually suppress each other (e.g., Rabin et al. 1986; Snapper and Paul 1987; King et al. 1993; Larner et al. 1993), there are also reports in disagreement with this antagonism. For example, it was reported that in HeLa cells IFNγ induced the expression of IL-6, which is a Th2 cytokine (Faggioli et al. 1997). In general, the balance between Th1 and Th2 cytokines depends on many factors (for review, see Romagnani 1999), among which are the nature of the antigen, the genetic background of the host, and the cytokines involved in the primary interaction of T cells with the antigen-presenting cells. In addition, it was discovered that the level of glutathione in these cells is also of importance (Peterson et al. 1998).

The interactions among the cytokines within the Th1 group are very important, too. As we shall see, the treatment of a number of malignant diseases includes a combination of Th1 cytokines such as IFNγ combined with IL-2 or IL-12. It appears that the effect of the two cytokines (IL-2 and IL-12) is partly due to the fact that they induce secretion of IFNγ. The Eta-1 cytokine, called osteoporin, also plays an important role in the development of a Th1 response by stimulating the synthesis of IL-12 and IFNγ and suppressing the expression of IL-10 (Ashkar et al. 2000). For the relationships within the network of cytokines, see Koyano-Nakagawa and Arai (1996).

Concerning the B cells in humans, the Th1 cytokines IFNγ and IL-2 have a stimulatory effect on the production of IgG2a, whereas the Th2 cytokines IL-10 and IL-4 (previously called B-cell growth factor, BGF-1) induce production of IgG1 and IgE.

Depending on whether one or another cytokine is dominant, the consequences are different. For example, defective production of IL-2 leads to a loss of the delayed-type immune hypersensitivity and increased level of IL-4 leads to an overproduction of IgE, whereas a high level of IL-5 results in hypereosinophilia (Clerici et al. 1997b; Di Piro 1997). The inhibition by IL-10 of the production of superoxide anions is found to be connected with the suppression of genes coding for the subunits of the NADPH oxidase (Kuda et al. 1996).

The activity of the Th1 subpopulation is connected with the expression of the surface antigen CD26 (dipeptidyl peptidase IV). Cultivation of peripheral

blood mononuclear cells in the presence of IFNγ or IL-12 results in expression of a Th1, whereas the presence of IL-4 determines a Th2 phenotype (Willheim et al. 1997). The transmembrane molecule CD30 is a marker for the Th2 subpopulation and it correlates with a low production of IFNγ (Leonard et al. 1997). These markers are used to determine the T helper cell phenotype.

The complete activation of the T cells requires two different signals: one through the T-cell receptor (TCR) and a second, costimulatory one through the binding between the CD28 and B7 surface antigens. Blockage of this interaction changes the differentiation of the Th1 and Th2 subpopulations both *in vitro* and *in vivo*. In experiments with *Schisostoma mansoni*-sensitized mice it was shown that blockage of the CD28-B7 interaction with the antagonist CTLA4Ig led to an increased level of IFNγ associated with a considerable decrease in the IL-4 and IL-5 levels, in the number of eosinophils in the bronchoalveolar lavage, and in the peribroncheolar infiltrates as well as in the hyperplasia of mucoid cells. Therefore, inhibition of the costimulatory signal leads to a decreased secretion of Th2 cytokines and an increased secretion of Th1 cytokines (Padrid et al. 1998).

The hypothesis was postulated that the shift from a Th1 to a Th2 phenotype was due to zinc deficiency (Sprietsma 1997) and that lead suppressed the Th1 and stimulated the Th2 response (Heo et al. 1998).

In general, disturbance of the cytokine production or disturbed expression of their receptors lead to serious disturbances in the cell growth and in the immune response.

Finally, it should be mentioned that the chemokines (a superfamily of polypeptide mediators which play a key role in T helper cell migration) determine the selective migration of Th1 and Th2 cells in response to the expression of different chemokine receptors (Bonecchi et al. 1998).

*chapter two*

# *Interferon gamma*

## *2.1 Differences between IFNγ and IFNα/β*

The main differences between IFNγ (type II) and IFNα/β (type I) are shown in Table 2.1 and are summarized as follows:

- Different primary structure.
- The IFNα/β genes are not split, whereas the IFNγ gene has introns.
- IFNα/β share a common receptor that is different from that of IFNγ.
- The two types of interferons induce or suppress the synthesis of different proteins (see Table 2.2 and Chapter 3, Section 3.4).
- IFNα/β stimulate mainly the activity of the natural killer (NK) cells, whereas IFNγ stimulates mainly the macrophages.
- IFNα/β are stable at pH 2.0; IFNγ is acid labile.
- Differences are in the signaling pathway leading to the corresponding activity.

The different receptors and the differences in the metabolic signaling pathways open avenues for a combined clinical use of IFNγ and IFNβ (Schiller et al. 1986, 1987, 1990a,b) or IFNγ and IFNα (Brunda and Wright 1986). In the latter case, the possible effect of IFNγ on the IFNα receptor has to be taken into consideration. This effect seems to depend on the cellular type. In the neuroblastoma cell line T98G, IFNγ suppresses the binding of IFNα to its receptor, possibly by affecting its dissociation constant (Kd) (Hannigan et al. 1984). In other tumor cell lines (melanoma HMV-1, kidney carcinoma ACHN, Daudi lymphoma), IFNγ induces the receptors of IFNα but only under the condition that IFNγ preceded the treatment with IFNα (Ishii and Tsukagoshi 1989).

## *2.2 INFγ-producing cells. Inducers and inhibitors. Effect of aging*

Judging by the presence of mRNA, IFNγ, unlike IFNα, is not constituitively produced (Tovey et al. 1987). In many experiments, however, the control

***Table 2.1*** Differences between interferon type I (IFNα/β) and type II (IFNγ)

| | IFNα | IFNβ | IFNγ |
|---|---|---|---|
| Main producers | B lymphocytes, macrophages | Fibroblasts, epithelial cells | T lymphocytes |
| Inducers | Viruses, dsRNA,[a] microbial proteins, organic polymers | Viruses, dsRNA,[a] microbial proteins, organic polymers | Viruses, mytogens, phorbol esters, Ca ionophores |
| Number of genes | More than 20 | 1 | 1 |
| Species specificity | Relatively specific | Higher species specificity | Species specific |
| Chromosomal localization | 9 | 9 | 12 |
| Number of amino acids | 143 | 145 | 143 |
| Molecular mass (kDa) | 17.5–23 | 18–23 | 17 |
| Acid stability | Stable | Stable | Labile |
| Hydrophobicity | + | ++++ | ++ |
| Induction of antiviral state | Rapid | Rapid | Slow |

[a] Double-stranded RNA.

***Table 2.2*** Modulation of gene activity by IFNγ

| Stimulated or induced activities | Repressed activities |
|---|---|
| MHC class I (human HLA -A, -B, -C; mouse H2) | Oncogenes (e.g., c-myc, c-ras, c-sis) |
| MHC class II (human HLA -DR, -DQ, -DP; mouse Ia) | Transferrin receptors |
| 2′,5′- oligo-A-synthetase | 50-kDa keratin |
| Protein kinase | Ornithin decarboxylase |
| Receptors of: e.g., TNF, IgE, Fc, IgG, IL-2 | Heavy chain of IgM |
| IgG2a | Collagen |
| Guanylate-binding proteins | Smooth muscle α-actin |
| Tissue transglutaminase | Stromelysine |
| IFNγ-inactivating factor | Collagenase |
| 67 kDa keratin | |
| TNF; IL-2; CSF-1; MCP-1 | |
| Indoleamine-2,3-dioxiganase | |
| NO synthase | |

organisms have shown a low level of IFNγ mRNA expression. This is due to the complex factors capable of inducing the synthesis of IFNγ. The latter is secreted by T lymphocytes (Th1 and Th0) and also by the large granular lymphocytes (Young and Ortaldo 1987) upon their activation by T-cell mitogens, antigens, interleukins IL-2, IL-12, phytohemaglutinin (PHA), phorbol myristate acetate (PMA), concavaline A (ConA), plant lectins, bacterial lipopolysaccharides (LPS), and other agents (Ennis and Meager 1981; Marcucci et al. 1981; Nathan et al. 1981; McKimm-Breschkin et al. 1982; Vilcek

et al. 1985a,b; Wada et al. 1985; Blanchard et al. 1986; Young et al. 1986; Croll et al. 1987; Heslop et al. 1989; Ye et al. 1995). There are data showing that B cells also can be activated to produce IFNγ (Dayton et al. 1992).

The synthesis of IFNγ is controlled mainly at the level of transcription. Cells primed with IFNγ produce more of this cytokine when treated with inducing agents (Waranowska-Stewart et al. 1980; Toth et al. 1985). Phorbol esters and Ca ionophores act synergistically to increase the expression of IFNγ (Albert et al. 1985). Some growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and fibroblast growth factor (FGF) also increase its production (Johnson and Torres 1985). A superinduction of IFNγ is observed after exposure to low doses of gamma radiation and treatment with cycloheximide. This suggests the presence of a protein which prevents at a posttranslational level the accumulation of IFNγ (Lebendiker et al. 1987). Prolactin at a concentration of $10^{-8}$ *M* can considerably increase the level of IFNγ (Cesario et al. 1994). It has been reported that aspirin also increases the production of IL-2 and IFNγ (Hsia et al. 1989).

When IFNγ is combined with bacterial lipopolysaccharides (which is often recommended because of their synergistic action), it should be taken into account that prolonged incubation of macrophages with this combination may lead to inactivation of NO-synthase, an enzyme important for their antibacterial activity (see Chapter 3, Section 3.5; Vodovotz et al. 1994).

In experiments with mice it has been shown that ethanol suppresses the function of the Th1 lymphocytes, whereas that of the Th2 lymphocytes remains unaffected or is increased. This is due to a direct effect of the ethanol on the antigen-presenting cells (APC) which determine whether the Th1 or Th2 phenotype will prevail (Waltenbaugh et al. 1998). Ethanol suppresses the production of IFNγ in mouse splenocytes stimulated *in vitro* with LPS and ConA (Chen et al. 1993b). Experiments with volunteers have shown that the effect of a moderate intake of ethanol on cytokine production is biphasic: There is an increase of Th1 cytokines (IL-12 and IFNγ) after 4 hours and a decrease of IFNγ after 16 hours (Szabo 1998).

Concerning the effect of smoking, a defective response of the lung macrophages to IFNγ was found in healthy smokers (Rose et al. 1986).

There are data showing that vitamin $D_3$ and its derivative 1,25-$OH_2$-$D_3$ inhibit the secretion of IFNγ without affecting the secretion of IL-4 (Lemire et al. 1995), although vitamin $D_3$ is used in combination with IFNγ to induce differentiation in some tumors.

As already mentioned, production of IFNγ is suppressed by cytokines produced by the Th2 lymphocytes (see Chapter 1). Natural inhibitors of IFNγ have also been reported which probably play a role in the local regulation of its activity (Lefkowithz and Fleischmann 1986a,b; Vedrenne et al. 1997). It has been shown that heparin inhibits the activity of IFNγ (Daubener et al. 1994), whereas protamin has a stimulatory effect (Daubener et al. 1996). There are data indicating that increased plasma concentrations of cortisol result in decreasing the IFNγ activity (Spath-Schwalbe et al. 1989).

Aging diminishes the ability of the T cells to produce IFNγ and IL-2 without affecting the production of IL-4 and IL-6, thus leading to an imbalance between Th1 and Th2 cells, i.e., between cellular and humoral immunity (Candore et al. 1993). It was observed in mice that the decreased production of IFNγ with age may be reversed by oral administration of zinc, which is explained by the activation of protein kinase C (PKC) and its binding to the membrane of the T lymphocytes (Grasso et al. 1992). In adult rats the decreased bactericidal activity of leukocytes can be recovered by IFNγ (Fu et al. 1994).

## 2.3 Structure and physicochemical properties of IFNγ

IFNγ was isolated and purified to homogeneity in 1982. In the body it is released in minute amounts, which seriously complicates the preparation of natural IFNγ for scientific and clinical purposes. This problem was solved by recombinant DNA technology (gene engineering) (Devos et al. 1982; Gray et al. 1982b; Jay et al. 1984) which made it possible to produce human IFNγ in large quantities for studying its structure, properties, biological activities, and medical applications.

### 2.3.1 The molecule of human IFNγ

IFNγ is synthesized as a protein precursor composed of 166 amino acids (aa), a part of which belong to the so-called signal sequence. The latter is cleaved off by specific proteases during protein synthesis and the mature form of IFNγ is secreted (Devos et al. 1982; Gray et al. 1982a). The exact length of the signal peptide (20 or 23 aa) has long been debated, but now the accepted belief is that it consists of 23 aa. This means that the mature form of IFNγ is composed of 143 aa. As seen in Figure 2.1, the human IFNγ is a rare case of a natural protein devoid of cysteine residues. This does not hold true for IFNγ of other animal species. Chicken IFNγ, for example, has in its molecule two cysteine residues located in its C terminus (Michalski et al. 1999).

The tripeptide Cys-Tyr-Cys, which was considered for a long time to be a part of the N terminus of the mature IFNγ, is now accepted to belong to the C terminus of its signal peptide. There are data showing that the presence of this tripeptide in the IFNγ molecule slightly decreases its antiviral activity (Hsu et al. 1986). The mature form of human IFNγ contains 26 basic aa (18 lysine and 8 arginine residues) which give the protein markedly basic properties.

Unlike IFNα and IFNβ, which are not glycosylated, there are two centers of glycosylation in the natural IFNγ — Asn-25 and Asn-97 (Sareneva et al. 1994). The glycan at Asn-25 is composed of hybrid structures and fucosyl complexes, whereas that at Asn-97 consists of a mixture of high molecular mass mannose and hybrid residues (Mrtz et al. 1996). Glycosylation of human IFNγ is important for its synthesis and stability in the cell but not for its activity (Arakawa et al. 1986a). Inhibition of glycosylation with

[1]Gln Asp Pro **Tyr Val Lys Glu Ala Glu Asn**[10]
**Leu Lys Lys Tyr Phe** Asn Ala Gly His Ser[20]
Asp Val Ala Asp •Asn Gly Thr **Leu Phe Leu**[30]
**Gly Ile Leu Lys Asn Trp** Lys Glu Glu Ser[40]
**Asp Arg Lys Ile Met Gln Ser Glu Ile Val**[50]
**Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe**[60]
**Lys** Asp Asp Gln Ser Ile Gln **Lys Ser Val**[70]
**Glu Thr Ile Lys Glu Asp Met Asn Val Lys**[80]
**Phe Phe** Asn Ser Asn Lys **Lys Lys Arg Asp**[90]
**Asp Phe Glu Lys Leu Thr •Asn Tyr** Ser Val[100]
Thr Asp Leu **Asn Val Gln Arg Lys Ala Ile**[110]
**His Glu Leu Ile Glu Val Met Ala Glu Leu**[120]
**Ser** Pro Ala Ala Lys Thr Gly Lys Arg Lys[130]
Arg Ser Gln Met Leu Phe Arg Gly Arg Arg[140]
Ala Ser Gln[143]

***Figure 2.1*** Amino acid sequence of human IFNγ. The bold-labeled amino acid residues form the six α-helices. • Sites of glycosylation *in vivo*.

tunicamycin sharply decreases the synthesis of IFNγ, but it does not affect its secretion (Sareneva et al. 1986a). Experimental data show that glycosylated and nonglycosylated IFNγ have the same antiviral activity (Arakawa et al. 1986a) and stimulate to the same extent the tumoricidal activity of macrophages (Varesio et al. 1986). According to some data, the recombinant nonglycosylated IFNγ has an even higher biological activity than the natural glycosylated IFNγ. Its antiviral activity against the Sindbis virus develops faster and is severalfold higher than that of the natural IFNγ (Gomi et al. 1985). It is possible that glycosylation prolongs the half-life of IFNγ in the blood (Sareneva et al 1993).

The three fractions described in the natural IFNγ with molecular masses of 17, 20, and 25 kDa (Kelker et al. 1983a,b; Braude 1984) correspond to proteins of different extent of glycosylation. There are data that the glycosylated IFNγ is more resistant to proteases (Sareneva et al. 1995).

The uneven distribution of basic aa in the IFNγ molecule is a prerequisite for specific protease attacks. This explains the heterogeneity in the molecular mass of the nonglycosylated IFNγ observed in natural and in recombinant IFNγ preparations. Together with the main fraction corresponding to a 143-aa protein, truncated forms are often found both in natural and in recombinant IFNγ preparations. The most typical of them are composed of 138, 131, or 128 aa. As it may be deduced from Figure 2.1, they all are products of proteolytic attacks at the sites of Lys and Arg corresponding to proteins having deleted 5, 12, or 15 C-terminal aa.

Obviously, the heterogeneity of the IFNγ molecule is due to two processes: (1) various degrees of glycosylation and (2) proteolytic cleavage at the C terminus (Bulleid et al. 1990; Curling et al. 1990; James et al. 1996).

Covalent dimers may also be found in preparations of IFNγ, and their content increases with the time of storage. Because the mature IFNγ does not

contain cysteine, it is obvious that the covalent dimer is not due to the formation of intermolecular disulfide bonds. Although the chemical nature of covalent dimerization of IFNγ is not completely elucidated, it is believed that it occurs during proteolysis. Upon proteolytic cleavage, the C terminus of one processed polypeptide chain can be covalently attached to the N terminus of another. According to some investigators, the C-terminal truncation occurs at Phe 137, leading to a covalent bond formation between this aa and the N-terminal methionine of another IFNγ molecule (Lauren et al. 1993).

Studies with artificial recombinant covalent dimers of IFNγ in which the N and C termini are linked by polypeptide linkers have shown that the covalent dimerization does not affect receptor binding and therefore does not interfere with IFNγ activity (Lunn et al. 1992a; Randal and Kossiakoff 1998; Landar et al. 2000).

Recently, we have observed accumulation of truncated forms as well as covalent dimers of IFNγ in highly purified (>99.5%) recombinant preparations on storage in solutions. The high degree of purity in this case is a reason to conclude that no proteolytic enzymes are involved. It also is worth mentioning that the same truncated dimeric forms are found in the producing cell lysates. This suggests that they also are formed *in vivo*. All these data led us to conclude that the processing events affecting recombinant human IFNγ are due to chemical rather than to enzymatic reactions.

Most likely these reactions are connected with a nonenzymatic glycosylation (glycation) involving the ε-amino groups of lysine residues. Subsequent reactions leading to the formation of Amadori products at the regions enriched in basic aa could result in cleavage of the polypeptide chain and a simultaneous formation of covalent dimers. A possible source of glycating agents could be the 3-deoxyglucasone as well as other highly reactive compounds from the carbohydrate metabolism (Mironova et al. 2001).

X-ray crystallography shows that 62% of the IFNγ molecule is organized in α-helices and no β-sheets are found. The α-helices form six domains (designated as A, B, C, D, E, and F) linked with unstructured regions (see Figure 2.2; Ealick et al. 1991). These studies also show that in aqueous solutions with physiological pH and ionic composition IFNγ forms noncovalent homodimers, which represent the biologically active form of IFNγ (Devos et al. 1982; Yip et al. 1982; Yphantis and Arakawa 1987). The presence of tetramers which dissociate to dimers has also been shown (Kudo and Kawano 1999). The dimers bind to the cellular receptors. Within the noncovalent homodimer the monomers are organized in an antiparallel fashion such that the N terminus of one molecule is in close proximity to the C terminus of the other. This structure is destroyed (denaturation) and the interferon is inactivated at temperatures higher than 52°C and pH values outside the range of 4.0 to 9.0 (Rinderknecht et al. 1984; Hsu and Arakawa 1985; Arakawa et al. 1987; Hoshino et al. 1987; Yphantis and Arakawa 1987). A reversible acid denaturation, however, has also been observed (Arakawa et al. 1990). Thermal denaturation at pH 6 leads to formation of aggregates, which depends on the

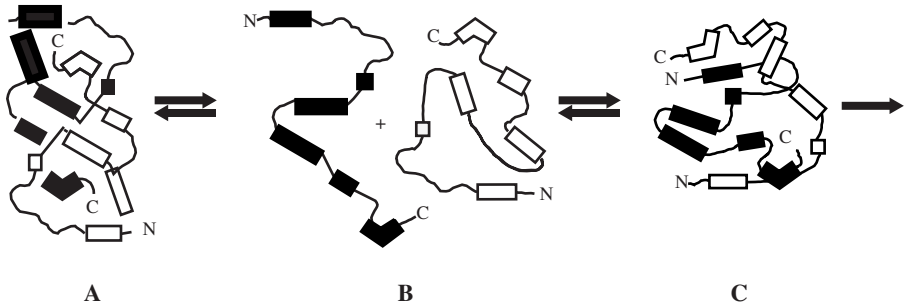


***Figure 2.2*** A model of the higher order structures of IFNγ upon dissociation and aggregation. The black and the white rectangles indicate the α-helical regions in the two monomers. (A) Scheme of the relatively rigid dimer (according to Ealick et al. 1991); (B) the dimer dissociated into two conformationally flexible monomers; (C) formation of irregular dimers prone to form inactive IFNγ aggregates.

partial or a complete unfolding of the IFNγ molecule. The latter is more stable at pH 5, which is possibly due to protonation of one or both histidine residues (Mulkerrin and Wetzel 1989). Other investigators have found that the reversibility of the thermal denaturation of IFNγ depends on the pH value and the buffer concentration. In high buffer concentrations and pH values between 2 and 9, IFNγ denaturation is irreversible. It becomes reversible at pH 3.5 to 4.5 and low buffer concentrations.

IFNγ also undergoes aggregation (and therefore inactivation) under mechanical stress (Zlateva et al. 1999). Our unpublished data show that the same events are induced by the surface tension of the solution. It has been suggested that aggregation is preceded by some intermediate form prone to aggregate (for details, see Kendrick et al. 1998). According to our observations, this form is represented by the monomers which are more susceptible to aggregation due to their greater conformational flexibility as compared to the more rigid dimers (see Figure 2.2). Such an assumption is supported by the fact that decreasing the flexibility of the monomers by an artificial disulfide bridge has a stabilizing effect on the IFNγ, including its acid denaturation (Kontsek et al. 2000).

The interaction between the monomers in the IFNγ dimer is very strong, and some investigators believe that the latter can be destroyed by denaturation only (Arakawa et al. 1987; Yphantis and Arakawa 1987; Nagata et al. 1993).

Measuring the intrinsic fluorescence of the single tryptophan residue in the IFNγ molecule, we have proven the existence of a dynamic equilibrium between the dimeric and monomeric forms of IFNγ with an equilibrium constant of the order of $10^{-6}$ *M* at room temperature (Boteva et al. 1996; Nandi 1998). Due to this equilibrium, the ratio between dimers and monomers depends on the temperature and IFNγ concentration. The equilibrium between dimers f2 and monomers f1 (f1 + f2 = 1) obeys the law of mass action, which makes it possible to determine the monomer fraction by the formula:

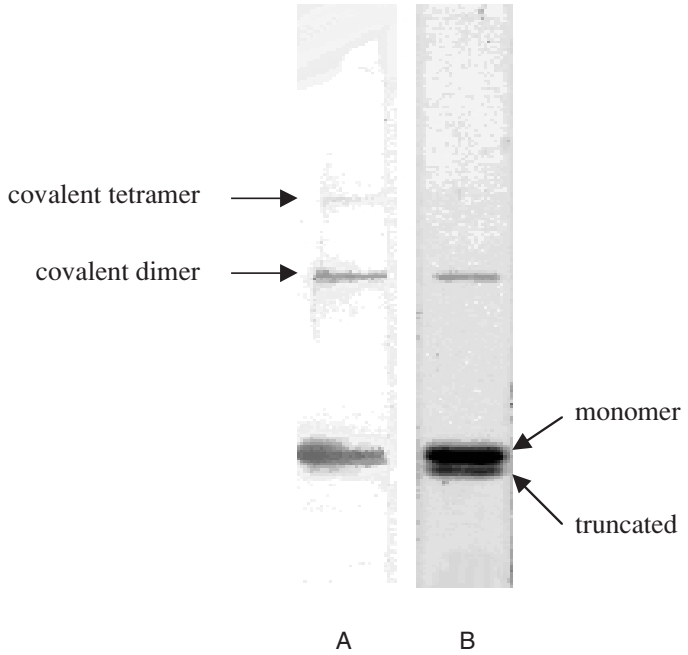


***Figure 2.3*** SDS electrophoresis of IFNγ preparation (Gammaferon) containing different forms of the cytokine: monomers, truncated monomers, covalent dimers, and tetramers. (A) Immunoblot of (B) (stained with Comassie) treated with a monoclonal antibody to IFNγ.

$$f_1 = \frac{1}{4n}\left(\sqrt{8n+1}-1\right)$$

$$n = \frac{M}{K_T}$$

where $M$ is the molarity of IFNγ (calculated as the concentration of monomers, regardless of whether they are in the dimeric or monomeric form), and $K_T$ is the equilibrium constant of the dimer-monomer transition at temperature $T$.

Besides truncated forms, monomers and dimers, a tetrameric form of IFNγ is often observed by gel electrophoresis (Langer et al. 1994; see Figure 2.3). It has been shown that the formation of IFNγ dimers does not depend on its glycosylation (Sareneva et al. 1995).

## 2.3.2 *The IFNγ gene*

IFNγ differs from the type I interferons in both structure and organization of its gene. Whereas the genes of IFNα and IFNβ are noninterrupted (intronless),

the gene of IFNγ contains four exons and three introns. Comparing the interferon genes with other eukaryotic genes, we could say that IFNα and IFNβ represent an exception, whereas the gene of IFNγ is a typical split eukaryotic gene. It spans over 6000 bp and is located on chromosome 12 (12q24.1) (Trent et al. 1982).

IFNγ mRNA is 1200-nt long and codes for a protein of 166 aa. It is detected in the cytoplasm of T lymphocytes 6 to 8 hours after induction, and it reaches its maximum level after 12 to 24 hours. The protein itself can be detected 8 to 12 hours after induction, and the maximum level is reached after 18 to 24 hours, which approximately coincides with the mRNA maximum level.

### *2.3.3 Molecular structure and biological activity of IFNγ*

In general, the protein precursors (the preproteins) are biologically inactive. In this respect, interferons, including IFNγ, are an exception. Nonprocessed interferons that contain a part of or the entire signal sequence are biologically active and also exhibit antiviral activity comparable to that of the mature forms. This is one of the reasons it was debated for such a long time whether or not the three aa Cys-Tyr-Cys belong to the signal peptide (see above). However, the deletion of any aa residue from the N terminus of human IFNγ leads to a sharp decrease or a complete loss of its biological activity. The latter is also suppressed by monoclonal antibodies specific to the N-terminal part of the IFNγ molecule. These data led to the conclusion that the N terminus of IFNγ is important for its binding to the receptor and manifestation of its biological effects (Hogrefe et al. 1989). However, both ends of IFNγ are important for receptor binding and for its biological activity (see Chapter 4).

The N-terminal part of IFNγ binds to the extracellular part of the receptor (aa 95 to 120) and the C-terminal end to the intracellular part (aa 253 to 287) (Szente and Johnson 1994). The role of the N-terminal α-helix A and the AB loop for protein stability has also been proven (Waschutza et al. 1996).

The role of the C-terminal region for IFNγ activity has been extensively studied, but the results obtained are controversial. This question is of great importance, since, as it was mentioned before, the C terminus of IFNγ is rich in basic aa and it contains "hot spots" for proteolysis. For this reason, natural and recombinant interferon preparations often contain C terminal truncated forms. Their content is shown to depend on the type of producing cells (Pan et al. 1987; Sano et al. 1987; Goldman et al. 1997; Vassileva et al. 2000; Mironova et al. 2001). There are conflicting results in the literature concerning the biological activity of the IFNγ truncated forms and therefore the role of the C-terminal aa for the IFNγ activity.

The role of the latter has been studied by four different approaches: (1) truncation of the C terminus by proteolysis; (2) blocking C-terminal aa by specific antibodies; (3) obtaining truncated forms by systematic deletion of the IFNγ gene and its expression in bacteria; (4) mutation analysis.

1. One group of investigators has found that proteolytic cleavage of aa from the C terminus affects the activity of IFNγ in a different way. For instance, removal of four C-terminal aa does not affect its activity. However, if 15 aa are cleaved off, there is a sharp decrease in the interferon-binding ability to its receptor, and therefore a decrease in activity (Arakawa et al. 1989). Elimination of 13 amino acids does not change the conformation and dimerization of IFNγ, but it decreases 1000-fold its antiviral activity (Arakawa et al. 1986b). In another experiment truncation of the C terminus by 7, 14, and 15 aa has shown that the lack of 7 aa does not affect activity, cleavage of 14 aa has a strong activity-decreasing effect, and deletion of 15 aa prevents IFNγ binding to its receptor and leads to a loss of activity (Haelewyn et al. 1997). Cleavage between the 129th and 130th aa residues (i.e., loss of 14 aa) is also related to a 1000- to 2000-fold decrease in receptor-binding ability at 4°C resulting in a 50-fold reduction in IFNγ antiviral activity (Leinikki et al. 1987). Opposite results were obtained by (Zhang et al. 1992) showing that IFNγ devoid of 15 C-terminal aa was still biologically active.
2. The use of antibodies specific to different C-terminal epitopes (Favre et al. 1989; Lord et al. 1989) has led to the conclusion that this end and especially the 15 C-terminal aa are not important for the biological activity of IFNγ. In contrast to these results, antibodies against the 132 to 146 aa domain were reported completely to neutralize the antiviral and antiproliferative activities of IFNγ (Seelig et al. 1988).
3. Experiments with recombinant IFNγ with various C-terminal deletions gave also conflicting results. According to some data, elimination of 14 or more aa from the C terminus decreased the IFNγ activity to 2% of that expressed by the wild-type molecule. However, deletion of 11 C-terminal aa increased the antiviral activity, especially if the C-terminal aa was leucine. It is interesting that a biologically inactive IFNγ variant truncated by 20 aa preserved its capability of forming dimers. Judging by the CD (circular dichroism) spectra, it had the same secondary structure as the full-size IFNγ (Slodowski et al. 1991). A nine-aa deletion from the IFNγ C terminus led to a sevenfold higher specific activity which remained unchanged after removal of the next two aa. Further truncation of the molecule, however, decreased its activity to 1% of the maximal level (Lundell et al. 1991).

   Using the same approach, other investigators have shown that the C-terminal part is of crucial importance for the antiviral, antichlamydial and antiproliferative activities of IFNγ (De la Maza et al. 1987a,b). In contrast to these results are the data of several Japanese investigators who used the same truncated recombinant constructs. They reported that elimination of 19 to 23 C-terminal aa did not affect the IFNγ antiviral activity (Sakaguchi et al. 1988). The data from another study (elimination of 21, 26, 32, or 37 C-terminal aa) indicated that truncation

of more than 26 aa led to a complete loss of IFNγ activity, whereas deletion of 21 aa decreased its activity two to three times only. From these data, it was concluded that the C-terminal 21 aa were not directly involved in the IFNγ function (Luk et al. 1990). Supporting results were obtained with a truncated at Lys-133 chicken IFNγ whose biological activity was found to be comparable to that of the full-length protein (Michalski et al. 1999).

4. Data obtained by mutational analysis have shown that the C-terminal domain (particularly positions 128 to 131) is important for the biological activity of IFNγ (Wetzel et al. 1990). This is a region which is affected by the deletion of more than 12 aa. The important role of His-111 for the binding of interferon to its receptor has also been shown (Lunn et al. 1992b).

As we shall see later (see Chapter 4), according to a number of data, the basic sequence at positions 125 to 131 plays a critical role for the biological activity of IFNγ. This sequence is responsible for the nuclear localization of the active complex of IFNγ and its receptor. This means that removal of more than 12 C-terminal aa should affect its biological activity. On the basis of an electrostatic study, it was assumed that the C-terminal part contributes to the low structural stability and propensity for aggregation of recombinant IFNγ (Altobelli et al. 2001).

From this summary of the relationship between structure and biological activity, it can be concluded that the presence of additional aa at the N terminus, the absence of a number of C-terminal aa (probably up to 12 aa), and also the loss of a polysaccharide component of the natural IFNγ does not essentially affect its biological activity. In other words, the biological activity of IFNγ is determined by the 130-aa-long core polypeptide in the mature form of the protein. According to one study, phosphorylation of serines 132 and 142 in the C-terminal domain increases only slightly the activity of IFNγ (Akinaga et al. 1987).

Although the role of the signal peptide (involved in the secretion of IFNγ) is clear, the role of the carbohydrate component can only be hypothesized. Taking into consideration the susceptibility of recombinant IFNγ to aggregation, it could be presumed that the glycosylation is related to its solubility and stability in aqueous solution which would affect its half-life in the organism (see Section 2.3.1). There are also data indicating that the recombinant IFNγ binds to the same receptor as the natural interferon, but it binds with higher affinity and is internalized more slowly (Akinaga et al. 1987).

As was already pointed out, the quaternary structure of IFNγ (represented by the noncovalent homodimer) is of great importance for its binding to the interferon receptor, i.e., for its biological activity. Since all higher structures are directly related to the primary structure of the protein, it is understandable that aa substitutions leading to drastic changes in the quaternary structure of IFNγ will have a negative effect on its activity.

## 2.4 IFNγ receptors

The biological activity of IFNγ is realized by its binding to specific receptors localized on the cell membrane (for review, see Langer and Pestka 1988). As was mentioned before, although IFNα and β share a common receptor, IFNγ has a different one (Branca and Baglioni 1981; Merlin et al. 1985). Binding to the receptor is a necessary but not sufficient condition for the expression of the IFNγ biological activity. The affinity of IFNγ for its receptor is not always a measure of its activity (Jabbar and Twentyman 1990). The receptor-interferon complex has to be internalized by endocytosis which initiates a cascade of processes reaching the nucleus (see Chapter 4).

During the last decade, in parallel with INFγ, the molecule of its receptor, IFNγRα, has also been extensively studied. Numerous immunochemical, radiochemical, and cytochemical studies have unambiguously shown that the cells have only one type of IFNγ receptor, and it is present in all cells with the exception of the anuclear erythrocytes. The number of IFNγ receptors on the cell surface depends on the cellular type and varies between 200 and 60,000, being usually about 2,000 to 4,000 (for review, see Branca 1988). It is interesting to point out that even thrombocytes carry up to 300 IFNγ receptors (Molinas et al. 1987), which is believed to be connected with their transport within the circulatory system. The number of IFNγ receptors is especially high on the surface of cells of the skin, nervous tissue, and trophoblasts of the placenta where their number is 10 to 100 times higher than that of hematopoietic cells. A rich and convenient source of IFNγ receptors is the placenta (Stefanos et al. 1989). There are data that dexamethasone increases the number of IFNγ-binding sites (Strickland et al. 1986; Diez et al. 1987). It is interesting to mention that prostaglandin E2 (PgE2) increases the number of IFNγ receptors on the CD8+ suppressor T lymphocytes and is necessary for their differentiation (ElMasry and Rich 1989).

Although the IFNγ receptor has a membrane localization, several studies have shown that it is distributed between the cell membrane and the cytoplasm in a 1:2 to 1:4 ratio (see Bach et al. 1997).

The human IFNγRα receptor is a glycoprotein composed of 472 aa, which correspond to a molecular mass of 52.5 kDa. Actually its molecular mass is larger due to glycosylation (the protein having five sites for this modification). Lower molecular mass products have been described as a result of degradation (Mao et al. 1989). Glycosylation is not of direct importance for IFNγ binding to the receptor, but it appears to be important for the correct conformation of the protein in the plasma membrane (Fischer et al. 1990).

Several fractions of IFNγRα corresponding to the different extent of glycosylation have been characterized. It has been determined that the completely glycosylated forms only (80 and 95 kDa) are localized on the plasma membrane. This polypeptide chain contains ten cysteine residues (Farrar and Schreiber 1993), but according to another study, there are eight cysteine residues in the polypeptide chain (Stuber et al. 1993). The cysteine residues form disulfide bridges, which are important for the interferon binding.

If they are disrupted by reducing agents, IFNγ is released from the complex. Structural analyses show that all potential sites for N-glycosylation are occupied. The IFNγRα also contains O-glycosylation sites, which have not yet been well characterized.

Three domains can be distinguished in the α-chain (see below) of the IFNγ receptor: extracellular (228 aa), intracellular (221 aa), and a transmembrane domain composed of 23 aa. The extracellular domain includes the N-terminal, the intracellular domain — the C-terminal part of the molecule. The glycosylation sites are localized mainly in the extracellular domain (Bach et al. 1997).

The gene of human IFNγRα (3000 bp) contains seven exons and six introns and is localized on chromosome 6 (q16 to q22) (Rashidbaigi et al. 1986; Bach et al. 1997). It produces a 2300-nucleotide (nt) mRNA which encodes a protein of 472 aa.

Employing an original approach, it was shown that the production of a functionally active IFNγ receptor depends not only on the normal functioning of its gene on chromosome 6 but also on other *trans*-acting genes localized on chromosome 21. To this end, hybrid mouse–human cell lines were used containing the complete mouse chromosome set and one or two human chromosomes.

The technique of cell hybridization was developed more than two decades ago. With the help of membrane-active substances, such as polyethylene glycol, capsids of the Sendai virus, and others, it is possible to fuse plasma membranes of two or more heterologous cells. After the first cell division, the nuclear material of the resulting heterokaryon is fused, and during the subsequent cell divisions, a systematic loss of chromosomes of one of the cell types occurs. Hybridization of human and animal cells usually leads to a selective loss of human chromosomes. After a certain number of cell divisions, stable cell lines are obtained containing the complete set of the animal chromosomes and one to two human chromosomes. The hybrid cells thus obtained are a valuable model for studying the function of a group of genes separated from the rest of the genes in a particular genome.

It has been shown that hybrid mouse–human and hamster–human cell lines bearing only human chromosome 6 produce nonfunctional human IFNγ receptor. Unlike these cells, cell lines containing both human chromosomes 6 and 21 produce a functional IFNγ receptor and respond to IFNγ. Mouse cells and hybrid cells containing human chromosome 6 only are not responsive to human IFNγ (Pestka 1992; Bach et al. 1997).

Thus, these experiments showed that two subunits of the IFNγ receptor are needed for the transmembrane signaling leading to changes in gene activity — one designated the α-chain (IFNγRα) and an additional protein factor called the β-chain (IFNγRβ). The gene of this factor coding for a 1800-nt mRNA is localized on chromosome 16 in mice (Hibino et al. 1991) and on chromosome 21 in humans (Jung et al. 1987; Farrar and Schreiber 1993; Soh et al. 1994; Bach et al. 1997). This factor — which possibly represents a whole family of factors — does not have an affinity for IFNγ by itself. However, it is mobilized by the interferon-receptor complex Rα, and thus a

new complex is formed. The β-subunit interacts with the extracellular part of the α-subunit (Hibino et al. 1992; Kalina et al. 1993). It is presumed that there are different β-subunits responsible for various biological effects in different cellular types by inducing different metabolic pathways (Pestka 1992). Thus, the receptor oligomerization in the cell membrane initiated by the ligand-receptor interaction leads to a cascade of reactions modulating the transcriptional activity of the nucleus (see Chapter 4).

It is important to point out that in some cells IFNγ was found to participate in the regulation of the β-chain expression. Thus, T helper 1 (Th1) lymphocytes which produce IFNγ are insensitive to it lacking a β-chain. Th2 lymphocytes which have a β-chain respond to IFNγ, and the latter can inhibit the expression of this chain. In this way, IFNγ can change the sensitivity of some cells to its action (see Bach et al. 1995, 1997). This could explain the appearance of IFNγ-resistant cells after their long-term treatment with this cytokine.

It has been shown that the additional β-subunit is necessary for relaying the signal for expression of the major histocompatibility complex (MHC) type I molecules, the 2′,5′-oligoadenylate synthetase and for inducing antiviral resistance (Kalina et al. 1993).

IFNγ binds to its receptor with an extremely high affinity constant Ka = $10^9$ to $10^{11}$ $M^{-1}$ (Cofano et al. 1986, 1989). Responsible for the ligand binding is the extracellular domain (Fountoulakis et al. 1991). This is confirmed by experiments with antibodies specific to different epitopes of the receptor (Garotta et al. 1990). This domain is active even when separated from the rest of the receptor (Littman et al. 1985). Unlike some data showing that the IFNγ dimer binds to the receptor in a 1:1 stoichiometric ratio (Fountoulakis et al. 1990a), newer data show that the ratio is 2:1, i.e., one IFNγ dimer binds two receptors each consisting of one α- and one β-chain (Fountoulakis et al. 1992; Bach et al. 1997). According to others, two dimers (equivalent to one tetramer) bind the receptor and participate in its dimerization in order to induce antiviral activity (Langer et al. 1994).

It seems that for binding to the receptor the N and C termini of IFNγ have to be juxtaposed, as they are in the dimer. This is supported by the fact that synthetic peptides imitating the end domains of the dimer inhibit the IFNγ binding to its receptor (Seelig et al. 1994). There are also data showing that antibodies to the N-terminal region of IFNγ block its binding to the receptor (Magazine et al. 1988). All these data prove the major role of the N-terminal domain in the process of receptor binding.

Binding of IFNγ to its receptors stimulates endocytosis, which leads to internalization of the IFNγ-receptor complex (see Kushnaryov et al. 1988; Finbloom 1990). In this way IFNγ starts a metabolic pathway partially overlapping the processes induced by the other interferons (IFNα and IFNβ).

Structure-function relationship studies (Aquet et al. 1988; Calderon et al. 1988; Fountoulakis 1989; Pestka 1992) have shown that the first six N-terminal aa are not important for the function of the extracellular IFNγRα domain, whereas deletion or substitution of aa in the remaining region (6 to 227 aa) leads to a decrease or a complete loss of its ligand-binding activity. Functional

regions are also found in the intracellular domain of the IFNγ receptor. The receptor itself has a unique and unusual aa composition (25% of its aa are serine and threonine) and does not share any homology to the known receptor proteins.

It has been proven that the first 48 aa of the intracellular domain of the IFNγ receptor (localized immediately next to the membrane) are responsible for the ligand-induced internalization, its disaggregation, and induction of the corresponding biological response. The 39 C-terminal aa are also important for induction of the IFNγ biological response, and Leu-270 and Ile-271 are crucial for the internalization and degradation of the receptor. Mutation analysis has shown that Tyr-440, Asn-441, and His-444 are of critical significance for the biological activity of IFNγ (Farrar et al. 1992). The functional importance of the 388 to 449 region is proven by specific monoclonal antibodies, which after intracellular microinjection block the cellular response to the receptor-bound IFNγ (Aguet 1990). Overexpression of either human or mouse receptors lacking the entire C-terminal domain or devoid of 39 C-terminal aa residues or having a functionally important Tyr substituted with Ala leads to loss of IFNγ function. Cells expressing both the mutated and the normal receptors in a 100:1 ratio do not respond to IFNγ even in doses 30,000 times higher than needed to induce a maximum response in normal cells (negative dominant mutations) (Dighe et al. 1993). Concerning the β-subunit, it has been shown that only the intracellular domain localized next to the membrane is important for its function (Kotenko et al. 1995; Bach et al. 1996).

The extracellular domain of the IFNγ receptor can be enzymatically cleaved off. This part of the receptor — soluble receptor (sIFNγRα) — is also capable of binding IFNγ with high affinity, thus inactivating it (Ozmen et al. 1993). It is interesting that some viral genomes (Alcami and Smith 1995 and references therein) code for this soluble receptor and induce its synthesis upon infection of the cells. These viral soluble receptors shedded in the surrounding medium bind IFNγ and deprive the cells of their antiviral protection. Evidently, this is an adaptation allowing the virus to avoid the antiviral activity of the IFNγ. The soluble receptor has also been cloned and purified (Fountoulakis et al. 1990b; Michiels et al. 1998). It may have a practical application for inactivating endogenous IFNγ in some diseases where this cytokine plays a pathogenic role, such as, for example, multiple sclerosis (see Chapter 11, Section 11.3).

A number of data show that the high specificity of IFNγ is due to the specific interaction of the extracellular part of the receptor with the N-terminal part of IFNγ. Three different kinds of experiments have shown that when IFNγ is directly introduced into the cytoplasm, its species specificity is lost: (1) human IFNγ introduced into mouse macrophages by liposomes stimulates their tumoricidal activity (Fidler et al. 1985); (2) human IFNγ expressed in mouse macrophages by a specific vector induces their antiviral activity (Sanceau et al. 1987); (3) microinjection of human IFNγ into mouse fibroblasts induces the expression of mouse Ia antigen (Smith et al. 1990; Johnson et al. 1998).

At first glance, these data contradict the idea that transmembrane signaling is necessary to achieve the IFNγ biological effects. However, they can be explained by the species-nonspecific interaction between the intracellular domain of the receptor and the C-terminal part of IFNγ when it enters the cytoplasm (Szente et al. 1994 a,b) (see Chapter 4).

Right after binding of IFNγ to the receptors, the IFNγ-receptor complex is rapidly internalized (in the course of minutes at 37°C) (Kushnaryov et al. 1988; Finbloom 1990). Once in the cytoplasm, the receptor can be recycled in some cellular types or it can be degraded in others. There is also some pool of receptors in the cytoplasm (Celada and Schreiber 1987). The appearance of new unoccupied receptors on the cell membrane occurs usually 4 to 12 hours after internalization depending on the cellular type. This should be taken into consideration in the clinical application of IFNγ.

Formation of the ligand-receptor complex initiates a cascade of processes which are discussed in Chapter 4.

*chapter three*

# *Biological activities of interferon gamma*

Although IFNγ is naturally produced in the organism in minute quantities, it has an extremely important biological activity which is manifested as six different effects — antiviral, antibacterial, antiproliferative, antitumor, immunostimulatory, and modulation of gene activity. These activities are species specific, which, as was previously mentioned, is due to the species-specific interaction of IFNγ with the N-terminal (extracellular) part of the receptor (see Chapter 2, Section 2.4). Interestingly, it has been reported that IFNα/β and IFNγ from different species act synergistically when used in combination (Piasecki 1987).

## *3.1 Antiviral activity*

The role of IFNγ in inducing an antiviral state of the organism is demonstrated by the fact that mice lacking interferon receptors have a strongly decreased resistance to viral infections (van der Broek et al. 1995). As an antiviral agent, IFNγ is considered to be 20 to 50 times less active than IFNα and IFNβ. However, this is not always the case, because this activity depends on the cellular type and the viral strain used. For example, the antiviral action of IFNγ against the Sindbis virus is severalfold higher and its onset is faster than that of IFNα and β (Gomi et al. 1985). The specific antiviral activity of IFNγ is of the order of $10^7$ to $5 \times 10^7$ IU/mg. When cells are exposed to this cytokine they become resistant to viral infections (Horisberger and de Staritzky 1985; Sedmak et al. 1985; Mistchenko et al. 1989). The combination of IFNγ and IFNβ results in a synergistic antiviral activity (Gomi et al. 1986).

The antiviral activity of interferons is determined experimentally by using standard cell lines (most often the human cell line WISH) and a suitable virus (for example, the vesicular stomatitis virus, VSV) which has a cytopathic lethal effect on the cells. The cells are cultivated in a suitable nutritional medium. They are treated with interferon in different concentrations and are then infected with the virus. The effect of the interferon is measured

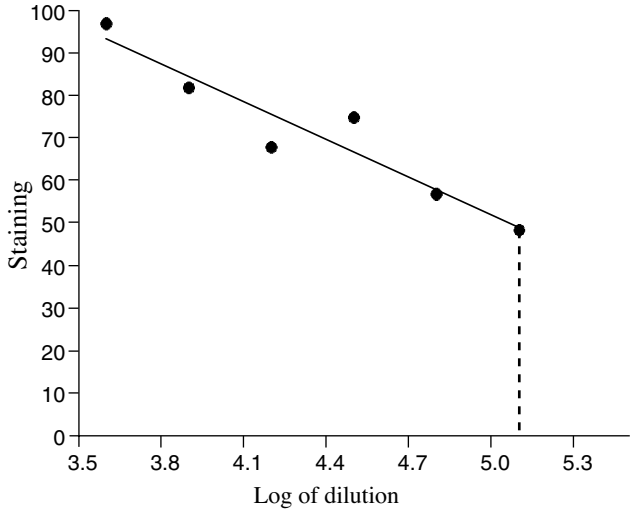


***Figure 3.1*** Testing the activity of IFNγ by staining with neutral rot. Average results from activity determination of four samples of Gammaferon eye drops. The dotted line at 50% staining indicates the log of the activity (in this case $1.25 \times 10^5$ IU).

as the percentage of dead cells. The reciprocal value of the largest dilution that protects 50% of the cells from the cytopathic effect of the virus is accepted to give the concentration of interferon in international units (IU) in 1 ml.

The percentage of living cells is determined visually or by staining the cells with neutralrot (which stains the living cells only) and measuring the amount of retained stain (Armstrong 1981; Johnston et al. 1981) (Figure 3.1).

As already mentioned, all cells (except the anuclear erythrocytes and some malignant cell lines) have IFNγ receptors, which means that they can exert their antiviral effects on all of them. The direct antiviral activity is due to different mechanisms among which, at present, the following have been reported (for details and references, see review by Pestka and Langer 1987):

1. Inhibition of protein biosynthesis through induction of the specific protein kinase PI, which requires for its activity the presence of double stranded RNA (dsRNA) (present during viral replication). This protein kinase phosphorylates and in this way inactivates the eIF2a translation initiation factor.
2. Inhibition of protein biosynthesis through induction of three highly specific enzymes: (a) 2′-5′-oligoadenylate synthetase, (b) 2′-5′-phosphodiesterase, (c) 2′-5′-oligo-A-dependent RNAse.
   a. The first of these enzymes, which also requires dsRNA for its activity, catalyzes the synthesis of nontraditional oligoribonucleotides, which have a 2′-5′- instead of a 3′-5′-phosphodiester bond. The abnormal oligonucleotides carry at their 5′ end a pyrophosphate or a triphosphate group and are composed of 3 to 12 monomers. The

synthesis of the 2′-5′-oligoadenylates is adenosine triphosphate (ATP) dependent.

b. The 2′-5′-oligoadenylates are not sensitive to cellular ribonucleases and a specific 2′-5′-phosphodiesterase is synthesized for their degradation. In this way an equilibrium is established between biosynthesis and degradation of the 2′-5′-oligoadenylates ensuring their transient effect.
c. The third enzyme which is induced by the interferon is a specific RNAse known as RNAse F or RNAse L, which requires 2′-5′-oligoadenylates for its activity. It degrades various viral and cellular RNAs. IFN$\gamma$ and IFN$\alpha$ also induce an adenosine-deaminase specific for dsRNA (Patterson et al. 1995).

The suppression of protein synthesis and the degradation of cellular RNA pose a question of how the cell achieves the transition into an antiviral state.

As has been mentioned already, the activation of two of the enzymes induced by IFNγ (protein kinase and 2′-5′-oligoadenylate synthetase) is dependent on the presence of dsRNA, which is produced as an intermediate during the replication of many viruses. The interferon induces the synthesis of all these enzymes, but they remain inactive in the cytoplasm until the appearance of dsRNA. In the presence of the latter, the latent enzymes are activated, as also is activated the nonspecific RNAse F. In parallel with cellular RNA, RNAse F also attacks the viral RNA. The end result of the activity of the interferon-induced enzymes is the inhibition of viral replication. This is due to the fact that at the beginning of viral infection the virus-specific RNA are few in number and they cannot be recovered after the enzymatic damage. Unlike the viral RNA, the cellular RNAs are present in numerous copies and are continuously synthesized in the nucleus. That is why damage inflicted to them can slow down cellular functions only temporarily, and they are recovered shortly after the effect of interferon is gone.

3. With adenoviruses, the antiviral activity of IFNγ may be partly due to a competition between signal transduction and transcription activator 1 (STAT-1), which is activated by IFNγ, and the adenoviral protein E1A. It has been shown that the C-terminal domain of STAT-1, which is phosphorylated by the IFNγ-kinase, interacts with a domain of the transcription cofactor CB/p300 (a member of the CREB [cyclic AMP response element binding] family of cofactors) which is the same domain involved in the binding of the adenoviral protein E1A. The latter is necessary for transcription of the viral genes, which cannot occur because of the competition with STAT-1 (Zhang et al. 1996).

A line of evidence shows that during evolution protective mechanisms have evolved that protect viruses from the antiviral effect of interferons (see Sen and Lenguel 1992). One of these mechanisms is the inhibition of the dsRNA-dependent protein kinase induced by IFNγ (e.g., in adenoviruses,

Epstein-Barr virus, human immunodeficiency 1 [HIV-1]). The vaccinia virus and retroviruses synthesize proteins which bind to dsRNA and prevent kinase activation. Other viruses inhibit RNAse L (e.g., in cells infected with the EMC [encephalomyocarditis] virus). In cells infected with Herpes simplex virus degradation products of 2′-5′-poly A are formed, which inhibit the activation of RNAse L by 2′-5′-poly A. There are some viruses encoding proteins which block the transcriptional activity of genes induced by interferon. Other viruses encode the extracellular soluble domain of the IFNγ receptor (Alcami and Smith 1995). When shedded in the surrounding medium, it binds IFNγ (Michiels et al. 1998; Puehler et al. 1998), thus competing with the cellular membrane receptors and preventing the onset of the antiviral state. A soluble analogue of the IFNγ receptor (MT7) which is produced by the myxoma virus has the same inhibitory effect (Upton et al. 1992; Mossman et al. 1995).

A number of viruses (such as human and mouse cytomegaloviruses, adenoviruses, herpes simplex virus) interfere with major histocompatibility complex (MHC) I-dependent peptide presentation (see Chapter 3, Section 3.3). The herpes simplex virus, for example, expresses the ICP47 protein which blocks viral peptide presentation on MHC I-dependent cells (Fruh et al. 1995; Hill et al. 1995). Expression of the hepatitis B terminal protein inhibits the cellular response to IFNα and IFNγ (Foster et al. 1991).

An interesting anti-immune mechanism is found during HIV infection. It turns out that HIV activates a methyltransferase which methylates the promoter of the IFNγ gene, thus blocking its synthetic activity (Mikovits et al. 1998).

The indirect antiviral effect of IFNγ is connected to the activation of effector cells of the immune system (see Section 3.3). The antiviral activity of CD8+ T lymphocytes also depends on IFNγ (Ruby and Ramshow 1991).

## 3.2 *Antiproliferative activity*

IFNγ suppresses the proliferation of a number of normal and malignant cells (Clemens and McNurlan 1985; Rossi 1985; Saito et al. 1986a; Pestka et al. 1987; Shearer and Taylor-Papadimitriou 1987; Mechti et al. 1988; Romeo et al. 1988; Schiller et al. 1988b; for malignant cells, see Chapter 12). Some examples are normal and malignant keratinocytes (Nickoloff et al. 1986; Schuger et al. 1990), normal and malignant endometrial cells (Tabibzadeh et al. 1987), vascular smooth muscle cells (Rubin and Gupta 1980; Hansson et al. 1989), human cells of bone origin (Beresford et al. 1990), and regenerating hepatocytes (Sato et al. 1993). Combination of IFNγ with the other two types of interferon potentiates its antiproliferative activity (Fleischmann et al. 1984). Its combination with dsRNA has also a synergistic effect (Chapekar and Glazer 1985).

In most cases, especially in malignant cells, the antiproliferative activity of IFNγ is accompanied by a differentiating effect (for reviews, see Fischer

and Grant 1985; Giovanni and Rossi 1985; Rossi 1985; Burke 1986; Moritz and Kirchner 1986). This has been shown in numerous publications in experiments *in vitro* with cell cultures of different cellular types, such as human epidermal carcinoma cell line A431, where IFNγ leads to a rapid terminal differentiation with 67-kDa keratin expression and cell death (Chang et al. 1987). In the mouse NIH 3T3 fibroblast cell line transformed with Ha-ras, even a short treatment with IFNγ results in a stable reversion to a normal phenotype (Seliger et al. 1991).

However, we should also mention that the inhibition of cellular proliferation is not always accompanied by cell differentiation. For instance, suppression of vascular smooth muscle fiber proliferation by IFNγ is associated with inhibition of α-actin synthesis (Hansson et al. 1989). In cells of bone origin, collagen synthesis is suppressed along with the proliferation (Beresford et al. 1990). In general, the relationship between cell proliferation and differentiation is complex and depends on many factors and on the cellular type. As an interesting paradox we shall mention the report that growth of the malignant ovarian cell line Caov-3 is stimulated by both IFNγ and tumor necrosis factor α (TNFα) (Mutch et al. 1990).

In some cell types the antiproliferative activity may lead to apoptosis, as is the case of transformed keratinocytes, where the key mediators of IFNγ-induced apoptosis are the G-proteins (Heck and Laskin 1994).

The direct antiproliferative effect of IFNγ may be due to several different mechanisms.

- The most widely accepted and discussed mechanism is the suppression of oncogene expression and more specifically the proto-oncogene c-myc which is involved in the regulation of the cell cycle (Clemens and McNurlan 1985; Peters et al. 1985; Harel-Bellan et al. 1988; Mechti et al. 1988; Bruchelt et al. 1990; Bennett et al. 1994a,b; Vairo et al. 1995). A number of studies find that the interferons inhibit the expression of this gene and accept that this is the reason of cell cycle arrest (Yarden and Kimchi 1983; Dani et al. 1985; Einat et al. 1985; Matsui et al. 1985; Watanabe et al. 1989a; Yan and Shen 1994). However, it appears that the interrelation between IFNγ, c-myc, and the cell cycle are much more complex (Knight et al. 1985). The absence of a causal connection between suppressed expression of c-myc and cell cycle arrest is suggested by the observation that cell cycle arrest and monocyte differentiation of HL-60 cells treated with IFNγ start at the 24th hour, whereas the inhibition of c-myc expression occurs at the 72nd hour (Sariban et al. 1987). Even more convincing are studies which show that cell cycle arrest in a human breast carcinoma cell line (Hamburger and Gayatri 1993) and in HeLa cells (Kelly et al. 1985) is accompanied by increased expression of c-myc. It is obvious that not in all cell types could cell cycle arrest be explained by a suppressed expression of the c-myc gene.

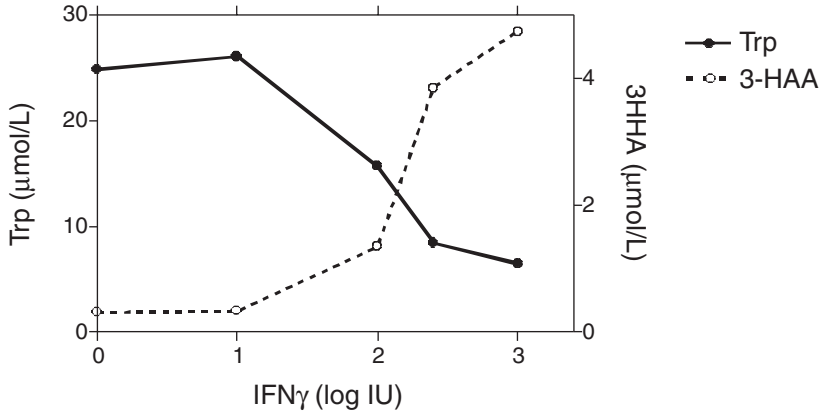


***Figure 3.2*** Degradation of triptophan (Trp) and secretion of 3-hydroxyanthranilic acid (3-HAA) in macrophages treated with IFNγ. According to data by Werner et al. (1987).

- Another mechanism is related to the effect of IFNγ on tryptophan metabolism (Figure 3.2), leading to tryptophan starvation. It has been shown that this cytokine induces the enzyme indolamine-2,3-dioxygenase (IDO) leading to degradation of tryptophan to kinurenin (Werner et al. 1987a,b, 1989; Dai and Gupta 1990), which is proportional to the IFNγ activity and can be used for quantitative determination of this activity (Daubener et al. 1994). There are data on keratinocytes that along with IDO induction IFNγ also induces the tryptophanyl-tRNA synthetase which balances the level of this important amino acid (Reano et al. 1993).

  The IDO gene is regulated in a different way by IFNγ and IFNα. Its induction correlates with the induction of the guanosine triphosphate (GTP)–cyclohydrolase, a key enzyme for pteridine synthesis (Taylor and Feng 1991).
- Cell cycle arrest may also be due to decreased expression of transferrin receptors caused by IFNγ and leading to iron deficiency (Bourgeade et al. 1988; Feelders et al. 1998).
- Induction of certain proteins such as DAP (death associated proteins) and calcium/calmodulin-dependent enzymes leading to apoptosis (for details and references, see Levy-Strumpf et al. 1997).

  Apoptosis is a programmed cell death. Unlike necrosis it is an active process, which occurs through gene induction, similar to the induction that occurs during the cell cycle, but leading to activation of proteases and nucleases and to cell death accompanied by typical biochemical and morphological changes. Cellular receptors of the TNF group, such as Fas, binding to the corresponding ligands lead to a metabolic pathway resulting in apoptotic cell death.

  This mechanism can be connected to the increase in cytoplasmic $Ca^{2+}$ due to its intracellular redistribution and influx of extracellular

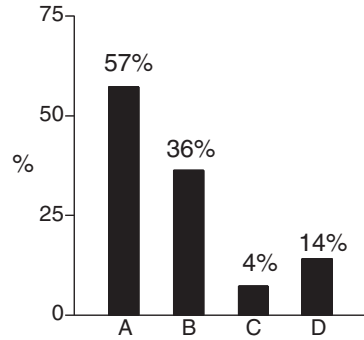


***Figure 3.3*** Growth suppression (%) of malignant melanoma cell lines by IFNγ (1000 IU/ml). (According to data by Jernberg-Wiklund et al. 1991.) (A) Line U-1958 (IL-6 dependent). (B) Line U-266-1970 (IL-6 dependent). (C) Line U-1996 (partially IL-6 dependent). (D) Line U-266-1996 (IL-6 independent).

$Ca^{2+}$ through the membrane channels caused by IFNγ (Klein et al. 1987). This activates the $Ca^{2+}$-calmodulin-dependent protease leading to apoptosis. It has to be taken into consideration that IFNγ can have a different effect depending on the receptor leading to apoptosis. For example, in the human myeloblastic leukemia cell line Eol-1 IFNγ triggers apoptosis by inducing Fas. At the same time it inhibits the TNF-induced apoptosis connected to the TNFR1 receptor, since IFNγ induces the expression of TNFR2 receptor (Horie et al. 1999).

- IFNγ can block the cell cycle at the end of the G1 phase. In mammary gland epithelial cells IFNγ induces the synthesis of the kinase inhibitor $p27^{kip1}$ which inhibits the activity of the cyclin-dependent kinases cdk2, cdk4, and cdk6 24 hours after treatment. At this time point, the kinases should hyperphosphorylate the retinoblastoma protein Rb in order to permit the cell to enter S phase. Inhibition of this process blocks the cell cycle at the end of the G1 phase. It is interesting and important to note that in some mammary carcinoma cell lines that are resistant to growth inhibition by IFNγ, $p27^{kip1}$ is not induced by this cytokine (Harvat et al. 1997).
- In some cases, the antiproliferative activity of IFNγ can be due to the inhibition of growth factor production. For example, this is the case of a myeloma cell line where proliferation is suppressed by IFNγ due to inhibition of interleukin-6 (IL-6), which is a growth factor for these cells (Palumbo et al. 1995a) (Figure 3.3).
- Another direct antiproliferative mechanism may be the inhibition of DNA synthesis (Nanes et al. 1989) and more specifically of the DNA polymerase, as it has been reported for this enzyme in mammals (Tanaka et al. 1987).
- IFNγ stimulates fibronectin synthesis in melanocytes and melanoma cells (Varani et al. 1989), and there is an inverse correlation between fibronectin synthesis and cell proliferation.

Most probably the direct antiproliferative activity of IFNγ is due to a combination of some of these mechanisms and maybe of some others yet undiscovered. It is possible also that different mechanisms may operate in different cell types.

## 3.3 Immunostimulatory activity

Stimulation of cellular immunity is one of the main biological functions of IFNγ (immune interferon). It is manifested by activation of effector cells of the cellular immunity (macrophages, NK/CD56 cells, CD8+ cytotoxic T lymphocytes, CD4+ T helper cells) which destroy foreign pathogens and tumor cells (Becker 1985a,b; Segal 1998).

This function is also manifested by the regulation of the expression of major antigens of the tissue compatibility complex (MHC).

MHC are a group of proteins denoted in humans as HLA (human leukocyte antigens). They are products of a network of interconnected genes in humans and in other mammals which control the immune response. They are localized on the cell surface and serve as molecular markers for differentiating between self and foreign tissue and for the presentation of peptides from foreign cells and pathogens. For example, MHC composition is the main factor which determines the fate of transplants and the formation of antibodies against foreign tissue. According to their composition, structure, and function, MHC are divided into two classes: I and II.

Unlike the MHC class I antigens (HLA-A, -B, -C in humans) which are expressed in almost all cells, class II antigens are expressed in a limited number of cellular types (B cells, macrophages/monocytes, and some activated T lymphocytes) involved in antigen presentation. They are heterodimeric glycoproteins on the cell surface and are encoded by three loci in the D region of human chromosome 6 (HLA-DR, -DQ, -DP). Their expression in macrophages is necessary for antigen presentation to T lymphocytes and for induction of cytolytic T-cell activity.

IFNγ stimulates the expression of class I MHC and induces class II MHC in a number of important cellular types (e.g., see Becker 1985a,b; Celada and Maki 1989; Griffiths et al. 1989a,b) such as phagocytes and endothelial and epithelial cells, but it suppresses the synthesis of class II MHC in B lymphocytes. In this way IFNγ participates in the antigen presentation process during the inductive phase of the immune response. In many cellular types IFNγ can regulate MHC expression by itself, but in a number of cases it acts synergistically with other cytokines, such as TNFα, for example, and with bacterial antigens and mitogens.

The major target cells of the immune system activated by IFNγ are the macrophages. It regulates the differentiation of monocytes into mononuclear phagocytes (macrophages), stimulates the antigen-presenting activity of macrophages, and facilitates their interaction with T cells. IFNγ also participates in the regulation of T helper (Th) cell differentiation by stimulating the proliferation of Th1 and inhibiting the proliferation of the antagonistic

Th2 type. IFNγ exhibits a stimulatory effect not only on CD4+ but also on CD8+ lymphocytes (Siegel 1988; Ruby and Ramshaw 1991). It is believed that IFNγ also participates in changing the immunoglobulin isotype in B lymphocytes (see Chapter 2). Bacterial lipopolysaccharides (LPS) are believed to be some of the most powerful macrophage activators, and they are often used in combination with IFNγ. However, it should be taken into consideration that the sequence in which the two stimulating agents are used is important. Treatment of monocytes with a priming dose of IFNγ followed by LPS after 18 to 24 hours leads to an increased cytotoxicity of the monocytes. However, even small doses of LPS during the preliminary treatment with IFNγ suppress the development of monocyte cytotoxicity (Chu et al. 1993a). The suppression is mainly due to products of the cyclooxygenase metabolic pathway, because large quantities of prostaglandin E2 (PgE2) are formed and indomethacin (an inhibitor of this enzyme) eliminates the cytotoxicity-suppressing effect.

The immunostimulatory activity of IFNγ is also connected with the induction of a number of membrane costimulatory molecules, such as ICAM-I/CD54, LFA-3/CD58, B7-1/CD80, B7-2/CD86, without which the cytotoxic activity of leukocytes cannot be realized (Zhang et al. 1998).

IFNγ activates the killer and cytostatic function of macrophages which is directed to various intracellular and extracellular parasites and tumor cells (Varesio et al. 1984; Landolfo et al. 1985; Fan et al. 1986; Sodhi et al. 1990), an effect occurring only in mature macrophages (Hosoi et al. 1985; Saito et al. 1986). In these cases it induces the expression of specific structures on the surface of the macrophages through which they recognize the target cells as well as the expression of specific cytotoxic agents such as TNFα and reactive oxygen and nitrogen derivatives (Murray et al. 1979; Nathan et al. 1979, 1985; Kaplan et al. 1986; Degling et al. 1993; Feng and Walker 1993; Lavnikova et al. 1993; Le Page et al. 1996). Among the latter, the most extensively studied is nitric oxide (NO), which is known to play an important role in the destruction of intracellular bacteria, parasites, and tumor cells.

NO is synthesized from L-arginine by a specific enzyme known as NO-synthase (NOS). The expression of one of the three isoforms of this enzyme is induced by IFNγ and other cytokines such as TNFα. In mouse keratinocytes it has been found that NOS is regulated at the posttranscriptional level (Heck et al. 1993).

Apart from increasing the nonspecific cytocidal activity of macrophages, IFNγ also increases their antibody-dependent cytocidal activity (ADCA). This occurs through the activation of different components of the complement, such as C2, C4, and factor B (Lappin et al. 1990; Vincent et al. 1993; Watanabe et al. 1995).

There are data indicating that under the effect of IFNγ the cytotoxic activity of the neutrophil leukocytes also can be stimulated (Shalaby et al. 1985; Steinbeck et al. 1986, 1989; Miyake et al. 1988; Livingston et al. 1989; Morrison et al. 1989; Cemerlic et al. 1991), and it can be also antibody dependent (Reali et al. 1994). IFNγ increases the hemotactic response (which

is weak in the newborn) and can be used as an immune defense against infections in pediatric practice (Hill 1993). It has been reported that thrombocytes can also exhibit an IFNγ-induced cytotoxic function (Pancre et al. 1987).

For natural killer (NK) and lymphokine-activated killer (LAK) cells, see Chapter 12, Section 12.1.

## 3.4 Modulation of gene activity

IFN$\gamma$ suppresses the activity of some genes, such as, for example, some oncogenes (Peters et al. 1985), genes coding for collagen (Giri et al. 1986; Duncan and Berman 1987; Smith et al. 1987; Clark et al. 1989; Granstein et al. 1990; Narayanan et al. 1992), and for transferrin receptors, and activates others like the genes of IL-2, IL-12, TNF$\alpha$ (e.g., see Schreiber et al. 1983; Peters et al. 1985), of IL-2 receptors (Rambaldi et al. 1987), of MHC I and MHC II (e.g., see Wallach et al. 1982; Becker 1985a,b; Berrih et al. 1985; Campbell et al. 1985; Carrel et al. 1985; Giacomini et al. 1986; Kameyama et al. 1987; Korber et al. 1988; Volc-Platzer et al. 1988).

There are, however, some malignant cells which do not respond to IFNγ with an increased synthesis of MHC I (Anderson and Berkowitz 1985). Tumor growth factor β (TGFβ) suppresses the induction of MHC II by inhibiting the mRNA accumulation of the transactivator CIITA (class II transactivator; Lee et al. 1997b), which is essential for the expression of these molecules. IFNγ also upregulates the induction of the non-variable chain connected with MHC II. This chain is a glycoprotein necessary for antigen processing and intracellular transport of MHC II molecules (Barr and Saunders 1991).

IFNγ also induces the intercellular adhesion molecule I (ICAM-I) (Dustin et al. 1988; Rothlein et al. 1988), which plays an important role in immunity. On the one hand, this molecule is important for the adhesion of loose cancer cells and the formation of metastases and, on the other hand, due to ICAM-I, tumoricidal lymphocytes can attach to cancer cells and destroy them. In the end, this molecule is very important for the defense of the organism against malignant cells, taking part in the immunological control accomplished by NK and LAK cells.

The ICAM-I ensures the adhesion of effector leukocytes to target cells that express it. The interaction is achieved with the help of the leukocyte function antigen LFA-1 (CD11a/CD18) (Marlin et al. 1987; Makgoba et al. 1988a,b; Fidgor et al. 1990; Becker et al. 1991).

As already mentioned, IFNγ induces some important enzymes such as IDO (see Section 3.2) and NOS (see Section 3.5). IDO induction is connected to the IFNγ-activated tyrosine protein kinase and PKC (Koide and Yoshida 1994). It is believed that in most cells IFNγ activates 15 to 20 genes (Gunther and Otto 1993), which usually occurs at the level of transcription (for review, see Revel and Chebat 1986; Williams 1991) (concerning the mechanism through which IFNγ affects gene activity; see Chapter 4). We shall only point out here that there are data indicating the participation of PKC in IFNγ-mediated gene activation (Fan et al. 1988; Mattila et al. 1989; Nezu et al.

1990). Some more important modulations of gene function are presented in Table 2.2. There are data that the mechanisms of gene activation by IFNγ are different from those of IFNα (Faltynek et al. 1985; Caplen and Gupta 1988).

It should be stressed that the effect of IFNγ on gene activity is not exactly defined and depends on a number of factors such as, for example, cellular type, animal species, and body region from which the cells have been isolated. For example, it was found that IFNγ stimulated the expression of stromelysine (a metalloproteinase that activates collagenase and is induced by IL-18) in bovine chondrocytes (Quintavalla et al. 1993), whereas in human fibroblasts the synthesis of this enzyme was inhibited (Unemori et al. 1991). Even cells of the same cellular type but isolated from different anatomical regions are affected in a different way by IFNγ. Fibroblasts isolated from the limb and from the abdominal region show a different pattern of *de novo* protein synthesis induced by IFNγ (Smith and Higgins 1993). Another example is the suppression of the gelatinase gene in human kidney carcinoma cells and the lack of this effect in fibroblasts (Gohji et al. 1994). The same holds true to the tumoricidal activity of mononuclear phagocytes isolated from different anatomical regions (Ahn et al. 1995).

These data could explain some apparently conflicting results in the literature regarding the biological effects of IFNγ.

## 3.5 *Antibacterial activity*

IFNγ kills a number of bacteria (see Chapter 10, Section 10.2.1) and intracellular parasites by activating macrophages to produce superoxide anions and nitric oxide as well as by increasing the chemotaxis (Murray et al. 1979; Nathan et al. 1983, 1984, 1985; Kaplan et al. 1986; Ding et al. 1988; Degling et al. 1991; Klein et al. 1991b; Martin et al. 1991; Lavnikova et al. 1993; Pendino et al. 1993; Xie et al. 1994). It stimulates macrophages to react to bacterial DNA (Sweet et al. 1998). Induction of IDO (see Section 3.2) also leads to antibacterial activity (MacKenzie et al. 1998).

## 3.6 *Antitumor activity*

The antitumor activity of IFNγ is due to both a direct and an indirect effect on tumor cells.

The direct effect is associated with its antiproliferative activity (see Section 3.2), whereas the indirect effect is due to the stimulation of the cellular immunity stimulation of competent effector T lymphocytes and macrophages (Baron et al. 1985) (see Section 3.3). Under the effect of IFNγ, macrophages become tumoricidal (Saito et al. 1986). It is interesting that macrophages can differentiate between tumor and normal cells. In experiments with mouse erythroleukemia cells it has been shown that the macrophages lyse undifferentiated cells only (Pak and Fidler 1989). The distinction between normal and malignant cells is also observed when IFNγ is combined with IFNα/β (Fleischmann et al. 1984). The cytotoxicity of macrophages toward tumor

cells varies and depends on many factors, such as the presence of a second signal (e.g., LPS) and the type of the target cells (Koestler et al. 1987).

The indirect antitumor activity of IFNγ depends on its effect on gene activity (see Section 3.4). With regard to the modulation of gene activity, new data show that IL-12 and IFNγ inhibit tumor growth by inducing tumor cells to produce an angiostatic activity (Coughlin et al. 1988; Angiolillo et al. 1996).

The generation of new capillaries (neoangiogenesis) is necessary for tumor growth. The use of compounds which inhibit angiogenesis (angiostatics) is an important approach in the treatment of solid tumors. Even when the tumor is in a stationary state, the continuous renewal of capillaries is necessary for its survival. A number of angiostatics are undergoing clinical trials.

This activity may be due to the production of the angiostatic chemokine IP-10, a -C-X-C- chemokine induced by IFNγ (Angiolillo et al. 1996; Arenberg et al. 1996). The local application of IFNγ also inhibits angiogenesis induced by basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) (Sato et al. 1990; Norioka et al. 1994) probably due to growth inhibition of the endothelial cells and suppressed synthesis of collagen by myofibroblast cells (Tsuruoka et al. 1988). A decreased vascularization under the effect of IFNγ was observed during wound healing in mice (Miles et al. 1994). At the same time it has been found that IFNγ can also act in the opposite way. It can increase angiogenesis through angiogenic factors released by IFNγ-stimulated macrophages (Kobayashi et al. 1994). Recently it has been reported that the human thymidine phosphorylase (TP) has angiogenic properties and is identical to the vascular endothelium growth factor (VEGF) produced by thrombocytes. High expression of TP is found in macrophages, Kuppfer cells, lymphocytes, and alveolar macrophages. TP may be responsible for the angiogenesis of a wide spectrum of solid human tumors (of the stomach, colorectum, ovary, and breast), where its activity is higher than that in the neighboring normal tissues. The expression of this enzyme is also increased in the advanced stages of cancer. On the other hand, TP increases the sensitivity of tumor cells to 5′-deoxy-5-fluorouridine. IFNγ, IFNα, and IL-1a induce TP in tumor cells (stomach cancer and colorectal cancer). Therefore, TP may be a possible target molecule for cancer therapy (Takebeyashi et al. 1996). Evidently, the different effect of IFNγ on angiogenesis — induced production of IP-10 or of TP — will be determined by different factors such as cellular type, macrophages, malignant type, and others. The increased activity of TP in solid tumors is used to accumulate preferentially 5-fluorourecil (5-FU) in malignant cells by applying the prodrug capecitabine, which under the effect of certain enzymes (including TP) is converted into 5-FU (Schüller et al. 2000).

In some tumors (e.g., B16 melanoma) IFNγ strongly increases the expression of the surface antigen Fas (CD95) and its ligand FasL (CD95L), which irreversibly programs the cells for apoptosis (see Section 3.2). Also of importance is the induction by IFNγ of TNFα and NO (Yoshimatsu et al. 1996). In some experiments it was found that NO kills malignant cells *in vitro*, but it

stimulates tumorogenecity and metastases *in vivo* (Edwards et al. 1996), which is in conflict with the other results.

There are data that not only activated macrophages but also vascular endothelial cells can produce NO either constitutively (Li et al. 1991) or together with smooth muscle cells upon induction by IFNγ and TNFα. In this way these cells can also participate in the antitumor defense. Thus, upon cultivation of erythroleukemic K562 cells together with endothelial and smooth muscle cells, these cytokines lead to NO production and death of the malignant cells (Geng et al. 1996).

An important effect of IFNγ in some tumors is the induction of the enzyme IDO, which leads to cell death due to tryptophan starvation (Werner et al. 1989; Taylor and Feng 1991; Leung et al. 1992) (see Section 3.2). Such a mechanism is supported by the fact that supply of tryptophan protects melanoma cells from the effect of IFNγ and TNFα (Wood et al. 1991). Tryptophan degradation produces 3-hydroxyantranylic acid (3-HAA), which is believed to have a cytotoxic effect (Werner et al. 1987; Figure 3.2).

Another antitumor effect of IFNγ seems to be related to the inhibition of oncogene expression (see Section 3.2). For example, overexpression of c-erbB-2 (Her-2/neu) in ovarian, mammary gland, and endometrial cancer (conditions with a poor prognosis [Esteller et al. 1995]) is suppressed by IFNγ and this leads to reduced cell proliferation (Marth et al. 1990).

Another tumor suppressor could also be the IFNγ-induced protein kinase PKR (p65 and p68 in mice and in humans, respectively), which is activated by dsRNA and inhibits protein synthesis by phosphorylating the eukaryotic initiation factor 2 (Meurs et al. 1993).

Thus, the antitumor activity of IFNγ is a result of the complex antiproliferative, immunostimulatory, and gene activity modulating effects of this cytokine. Evidently in different types of malignant cells, one or another activity may be dominant.

*chapter four*

# *Molecular mechanisms of interferon gamma activities. Transmembrane signal transduction*

As already pointed out, one of the important functions of IFNγ is its effects on gene activity. Before discussing the mechanisms underlying this activity, we should mention that gene activation is realized through a whole family of proteins called signal transducers and activators of transcription (STAT). The transcription-activating proteins should contain a specific basic amino acid sequence called the nuclear localization sequence (NLS) that is necessary for their entering the nucleus. IFNγ also contains such a sequence (RKRKRSR) at the C terminus, which makes this cytokine a "chaperone" for transporting protein complexes into the nucleus. This has been proven by experiments where a peptide corresponding to the C-terminal domain of IFNγ (containing an NLS) was covalently bound to the fluorescent protein R-phycoerythrin. This construct was then found in the nucleus of rabbit reticulocytes (Johnson et al. 1998).

Models of the signaling cascade triggered by IFNγ were recently proposed based on experimental data from the last few years (Bach et al. 1997; Johnson et al. 1998, and references therein). The molecular events considered by these models (JAK-STAT metabolic pathways) can be summarized as follows:

1. IFNγ binds to the extracellular domain of its receptor (IFNγRα) through its N-terminal part including amino acid residues 1 to 39. It has been demonstrated that truncation of this end and also a free peptide corresponding to this sequence prevent IFNγ binding to the receptor.
2. Binding of IFNγ to the α-chain of the receptor leads to mobilization of the β-chain (Greenlund et al. 1991) and to the formation of a receptor complex composed of one IFN dimer and two α- and two β-chains.
3. The receptor-IFNγ complex is internalized through endocytosis.

4. The C-terminal domain of IFNγ binds to the cytoplasmic part of the receptor (amino acid residues 252 to 291 of the α-chain). This region includes a leucine-isoleucine sequence (270 to 271) that is needed for the translocation of the protein complex in the cytoplasm and also the LPKS sequence (266 to 269), necessary for binding the tyrosine kinases JAK1 and JAK2. The NLS sequence (necessary for the transport of the whole complex into the nucleus) is localized in the IFNγ C-terminal domain. A peptide corresponding to the C-terminal part of IFNγ (95 to 133) which contains the NLS has been found to be biologically active, increasing severalfold the antiviral resistance of the cells (Szente et al. 1994a). If this model is correct (as it seems to be), the presence of an NLS in the C-terminal part of IFNγ makes doubtful the data that IFNγ remains active after deletion of more than 11 C-terminal amino acid residues (see Section 2.3.3).
5. Following the internalization of the IFNγ-receptor complex, the JAK1 and JAK2 kinases bind to the LPKS sequence. This binding is crucial for further activation of the subsequent cascade.
6. The bound JAK kinases are activated through phosphorylation (it is believed that this occurs through a mutual cross-phosphorylation when the two cytoplasmic ends of the associated α- and β-chains of the IFNγ receptor are in close proximity).
7. The activated JAK kinases phosphorylate Tyr-440 of the α-subunit, and this leads to the binding of two molecules of the transcription factor STAT-1 to the cytoplasmic domains of the two receptor chains. According to some data, STAT activated by IFNγ has a molecular mass of 91 kDa and is Tyr phosphorylated (Shuai et al. 1993).
8. The complex formation between IFNγ and the C-terminal part of its receptor leads to binding of the complex to the α-subunit of the protein importin followed by the subsequent binding of the β-subunit of the same protein. This transfers the whole complex in the nuclear pores. The transport of the complex through the nuclear pores is energy dependent. The energy is supplied by guansine triphosphate (GTP) hydrolyzed by the GTPase Ran.
9. Once in the nucleus, the complex disaggregates and the STAT-1 transcription factor is released and forms homodimers which are phosphorylated at the C-terminal Ser 723.
10. The activated STAT-1 homodimers bind to specific DNA sequences, gamma-activated sequences (GAS), inducing the corresponding gene. The specificity of the response is determined by these sequences and by the nature of the STAT protein.

This model explains why IFNγ introduced directly into the nucleus (see Chapter 2, Section 2.4) is active, losing its species specificity. Unlike the species-specific interaction between the extracellular part of the receptor and the N terminus of IFNγ, the interaction between its C terminus and the cytoplasmic part of the receptor turns out to be species nonspecific.

*chapter five*

# *Production of interferon gamma*

Human IFNγ, as well as the other interferons, can be prepared by two different approaches — from human cells after suitable induction and by recombinant DNA technology. The main difference between the two interferons — natural and recombinant — is that the first is glycosylated, whereas the second is not.

Production of natural IFNγ has the following disadvantages: low yield and the potential danger of contamination with oncogenic or viral DNA as well as with other pathogens. This requires additional methods of purification and control making the production process more expensive.

After it has been proven that the glycosylated and nonglycosylated IFNγ have the same biological activity (see Chapter 2, Section 2.3.1), IFNγ is now produced mainly by the methods of recombinant DNA technology (genetic engineering). Eukaryotic (Devos et al. 1982) as well as prokaryotic (Gray et al. 1982; Perez et al. 1990) cells may be employed as producers. The use of eukaryotic cells runs the risk of contamination with potentially dangerous viral and oncogenic DNA. That is why prokaryotic producers are preferred. The most widely used microorganism is *Escherichia coli* transformed with a plasmid containing the gene of IFNγ. The gene itself can be obtained by either reverse transcription from IFNγ mRNA or by a total chemical synthesis.

Usually the production cycle of human recombinant IFNγ includes the following steps:

1. Transformation of an appropriate strain with an expression plasmid containing the human IFNγ gene
2. Fermentation
3. Collection of the bacterial biomass
4. Disintegration of the transformed cells where rIFNγ is usually accumulated as inclusion bodies (Figure 5.1)
5. Disolving the inclusion bodies in a denaturing solution
6. Purification of IFNγ by chromatography (Figure 5.2)
7. Control of purity, activity, and identity

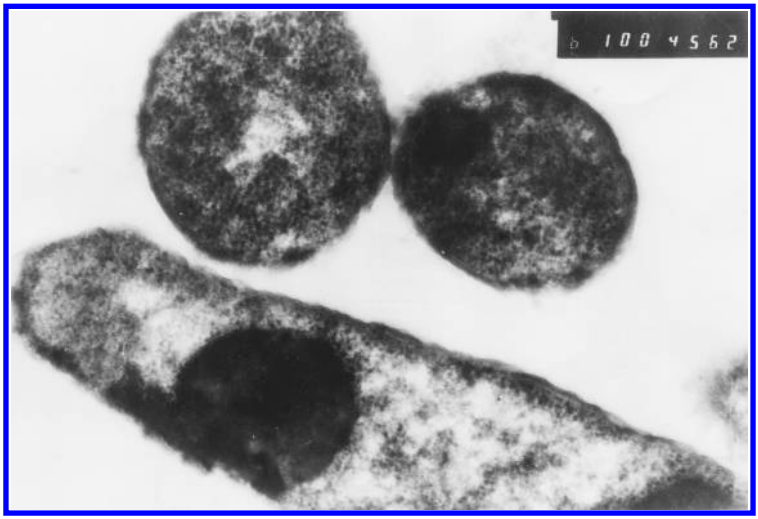


***Figure 5.1*** Electron micrographs of *E. coli* sections containing IFNγ stored as inclusion bodies (black spherical formations).

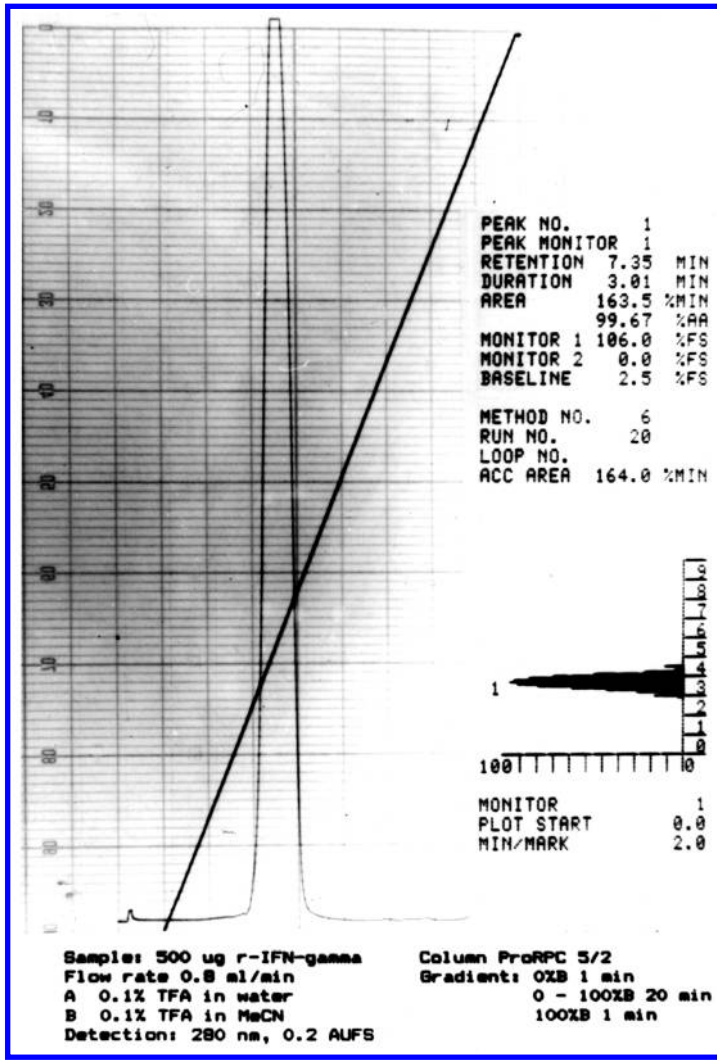


***Figure 5.2*** High-performance liquid chromatographic profile of purified IFNγ (Gammaferon) indicating a purity of 99.67%.

# *chapter six*

# *Interferon gamma preparations*

Several types of IFNγ with different structures are used in experimental studies and clinical trials: (1) natural IFNγ; (2) IFNγ with a conserved N-terminal methionine and/or with only the leader sequence Cys-Tyr-Cys; (3) IFNγ without the Cys-Tyr-Cys leader sequence; (4) IFNγ 1b with altered amino acid (aa) composition (composed of 140 aa instead of 143); (5) IFNγ with an artificial disulfide bridge in the monomer (see p. 15). We shall mention IFNγ preparations which are available on the pharmaceutical market or are used in clinical trials.

## *6.1 IFNγ-1b*

The technology for the production of IFNγ-1b was developed by Genentech, Inc. (460 Point San Bruno Blvd., South San Francisco, CA 94080). It is produced in *Escherichia coli* by recombinant DNA technology. Unlike natural IFNγ, it has 140 aa. It is highly purified and has a specific activity of $30 \times 10^6$ IU. IFNγ-1b is produced under the trade name Actimmune® by InterMune Pharmaceuticals, Inc. (3294 West Bayshore Road, Palo Alto, CA 94303). Boehringer Ingelheim (900 Ridgebury Road, Ridgefield, CT 06877) produces IFNγ-1b $ADN_r$ under the trade name Imukin®.

Actimmune is a clear solution in ampules of 0.5 ml containing 100 μg human rIFNγ-1b (i.e., $3 \times 10^6$ IU), 20 mg mannitol, 0.36 mg Na succinate, 0.05 mg polysorbate 20, and sterile water for injections.

Imukin is also a clear solution in ampoules of 0.5 ml containing 100 μg of human rIFNγ-1b $ADN_r$ ($2 \times 10^6$ IU) with excipient disodium succinate, polysorbate 20, and sterile water for injections.

## *6.2 Gammaferon®*

This is a recombinant human IFNγ composed of 143 aa and is devoid of the initial methionine (it appears that this is due to an enzyme activity of the

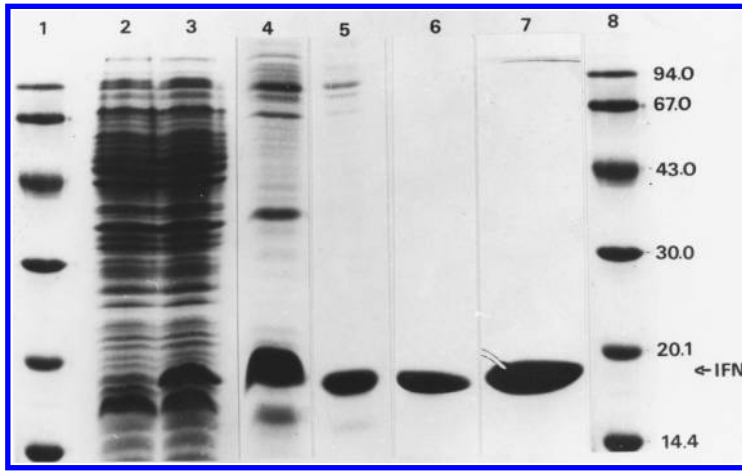


***Figure 6.1*** SDS-polyacrylamide electrophoresis of different protein fractions obtained during purification of IFNγ (example with Gammaferon). (1 and 2) Protein markers; (2) protein pattern of the *E. coli* strain; (3) protein pattern of the *E. coli* strain expressing IFNγ; (4) protein pattern of the inclusion bodies; (5, 6, and 7) protein pattern of the IFNγ preparation at three successive steps of chromatographic purification.

bacterial producer strain (Vassileva-Atanassova et al. 1999), and the Cys-Tyr-Cys signaling sequence. It is produced in *E. coli* (see Chapter 5, Figure 5.2) and the technology is protected by a European patent 0446582 B1/4.01.1995 for France, Germany, Italy, Great Britain, and Spain and patent 2054044/24.01.1998 for Russia.

The purity of Gammaferon is higher than 99.5% and its specific activity is $50 \times 10^6$ IU/mg (see Figure 6.1; Chapter 5, Figure 5.2).

So far, three pharmaceutical forms of Gammaferon have been developed and tested:

1. Virogel G5: a 4% hydrophilic gel (polyethylene glycol with a molecular mass of about $4 \times 10^6$, Badimol) containing $10^5$ IU/g (about 2 μg/g) Gammaferon, 0.2% dextran (Hemodex), 0.9% NaCl, pH 7.0 to 7.5. It is stable for 1 year at 4°C. It is used for topical application on the skin and mucous membranes. The advantage of the viscous gel is that it slows down the process of IFNγ aggregation. Besides this, according to some literature data polyethylene glycol facilitates the correct folding of the IFNγ molecule (Cleland et al. 1992).
2. Gammaferon-collyrium (eye drops): a vial containing lyophilized Gammaferon $5 \times 10^5$ IU (about 10 μg), dextran (Hemodex) 0.020 g, NaCl 0.045 g. To be dissolved in 5 ml apyrogenic bidistilled water. It is stable for at least 5 years in the lyophilized condition and for 10 days at 4°C after dissolving.
3. Gammaferon for parenteral application: an ampule containing $1 \times 10^6$ IU (about 20 μg) lyophilized Gammaferon, dextran (Hemodex)

0.040 g, NaCl 0.018 g. It is dissolved in 2 ml apyrogenous double-distilled water and is used for subcutaneous, intramuscular, and intravenous injections. Stable for at least 5 years in the lyophilized form and for 10 days at 4°C after reconstitution.

## 6.3 Others

Recombinant human IFNγ mainly for research purposes (as a chemical) is also available from the following companies: Chemicon, Endogen, ICN Biomedicals, PeproTech, Pharmingen, Roche, Sigma. (See http://www.biocompare.com/molbio.asp?catid/ and also listing below.)

As a rule, all IFNγ preparations should not be subjected to repeated freezing and thawing or to vigorous shaking (see p. 15).

Research Diagnostic, Inc.
Pleasant Hill Road
Flanders, NJ 07836

PBL Biomedical Laboratories
100 Jersey Avenue — Building D
New Brunswick, NJ 08901

Advanced Immuno Chemical, Inc.
105 Claremont
Long Beach, CA 90803

Pepro Tech EC, Ltd.
Princeton Business Park
5 Crescent Avenue
P.O. Box 275
Rocky Hill, NJ 08553

Pierce Endogen
P.O. Box 117
Rockford, IL 61105

Chemicon International
28820 Single Oak Drive
Temecula, CA 92590

Inter Mune
3280 Bayshore Boulevard
Brisbane, CA 94005

Boehringer Ingelheim
P.O. Box 368
900 Ridgebury Road
Ridgefield, CT 06877

*Part two*

# *Interferon gamma in pathological processes and its clinical application*

In this part we shall discuss the involvement of interferon gamma (IFNγ) in different pathological processes regardless of whether its role is or is not favorable and what are the results of its clinical application in different diseases.

*chapter seven*

# *Indications and contraindications*

Briefly, the indications for IFNγ clinical use are determined by its various activities — antiviral, antibacterial, and immunostimulatory and its modulatory effect on gene expression. This makes it a possible therapeutic agent in a number of diseases caused by intracellular and extracellular pathogens, cell malignization, and metabolic disorders associated with disturbed gene expression.

It is important to point out some contraindications. IFNγ should not be used during pregnancy due to the risk of damaging the fetus (Kato et al. 1990; Vassiliadis and Athanassakis 1992). It is also contraindicated in diseases where it plays a pathogenic role. At present, it is known that such diseases are multiple sclerosis (see Chapter 11, Section 11.3), Kaposi's sarcoma (see Chapter 12, Section 12.15.1), thrombocytopenia purpura (Boshkov et al. 1992), and possibly psoriasis (see Chapter 11, Section 11.4) as well as some lymphatic leukemias (see Chapter 12, Section 12.18.1) where the problem is not very clear. Due to suppression of erythropoiesis by IFNγ (see Chapter 9, Section 9.2), its use is not recommended in hemoglobinopathies (Miller et al. 1990).

# *chapter eight*

# *Routes of administration, pharmacokinetics, dosage*

Depending on the disease and the localization of the pathologic process, different routes of IFNγ administration are used: (1) parenteral, (2) by sonophoresis (phonophoresis), (3) topical, (4) by inhalation, and (5) as liposomes.

## *8.1 Parenteral*

The parenteral route of administration (subcutaneous, intramuscular, and intravenous) is used in malignancies and other systemic diseases, such as, for example, rheumatoid arthritis, chronic granulomatous disease, and systemic sclerosis. Intermittent injections as well as continuous infusion for several hours have been used. Taking into account the dynamics of the ligand-receptor complex, continuous and high-frequency infusions do not seem justified. The IFNγ-receptor complex is internalized (see Chapter 2, Section 2.4; and Chapter 4) and new functional receptors appear on the cell membrane after 4 to 12 hours. Therefore, administration of interferon before the appearance of new receptors would be useless. In clinical trials the intermittent administration has given better results than the continuous infusion (e.g., Rinehart et al. 1986; Machida et al. 1987; Quesada et al. 1987; Takaku et al. 1987; Kobayashi and Urabe 1988; Satake et al. 1993).

The pharmacokinetics of IFNγ according to many investigators are not very different from those of IFNα and β. The maximal concentration in the serum depends on the dose and the route of parenteral administration. It was found that in humans the serum concentration decreases according to a first-order exponential kinetics (Bocci et al. 1984; Furue et al. 1987). After intravenous administration its half-life is 120 min according to some investigators (Furue et al. 1987), and according to others it is 20 to 35 min (Erez et al. 1988; Will 1990). After subcutaneous administration the half-life was also found to be about 30 min (Yamasaki et al. 1995). After subcutaneous

administration of $0.5 \times 10^6$ to $8 \times 10^6$ IU/m²* of rIFNγ, other investigators observed a maximum level in the serum after 6 to 13 hours with an average level up to 17 ng/ml (Thompson et al. 1987). In other clinical studies the maximum serum concentrations were 1.0, 2.4, and 4.9 ng/ml following subcutaneous administration of 0.1, 0.25, and 0.5 mg/m², respectively (Digel et al. 1991).

As we have already mentioned (see Chapter 2, Section 2.3.1), natural IFNγ is glycosylated at positions 25 and 97. Experiments with rIFNγ lacking glycosylation on either one or both sites have shown that the unglycosylated IFNγ persisted longer in the blood than glycosylated recombinant forms. The natural product, however, turned out to have the longest half-life (Sareneva et al. 1993).

As far as dosage is concerned, we have to point out that many clinical trials attempt to reach the maximum tolerated dose (MTD). These doses varied within a wide range in different trials: from $10^6$ IU/m² (Kurzrock et al. 1985, 1986), $3 \times 10^6$ IU/m² (Schiller et al. 1987), $8 \times 10^6$ IU/m² (Thompson et al. 1987) to $75 \times 10^6$ IU/m² (Rinehart et al. 1987). Other investigators report an MTD of 0.5 to 1 mg/m² per day (Perez et al. 1988). High toxicity has been noted after intravenous administration of 0.05 and 0.5 mg/m² after intramuscular injection (Quesada et al. 1987). In other experiments the toxicity limited dose was found to be 15 mg/m² after intravenous infusion (Paulnock et al. 1989) and $8 \times 10^6$ IU/m² after subcutaneous injection (Thompson et al. 1987).

Concerning the dosage, we have to point out that the MTD is hardly the most suitable dose for IFNγ. Regardless of the high toxicity, experimental data show that this cytokine has an optimal concentration in order to manifest its biological activity, and therefore concentrations exceeding it lead to an opposite effect (Kleinerman et al. 1986). The lower doses are preferable, and this can be seen in the treatment of rheumatoid arthritis, which has also been confirmed by clinical trials with Gammaferon conducted in Bulgaria (see Chapter 11, Section 11.7). In general, the parenteral dosage depends on the disease and also on individual features, so that a dosage range only could be indicated for a particular disease. The great variety of doses is probably also due to the differences in the interferon preparations used. The differences may also depend on the fact that — as control tests have shown — in some preparations big differences are found between labeled and actually measured activity (Iyer et al. 1986).

It is of great interest whether parenterally administered IFNγ can reach tumors in the brain and in the lung. One experiment has shown that in a brain metastasis of kidney cancer, maximum concentration of subcutaneously injected IFNγ reached the tumor after 6 hours with a 30-min half-life in the serum (Yamasaki et al. 1995). It turns out that the blood/lung barrier (Jaffe et al. 1991; Halme et al. 1995) and the blood/pleural barrier (Douillard

* In oncological practice it is accepted as more accurate to base the dosage of cytostatics on body surface in square meters (m²). In most studies the dose of interferons is also expressed in the same way. There are nomograms which give the body surface of adults and of children separately from their weight and height.

et al. 1992) are difficult for IFNγ to penetrate (see Section 8.4; Chapter 12, Section 12.4).

## *8.2 Sonophoresis/phonophoresis*

A method that is not commonly used for local as well as for systemic administration of IFNγ is by the application of ultrasound (sonophoresis/phonophoresis), which can introduce different compounds through the pores of the skin by inducing cavitation. The use of a lower frequency than the therapeutic one (1 MHz) significantly increases the amount of the compound which is introduced (Mitragotri et al. 1995). However, it runs the risk of tissue damage. Encouraging results in the treatment of rheumatoid arthritis were obtained in Bulgaria when therapeutic frequency was used (see Chapter 11, Section 11.7).

## *8.3 Topical*

Topically, IFNγ is applied to the skin and mucous membranes in the form of a gel (see Sections 6.2 and 10.1) or of liposomes (Egbaria et al. 1990; du Plessis et al. 1992), which facilitates its penetration into the skin (see also Section 8.5); it is also applied to the eye as eye drops (see Sections 6.2 and 10.1.3). A solution of IFNγ is used for treatment of the oropharyngeal cavity. It is also administered directly into, for example, lesions (tumors, condylomas, papillomas), peritoneum, pleural cavity, and bladder.

The stability of IFNγ in unguents and gels is increased due to the high viscosity that decreases the rate of monomer aggregation inactivating the protein. The necessary concentration of IFNγ in the gels depends on the disease. Our experience shows that $10^5$ IU/g gel is enough for the successful treatment of herpetic skin infections (see Chapter 10, Section 10.1). The same concentration — $10^5$ IU/ml — is effective for viral eye infections. However, for the topical treatment of condylomata acuminata and cervical lesions, the concentration should be much higher — of the order of $10^6$/g gel.

IFNγ-containing liposomes bound to asafetuitin were better absorbed by liver cell lines (Ishihara et al. 1990) and were more effective than free interferon in inhibiting the replication of the hepatitis B virus (Ishihara et al. 1991).

IFNγ administered subcutaneously for treatment of malignant pleural effusions reached its maximum serum concentration of $10^2$ IU/ml after 4 hours, but a very small amount (1.14 IU/ml) penetrated into the pleural cavity after 24 hours. Upon intrapleural injection, high concentrations — 1984 IU/ml and 173 IU — were reached at the end of the infusion after 96 hours. In this case, a very small amount of INFγ (4.7 IU/ml) diffused into the serum. The conclusion is that the diffusion of IFNγ between the blood and the pleural cavity is very small (Douillard et al. 1992).

Taken orally, IFNγ cannot be absorbed through the stomach where it is subjected to proteolytic attack. However, its contact with the mucous membrane of the oropharyngeal cavity, especially after a longer contact,

leads to diffusion of the protein into the blood circulation. In experiments with mice the peroral application of IFNγ in aqueous solution of 0.1% gelatin led to a decrease of the blood leukocyte numbers proportional to the administered dose (Fleischmann et al. 1991). The perorally administered IFNα exhibited an antiviral effect in cats (Cummins et al. 1988) as well as in humans (Hutchinson and Cummins 1987; Koech et al. 1990), whereas IFNβ taken orally was ineffective (Witt et al. 1992). These data are interesting and important but they need confirmation, and the problem with peroral administration needs further investigation.

Our preliminary unofficial observations show increased resistance to respiratory viral infections after throat treatment of the oropharyngeal cavity with an IFNγ solution (about $3 \times 10^4$ IU/ml).

## 8.4 Inhalation

Inhalation formulation (aerosolic IFNγ) has recently been used for treatment of resistant forms of lung tuberculosis (Chatte et al. 1995; Condos et al. 1997) (see also Chapter 10, Section 10.2.1.1.1) and lung cancer (Kawata et al. 1994; Kessler et al. 1994; Yano et al. 1994; Halme et al. 1995; Mizutani et al. 1996, 1997) (see also Chapter 12, Section 12.4). It has been shown in humans that IFNγ inhaled as an aerosol effectively reaches the alveoli (Martin et al. 1993). In a mouse model it was found that aerosolic IFNγ only, but not parenterally administered, is active in lung allergic sensitization (Lack et al. 1994). Data that the parenterally administered IFNγ does not reach the alveoli and conversely — IFNγ inhaled in the lung does not reach the blood — have also been obtained in humans (Jaffe et al. 1991; Halme et al. 1995). This provides evidence for the existence of a barrier for IFNγ between the lung and the blood. After inhalation of 0.6 mg of IFNγ, it is found in the bronchoalveolar lavage after 3 hours (Halme et al. 1995). In contradiction to the existence of a hematoalveolar barrier are data showing that after intramuscular injection, as well as after inhalation, IFNγ in the blood increases five to ten times (Sokolova et al. 1993).

It has been shown in mice that aerosolic IFNγ increases the cytotoxicity of the alveolar macrophages (Eisenberg et al. 1991), decreases the production of IgE, and normalizes the function of the airways (Lack et al. 1994). Also, aerosolic IFNγ increased production of NO in rats (Pendino et al. 1993). More effective stimulation of the 2′-5′-oligoadenylate synthetase was found in humans following aerosolic IFNγ as compared to its intramuscular administration (Sokolova et al. 1993).

A disadvantage of the aerosolic form of IFNγ is the possibility for denaturation of the interferon exposed to the action of the surface tension at the solution/air boundary. In addition, the strong mechanical stress for the aerosol production adds to the denaturation effect. The inactivation of IFNγ by this procedure is confirmed by experimental data showing that during aerosolization only 0.4% of the initial activity of IFNγ remains in the aerosol. The addition of small liposomes increased this value to 27.7% (Kanaoka et al. 1999).

In order for the droplets of the aerosol to reach the alveoli, their size should not be larger than 5 μm. Thus, a huge surface is created and a large protein concentration is needed to ensure therapeutic activity. This makes necessary the administration of large doses, which makes the treatment considerably more expensive. Calculations show that if the diameter of the droplet is 5 μm and the surface occupied by one molecule of interferon is about 20 $nm^2$ (Ealick et al. 1991), a concentration larger than 3.4 mg/ml is needed to keep some interferon in the droplet unexposed to the surface tension. This disadvantage could be avoided by using an aerosol of liposomal IFNγ (Goldbach et al. 1996) or, as mentioned above, by addition of liposomes. The effect of surface tension on IFNγ activity needs further experimental investigation. This is well justified, because the administration of drugs via the respiratory tract is considered to be especially promising (Byron and Patton 1994).

## *8.5 Liposomes*

The application of IFNγ encapsulated in liposomes leads to increased antiviral, antibacterial, and tumoricidal activities of the macrophages as compared to the effect of free IFNγ (Kleinerman et al. 1985; Koff et al. 1985, 1994; Sone et al. 1986b; Stukart et al. 1987; Philips et al. 1988, 1989; Rutenfranz et al. 1990; Fidler 1992; Saravolac et al. 1996) and a stronger stimulatory effect on the natural killer (NK) cells (Rutenfranz et al. 1990).

In mice intravenously administered liposomal IFNγ has also activated the alveolar macrophages, which shows that in this form it could penetrate the hematopulmonary barrier. The liposomal IFNγ also penetrates the skin more easily, as shown in experiments with nude mice (Short et al. 1996) and humans (Short et al. 1995).

The macrophage-activating effect of liposomal IFNγ was increased severalfold in combination with muramyl dipeptides or tripeptides and acyltripeptides, which act synergistically with IFNγ (Sone et al. 1986a; Utsugi et al. 1986, 1988; Stukart et al. 1987; Fidler et al. 1989; Philips et al. 1989; Melissen et al. 1994; Goldbach et al. 1996).

The combination of IFNγ and muramyl peptides very strongly increased the antimicrobial resistance in mice, and this could also be used in humans to decrease opportunistic infections after transplantations (Ten Hagen et al. 1995).

It should be taken into consideration that the lipid composition of the liposomes affects the antiviral and antiproliferative activity of the included IFNγ (Smith et al. 1990; Yoshimura and Sone 1990; Anderson et al. 1994). Negatively charged liposomes composed of phosphatidylic acid or of phosphatidylserine inhibit the induction of NO production by IFNγ. Such a negative effect is not observed in liposomes composed of phosphatidylcholine (Aramaki et al. 1996). Negatively charged phosphatidylcholin liposomes strongly stimulate the secretion of IFNγ as compared to neutral liposomes (Aramaki et al. 1995).

## 8.6 Other methods of delivery

Recently IFNγ use has been associated with biodegradable polymers, such as lamellar particles of poly-L-lactides and poly-DL-lactyl-coglycolides (Venkataprasad et al. 1999), microspheres of polylacto-coglycolic acid (Yang and Cleland 1997), and polyvinyl-pyrolydon complexes (Mendiratta et al. 1999). This approach deserves further study and development.

Another delivery method is the use of adenoviral or retroviral recombinant vectors that express IFNγ or cytokines (Stoeckle et al. 1996; Xu et al. 1997; Karavodin et al. 1998; Osaki et al. 1999).

# *chapter nine*

# *Side effects*

The clinical use of IFNγ started in 1983. Even the early experiments showed that its parenteral administration has some side effects similar to those of IFNα/β (e.g., see Gutterman et al. 1984; Bottomley and Toj 1985; Kurzrock et al. 1986; Vadhan-Raj et al. 1986a,b; Reynolds et al. 1987; Schiller et al. 1987; Kobayashi and Urabe 1988).

No side effects were observed after local application of interferons in the form of a gel or unguent. The same holds true for inhalation of IFNγ as an aerosol, although experience with this sort of treatment is very limited.

Briefly, the side effects can be summarized as follows:

## *9.1 Frequent side effects*

With low and moderate doses, fever with a moderate elevation of body temperature, fatigue, headache, myalgia, sweating, loss of appetite, nausea, diarrhea, and leukopenia (after long-term treatment) are frequent side effects.

## *9.2 Rare and very rare side effects*

These include local erythema, local lymphadenopathy, cardiovascular disturbances (hypotonia, arrhythmia, heart weakness); neuropsychological and neurophysiological disturbances (Farkkila et al. 1988; Born et al. 1989); respiratory disturbances, alopecia, and disturbed liver and kidney function (Tashiro et al. 1996); even acute kidney insufficiency 19 days after treatment with IFNγ for acute lymphogenous leukemia (ALL; Ault et al. 1992). It is believed that some cases of glomerulonephritis may be due to increased secretion of interleukin-1 (IL-1) induced by lipopolysaccharide (LPS) and additionally increased by IFNγ (Newton et al. 1985; Kawasaki et al. 1993).

In very rare cases bone marrow hypoplasia and aplasia are observed during treatment of chronic myelogenous leukemia (Talpaz et al. 1992). Also, the appearance of antierythrocyte antibodies (Perez et al. 1991) and suppression of erythropoiesis (Mamus et al. 1985; Raefsky et al. 1985, Means et al. 1994) have been observed. IFNγ suppresses erythropoiesis by inhibiting proliferation and inducing the Fas receptor, which leads to apoptosis of the hematopoietic precursors (Sato et al. 1997).

In general, in myeloproliferative diseases development of antinuclear antibodies and rarely of systemic lupus erythematosus (SLE) are observed after long-term IFN application (IFNα with low doses of IFNγ) (Wandl et al. 1992a). There is high toxicity when IFNγ is combined with tumor necrosis factor-α (TNFα) (e.g., see Smith et al. 1991). When IFNγ is combined with IL-2, the toxicity was not higher than that of IL-2 alone (Viens et al. 1992).

Long-term subcutaneous or intramuscular administration of IFNγ may cause necrotic vasculitis with nonhealing wounds (Krainick et al. 1998).

All this requires that the parenteral application of IFNγ (as well as the other interferons) be performed in a clinical setting where possible cardiovascular disturbances can be treated. Even though they are very rare, such disturbances could occur due to hypersensitivity to interferons, especially with higher doses and repeated injections.

It is recommended to check the state of the kidney and liver functions.

Upon treatment with recombinant IFNγ, induction of antibodies has not been observed (Liang et al. 1985; Jaffe et al. 1987; Dummer et al. 1991). However, there are data that antibodies against natural IFNγ are present in the serum of patients with viral infections and in some cases those with active tuberculosis (Einhorn and Grander 1996) as well as in healthy individuals, but with a lower titer (Turano and Caruso 1993). It is interesting to note that in women with antispermal antibodies an elevated level of IFNγ has been found, and it has been suggested that this could be a factor for infertility (Witkin and Chandby 1989).

When parenteral administration of interferon is used, it is necessary that the physician and the patient sign a declaration that they are aware of the possible side effects.

# chapter ten

# Infectious diseases

## 10.1 Viral infections

The antiviral effect of interferon gamma (IFNγ) is very well manifested in a number of viral infections such as herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2, respectively) (see Section 10.1.1); herpes zoster (see Section 10.1.2); viral infections of the eye (see Section 10.1.3); condylomata accuminata, common warts; and human papillomaviruses (HPV) (see Section 10.1.5). Other viruses sensitive to IFNγ are the encephalomyocarditis virus, the influenza virus (see Section 10.1.4), the meningovirus, the vesicular stomatitis virus, enterovirus 70, CVA 24, the hepatitis A virus (see Section 10.1.7), rhinoviruses, cytomegalovirus (CMV) (see Chapter 11, Section 11.2), human immunodeficiency (HIV) (see Section 10.1.6), adenoviruses (see Section 10.1.3), and others. The antiviral effect is accomplished by activated T helper 1 (Th1) lymphocytes, where the interaction between the virus-specific CD4+ and the CD8+ helper lymphocytes is of importance especially for persistent infections (Berger et al. 2000).

### 10.1.1 Herpes simplex virus type 1 and type 2 (herpes genitalis)

These viral infections are extremely frequent and show a tendency to recurrences. It has been reported that herpes labialis (HSV-1) affects almost 50% of the population of the United States and more than 25% of the infected individuals often have severe recurrent infections (U.S. Department of Health 1980). Every year 19 million people are infected in France. In Bulgaria (Karagyozov et al. 1989) genital herpes is presently manifested as an epidemic sexually transmitted disease. The problem with genital herpes (and with herpes 1 infections) is the high frequency of recurrences, which are due to a latent form of the virus in the sensory neural ganglia. These patients usually have insufficiencies in their cellular immunity. On average genital herpes can recur four times a year in 80 to 90% of patients who have been infected once (Corey et al. 1983).

The best antiviral drug, approved for oral, systemic, and local application, is the nucleoside Acyclovir (Zovirax). It can decrease the number of

recurrences if it is constantly taken per os. Discontinuation of the prophylactic daily intake increases the number of recurrent infections (Straus et al. 1984, 1986). Applied topically, Acyclovir suppresses viral replication and accelerates healing, but it does not prevent recurrrences (see, e.g., Corey et al. 1982; Bryson et al. 1983).

It has been concluded that Acyclovir "is not curative, and the high cost of treatment, as well as the unknown toxicity with long-term administration make it a less than satisfactory solution to the problem" (Whittington et al. 1984; Mandelson et al. 1986). Nevertheless, Acyclovir is widely used, which is an indication of how widespread these infections are. For example, in France, the annual sales of this medication amount to 771 million Fr, and in 1991, Acyclovir sales in the world were estimated to 4 billion Fr. (around U.S. 600 million).

The need of continuous intake of the drug, its high price, and its toxicity demanded the search for new therapeutic agents. The first area of research was aimed at the interferons.

Experimental data show the inhibitory effect of all three types of interferon on the replication of the herpes simplex virus and the induction of viral resistance in the treated cells (Zawatsky et al. 1982; Chatterjee et al. 1985; Overall et al. 1985; Kawaguchi et al. 1986; Arvin et al. 1992). Replication of HSV-1 in mouse macrophages is suppressed by the interferons (Domke et al. 1985). The sensitivity of 19 herpesvirus strains to interferons was tested, and it was found that each strain had a characteristic sensitivity to each of the three types of interferon (Arao et al. 1987).

Experiments with knockout mice (with a damaged, not functional IFNγ gene) have shown that during infection of the central nervous system with HSV-1 IFNγ protects the neurons from the destructive encephalitis caused by this virus (Geiger et al. 1995a, 1997; Dalton et al. 1997). These knockout mice are more sensitive to skin infections caused by HSV-1 (Yu et al. 1996b). It was also shown in normal mice that the elimination of skin infections caused by HSV-1 depends on IFNγ (Smith et al. 1994). In mice with the knocked out IFNγ gene there is an increased replication of the virus in the ganglia of the trigeminus (Cantin et al. 1995). IFNγ protects newborn mice from lethal HSV-1 infection by activating the antibody-dependent toxicity of the macrophages and the production of superoxides (Kohl et al. 1989). Blocking IFNγ with antibodies prior to infection of mice with the herpes virus increased mortality (89% compared to 37% in the controls). The antibody had the strongest effect if the interferon was blocked during the first 24 hours of infection (Stanton et al. 1987). The role of the immune response and the synthesis of IFNγ in the elimination of herpes infection (HSV-1) also is revealed by the role of physiological stress which suppresses this immune reaction (Bonneau et al. 1997).

In 30% of individuals with frequent HSV-1 infections defective production of IFNγ is found when isolated macrophages are induced with the virus *in vitro* (Klieman et al. 1985). IFNγ does not play a role in the reactivation of the virus, but it contributes to its rapid suppression when it is reactivated (Cantin et al. 1999a).

In neonates a lack of response to IFNγ was observed with an enhanced cytotoxicity of the natural killer (NK) cells toward HSV under the effect of recombinant IFN (Oh et al. 1986). Many patients react to HSV-1 infections with an increased level of serum IFNγ (Yamamoto et al. 1993), which is probably a protective reaction.

It is interesting that HSV-1 mortality in mice is greater after inactivation of the IFNγ receptor gene than after inactivation of the IFNγ gene itself. This suggests the presence of an alternative ligand for the IFNγ receptor which ensures antiviral protection (Cantin et al. 1999b).

For genital herpes (HSV-2) in mice, it was also shown that the virus was sensitive to IFNγ, which also provided viral resistance (Pinto et al. 1990; Parr and Parr 1999), and its neurovirulence was due to defective production of IFNγ (Lewandowski et al. 1998). Again in experiments with mice it was found that IFNγ helped to eliminate the infection of the genital tract with HSV-2 by stimulating the CD4+ and CD8+ T cytotoxic lymphocytes (Milligan and Bernstein 1997). It appears, however, that this is not the most important mechanism and that the macrophages stimulated by IFNγ play the major role (Seid et al. 1986). SCID mice (which lack functional B and T lymphocytes) infected with herpes virus and cytomegalovirus develop macrophage infiltrates as normal mice (Heise and Virgin 1995). Both IFNγ and tumor necrosis factor α (TNFα) play a major role in macrophage activation independently of the B and T cells. Human blood monocytes activated *in vitro* with liposomal IFNγ kill cells infected with HSV-2 without affecting the noninfected cells (Koff et al. 1984). There are data that the suppression of viral replication by IFNγ is connected with the induction of nitric oxide (NO) in the macrophages (Croen et al. 1993; Karupiah et al. 1993; Komatsu et al. 1996; Baskin et al. 1997; Paludan et al. 1997). Despite these data it is believed that the mechanisms of the antiviral activity of interferons are not completely elucidated.

It has to be considered that in order to avoid the immune system HSV-1 expresses an early protein, ICP47, which blocks the presentation of viral peptides by cells with major histocompatibility (MHC) class I antigen. This protein binds to TAP (transporter for antigen presentation) and interferes with the translocation of peptides within the endoplasmic reticulum (Hill et al. 1995).

It is known that the interferons increase the expression of MHC I. Besides this, both IFNα and -γ inhibit the early protein ICP4 of the IE3 gene but through different mechanisms. IFNγ inhibits it at the level of translation, whereas IFNα inhibits it at the level of transcription. Thus, inhibition occurs at the early stages of infection, but late genes are also affected (Klotzbucher et al. 1990).

Some data show that the activity of the transactivating protein, VP16, or a complex of it with the host protein(s), is attenuated by the interferons, whereas the adsorption, penetration, uncoating, and transport of viral DNA are not affected (De Stasio et al. 1990).

Despite the antiviral activity of all three types of interferon, comparative experiments show that they differ in their inhibitory effect on herpes virus

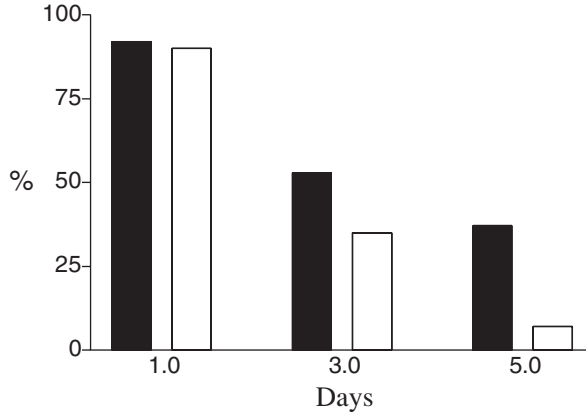


***Figure 10.1*** Involution of the vesicles in herpes simplex 1 infection treated with Virogel G5 (□) and placebo (■).

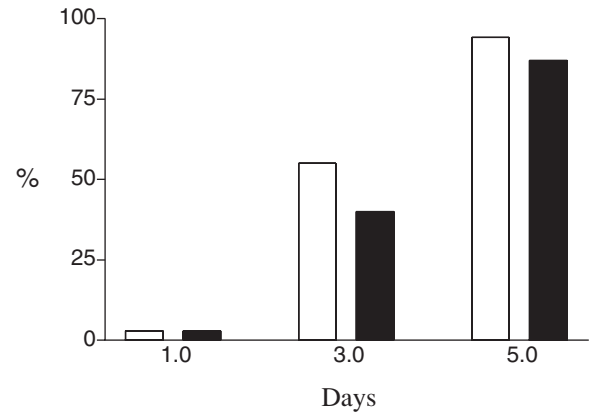


***Figure 10.2*** Course of epithelization of patients with herpes simplex 1 infection treated with Virogel G5 (□) and placebo (■).

replication. In cultured human corneal cells the interferons are arranged in the following way based on their antiviral activity: IFNβ>rIFNγ>IFNα (lymphoblast)>rIFNα2a>rIFNαA/D and in monkey kidney cells Vero: rIFNγ>IFNβ=IFNα (lymphoblast) > rIFNαA/D > rIFNα2a (Taylor et al. 1998). In human WISH and Hep-2 cells it has also been found that IFNγ inhibits to a greater extent than IFNα. An antiviral effect of IFNγ is not found in human T cells infected with HSV-1 (Thiele and Kirchner 1988). The synergistic antiviral effect of the combined application of IFNα and IFNγ has been shown in many studies. All of these data are in contradiction with the popular belief that IFNγ has a lower antiviral activity than IFNα.

Several clinical trials have been conducted with different interferons — natural leukocyte IFNα (intramuscularly) (Levin et al. 1982; Pazin et al. 1987),

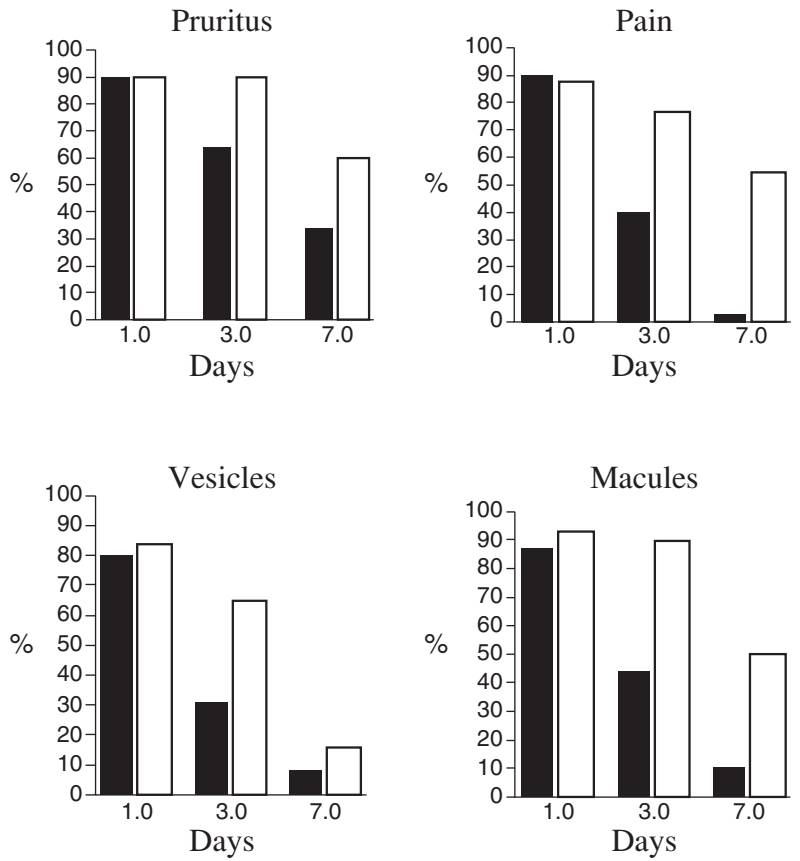


***Figure 10.3*** Evolution of the symptoms of patients with *H. simplex* 2 (*H. genitalis*) treated with IFNγ gel (Virogel G5) (■) and with placebo (□) (% of patients with the corresponding symptom).

recombinant IFNα-2a and 2b (subcutaneously) (Eron et al. 1986; Kuhls et al. 1986; Mandelson et al. 1986; Lassus et al. 1987). The results of these trials revealed different antiviral activities and various side effects. Contradictory results were obtained with topical application of IFNα (Rapp and Wroz 1985; Batcheler et al. 1986; Friedman-Kien et al. 1986; Eron et al. 1987; Glezerman et al. 1988; Sacks et al. 1990). There are reports for topical applications of IFNγ locally in recurrent infections with HSV (Torseth et al. 1986).

Trials conducted in Bulgaria in different clinics as well as with outpatients show a curative effect of the treatment of HSV infections with Gammaferon gel (Virogel G5) applied topically (Figures 10.1 to 10.3; Table 10.2). As can be seen in Table 10.2, in 63% of all cases the effect was assessed as very good and in 37% as good. Lack of effect was not observed. In outpatients, however, in some rare cases, mostly upon late treatment, a lack of effect was observed.

***Table 10.1*** Effect of Virogel G5 on the time for disappearance of the corresponding symptom in herpes zoster infection (average data)

| Symptom | Virogel G5 (days) | Number of patients | Common treatment (days) | Number of patients |
|---|---|---|---|---|
| Pain relief | 3.9 | 29 | 13.6 | 22 |
| Crust formation | 3.8 | 30 | 12.3 | 30 |
| Epithelization | 6.8 | 30 | 15.6 | 30 |

***Table 10.2*** Effect of Virogel G5 and placebo on herpes simplex I virus infection (average data)

| Symptoms | Virogel G5 (days) | | | Placebo (days) |
|---|---|---|---|---|
| Erythema disappearance | 2.16 | 2.16 | — | 2.8 |
| Fresh vesicles | 1.17 | 2.3 | — | 2.0 |
| Crust formation | 1.64 | 2.0 | — | 2.3 |
| Crusts only | 2.80 | 4.5 | — | 5.2 |
| Number of patients | 17 | 10 | 0 | 30 |
| % of patients | 63 | 37 | 0 | 30 |
| Evaluation of effect | Very good | Good | Absent | — |

In infections with genital herpes both natural and recombinant IFNα have been applied topically as a cream (20,000 IU/g) (Ghyka et al. 1989) or as parenteral injections ($5 \times 10^6$ IU of IFNα2 once daily for 5 days followed by $10^6$ IU three times a week for 3 months) (Mendelson et al. 1986). After topical application a reduction in the healing time was noted, especially if applied during the first 2 to 3 days of the infection. No effect of parenteral application was observed in women, whereas in men the recovery period and shedding of the virus in recurrent infections were accelerated.

The effect of the topical application of Gammaferon gel (Virogel G5) on genital herpes infections in clinical trials as compared to placebo is shown in Figure 10.3. A significant pain relief and reduced clinical symptoms were found as compared to the placebo.

### *10.1.2 Herpes zoster*

The infection caused by the varicella-zoster virus (VZV) is believed to occur when the cellular immunity is impaired or after trauma. In people with latent VZV a suppressed immune function was found during the early phases of the infection upon its reactivation. The late phases of healing are accompanied by the reestablishment of a normal immune function (Saibara et al. 1993b).

VZV infects more than 90% of the population by the age of 15 and affects annually up to 300,000 people in the United States. The incidence of the infection varies in different age groups — from 0.3 to 0.6% in children, 1.6% in adults under the age of 40 years, and 10% in adults of 80 years or older.

Among the different complications the most common are neurological, especially postherpetic neuralgia, which according to some investigators occurs in 50% of elderly patients over the age of 60 years and may become a chronic pain syndrome (Lee and Annunziato 1998).

The role of the interferons in this infection is revealed by the fact that mice lacking interferon receptors have a strongly decreased resistance to vaccinia virus with a large shift of the IgG distribution to IgG1 (van den Broek et al. 1997), which suggests a shift to Th2 profile (see Chapter 2).

There are data that VZV is more sensitive to the antiviral activity of all three types of interferon. In comparative studies some investigators find that IFNα and IFNβ are more active than IFNγ (Balachandra et al. 1994). The inhibition of VZV infection occurs through induction of NO synthase leading to increased synthesis of NO, which does not affect early proteins, but inhibits the synthesis of late viral proteins, viral replication, and the formation of viral particles (Harris et al. 1995). There are mutant strains of the virus that are resistant to Acyclovir, phosphonoacetic acid, and bromodeoxyuridine but are sensitive to IFNβ, which has a significant effect before the synthesis of the early viral proteins (Balachandra et al. 1994). For the application of different chemical compounds see elsewhere (Lee and Annunziato et al. 1998).

Effective treatment with a combination of acyclovir and leukocyte interferon has been reported (Baba et al. 1984). In several cases patients with immune deficiency with heavy VZV infections recovered rapidly upon treatment with IFNα (Levin 1984). There is a report that three cancer patients were cured from VZV infection after subcutaneous administration of IFNγ (Kabayashi and Urabe 1988). The effect of this cytokine on postherpetic neuralgia has also been reported (Usuki et al. 1988).

The results of clinical trials conducted in three Bulgarian clinics* with patients 20 to 77 years old show a strong healing effect after topical application of Gammaferon-gel (Virogel G5). The results of one trial are shown in Table 10.1. The distribution of patients according to days necessary for an improvement of the clinical symptoms is shown in Figure 10.4.

Following treatment with Virogel G5, postherapeutic neuralgia has not been observed even in elderly patients. Our conclusion is that the local application of IFNγ in the form of a gel is an effective, harmless, and relatively inexpensive treatment for this serious infection.

### *10.1.3 Herpetic and adenoviral eye infections*

The antiviral activity of interferons supports their application for treatment of viral infections of the eye.

* National Institute of Dermatology and Venerology, Sofia (Prof. Dr. A. Durmishev, Prof. Dr. C. Berova, Dr. A. Nikolova); University Clinic of Gynaecology and Obstetrics, Sofia (Prof. Karagjozov et al.); Medical University, Department of Dermatology, Plovdiv (Prof. Dr. J.K. Savova, Assoc. Prof. E.S. Teodossieva, Dr. R.K. Yankov).

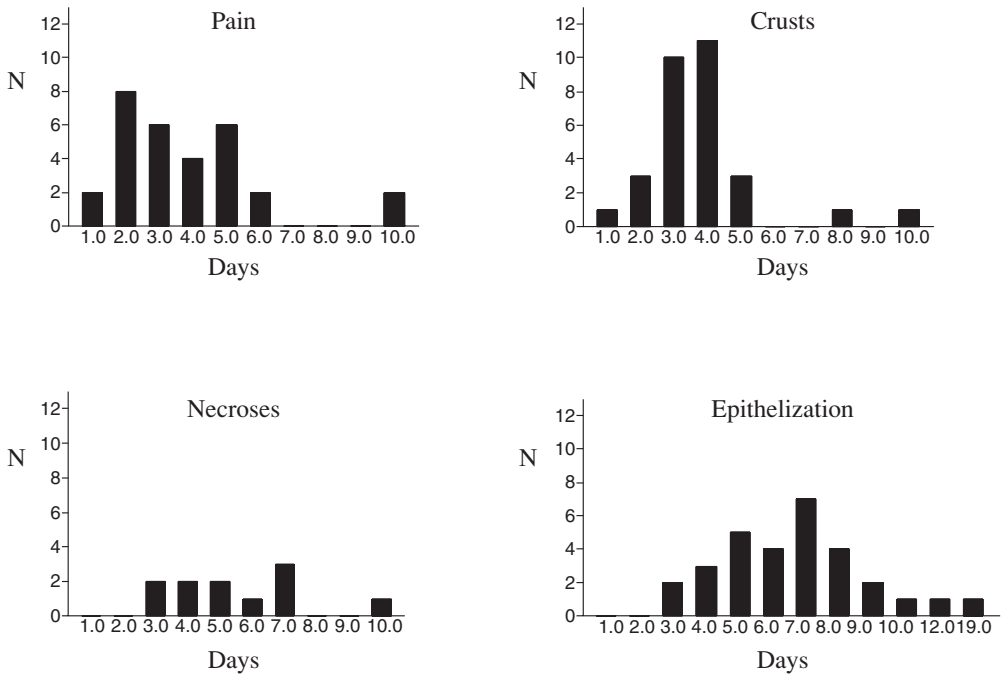


***Figure 10.4*** Distribution of 30 patients with *H. zoster* according to the symptoms during treatment with IFNγ gel (Virogel G5). N — number of patients.

This was first confirmed by experimental data in mice. Intraocular infection of mice with HSV-1 leads to bilateral retinitis. This infection is prevented by IFNγ which turns out to be independent of MHC class I or class II expression (Geiger et al. 1995b).

Transgenic mice which express the IFNγ gene in their photoreceptor cells can survive a HSV-2 infection in both eyes, in the optic nerve, and in the brain, an infection which is lethal to the control mice (Geiger et al. 1995a). The survival correlates with a considerably smaller number of apoptotic neurons in the central nervous system (CNS) which is due to the induction of the antiapoptotic proto-oncogene Bcl-2 in the brain of the transgenic mice.

Knockout mice with an inactivated IFNγ gene develop stromal keratitis after infection with HSV-1. The infection develops in the same way as in control mice, but the virus is retained for a longer period in the cornea and the mice are much more susceptible to encephalitis due to a reactivity shift from Th1 to Th2 phenotype (Bouley et al. 1995). It was shown that the combination of IFNα and IFNγ suppressed the replication of the virus in infected epithelial and fibroblastic cells of the cornea (Neumann-Haefelin et al. 1985; Balish et al. 1992; Chen et al. 1994), which was found to be connected with the induction of IFNβ (Chen et al. 1994). After an HSV-1 eye infection, the virus remains in a latent state in the ganglia of the trigeminus (Halford et al. 1996). IFNγ plays an important role for elimination of the virus from the cornea and the trigeminus (He et al. 1999; Leib et al. 1999).

A study of tear cytokines in spring conjunctivitis has shown that this allergic reaction is associated with elevated concentrations of Th2 cytokines, which play a major pathophysiological role in this reaction (Leonardi et al. 1999; Uchio et al. 2000). This justifies the application of Th1 cytokines, such as IFNγ.

Attempts to treat eye infections with local application of interferons gave varying results. We have found few literature data on the use of IFNγ for treatment of viral infections of the eye in humans. Contrary to experimental data on mice that the inflammatory processes in stromal keratitis caused by HSV-1 are due to Th1 cytokines (IFNγ and IL-2) (Hendricks 1997; Tang et al. 1997), in practice IFNγ shows a good healing effect (see below), which is in agreement with the experimental data described above. In the African green monkey it has been shown that human IFNγ applied locally in concentrations $> 3 \times 10^5$ IU/ml prevents the development of HSV-1–induced keratitis (Neumann-Haefelin et al. 1985). Early experiments with keratitis dendritica showed a similar healing effect of IFNγ and IFNα. However, very high doses were used ($30 \times 10^6$ IU/ml) upon interferon monotherapy in combination with trifluorothymidine or $1.5 \times 10^6$ IU/ml of each of the two interferons when used in combination (Sundmacher et al. 1987).

Because of the scarce literature data on the use of IFNγ for treatment of eye infections in humans, we will present several cases from the clinical trials conducted in Bulgaria (Table 10.3).

***Table 10.3*** Effect of Gammaferon on viral eye infections

| Before Gammaferon | | | After Gammaferon | | |
|---|---|---|---|---|---|
| Diagnosis | Duration (days) | Treatment | Subjective improvement (days) | Objective improvement (days) | Complete healing (days) |
| Herpes simplex | | | | | |
| Keratitis dendritica | 2–3 | — | 1–2 | 2–3 | 6–7 |
| Keratitis geographica | 7–9 | Corticosteroids | 3–4 | — | 11–12 |
| Keratitis stromatica | 11 | Corticosteroids; solkoseril | 2 | 4 | 16 |
| Kerato uveitis | 7–16 | Acyclovir; keracid | 2 | 3–4 | 10–15 |
| Keratitis trophica (metaherpes) | 7 | Acyclovir; keracid | 2 | No effect | No effect |
| Herpes zoster | | | | | |
| Blepharoconjunctivitis | 2 | — | 2 | 3 | 6 |
| Keratoiritis | 3–5 | Corticosteroids | 2 | 2–3 | 9–11 |
| Adenoviruses | | | | | |
| Conjunctivitis | 2–4 | Sulfonamids | 1 | 1 | 4–5 |
| Keratoconjunctivitis | 6–12 | Depresolon; Ophtalmoseptonex | 1–2 | 2–4 | 6–11 |

Similar results were also obtained in the other two hospitals. We should draw attention to the curative effect of Gammaferon at a 300 times lower concentration ($10^5$ IU/ml) as compared to the doses cited above.

The effect of Gammaferon is especially outstanding in adenoviral infections where the ophtalmological practice has limited capacities. Adenoviruses are believed to be relatively insensitive to interferons (Tiemessen and Kidd 1993). However, it has been shown that they are sensitive to recombinant IFNγ (Mistchenko and Falcoff 1987; Mistchenko et al. 1987).

In mice, IFNγ eliminated the virus from infected liver cells by activating the antigen-specific cytotoxic T lymphocytes (CTL) and increasing the expression of MHC class I molecules on target cells (Yang et al. 1995). Some data show that the suppression of adenoviral replication is due to a blockade of protein assembly and therefore a blockade of viral maturation (Mayer et al. 1992).

The conclusion from the clinical trials is that "Gammaferon eye drops" are well tolerated, and no side effects have been observed after their local application. A very good therapeutic effect was obtained in viral eye infections caused by HSV, herpes zoster virus, and adenoviruses. There was no effect in trophic herpes keratitis (metaherpes), where the pathogenesis does not justify the application of IFNγ.

Based on these results, Gammaferon-collyrium was approved for production and for use in the ophthalmological practice in Bulgaria.

### *10.1.4 Respiratory viral infections*

The epidemic incidence of respiratory viral infections (including influenza) affects a large percentage of the population and its economical and social importance is well known. IFNα and IFNβ have been used with varying success for prophylactics and treatment of such infections.

The use of interferons is justified by their antiviral activity and by experimental data on animals. IFNγ considerably inhibits the replication of a number of viruses, including the influenza virus (Figure 10.5) (Horisberger and Zeller 1987; Komatsu et al. 1996).

Mice with a knocked out IFNγ gene show a considerably decreased survival rate after infection with the influenza virus (Bot et al. 1998). The most recent data show that IFNα, IFNβ, and IL-18 (an IFNγ-inducing factor) produced by macrophages during viral infections induce IFNγ and thus play an important role for the establishment of a protective Th1-type immune response (Bot et al. 1998; Sareneva et al. 1998). The influenza virus induces the production of IFNγ by both T and non-T cells of the respiratory tract (Baumgarth and Kelso 1996). The IFNγ-producing T cells in the nose participate in a specific acceleration of the recovery from influenza infections (Tamura et al. 1996). Liposome-included IFNγ showed a twofold stronger macrophage-stimulating effect than free IFN (Saravolac et al. 1996). When administered intranasally in mice infected with the influenza virus, liposome-included IFNγ protected 70% of the animals as compared to 20% for

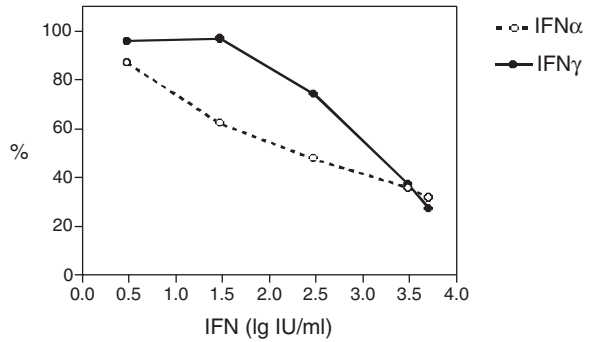


***Figure 10.5*** Inhibition of protein synthesis of the influenza virus by IFNγ end IFNα. (According to data by Horisberger and Zeller 1987.) Ordinates — % of synthesized viral protein.

the free IFNγ. Also during influenza virus infection, IFNγ induces NO synthesis. However, some data indicate that in mice the nitric oxide, by interacting with oxygen radicals, may be the reason for the development of viral pneumonia (Akaike et al. 1996).

There are no data in the literature concerning the use of IFNγ for prophylaxis and treatment of respiratory viral infections in humans. Our unofficial observations show the possibility of prophylaxis (and to a certain extent for a healing effect upon early application) through repeated treatment of the nasopharyngeal cavity with a $10^4$ to $10^5$ IU/ml Gammaferon solution.

## *10.1.5 Human papillomaviruses*

See also Chapter 12, Section 12.9 and review by Kirby and Corey 1992.

### *10.1.5.1 Condylomata acuminata*

These anogenital warts are caused by 14 types (of the more than 60 types) of human papillomaviruses (HPV). Some types of HPV, such as HPV16 and HPV18, have a transforming ability that can cause cervical cancer. Some of these viruses are sexually transmitted, and the cases of genital warts are increasing, affecting about 1% of women of reproductive age (Schonfeld et al. 1984). Seventy percent of the partners of patients with genital papillomas are also infected. It is believed that the risk of infection is 50% at first contact (Beutnar 1989), which explains the increasing number of cases of condylomata acuminata in developing countries.

This is illustrated by the increased number of cases in England — from 21,959 in 1976 to 67,078 in 1986 (*Statistical Bulletin* 1988). Similar results are also reported for France, Canada, Finland, Japan, and other countries (Karagyozov et al. 1989). In the United States 1 to 10% of women in the reproductive age have genital papillomas, and there are 1.3 to 1.4 million clinical consultations for this disease each year (Centers for Disease Control, 1983).

Condylomata acuminata is one of the hardest problems to deal with in dermatology. There are reports of cases with a 26 to 30-year duration. Traditional therapy includes podophylin, trichloroacetic acid, 5-fluorouracil, and surgical removal through electrocauterization, cryosurgery with carbon dioxide, laser, and excision with a scalpel. Nevertheless, recurrences are frequent due to difficulties in eradication of the whole lesion and to the possibility of the virus remaining in a latent form (viral reservoirs) in neighboring epithelial cells and mucous membranes. Also, these treatments have side effects such as pain, local irritation, and ulcerations. There has also been a report of a case of death due to high doses of podophylin (see Margolis 1982 and references therein).

It has been shown that the interferons, including IFNγ, selectively suppress the expression of HPV18 mRNA in infected HeLa cells (Nawa et al. 1990).

Clinical trials on condylomata acuminata patients have been conducted using natural and recombinant IFNα and IFNβ; in most cases in the form of local or systemic injections (e.g., Schonfeld et al. 1984; Weck et al. 1988; Horowitz 1989; Panici et al. 1989; Stadler et al. 1989; Douglas et al. 1990; Vance and Davis 1990; Welander et al. 1990; Condylomata Intern Study Group 1991, 1993; Cirelli and Tyring 1994). For IFNα administered through systemic or local injections at relatively high doses, the complete and partial effect varied between 36% and 80% accompanied by side effects.

Natural IFNα was applied topically for the first time in 1975 with a therapeutic effect (Ikic et al. 1975). Topical application of IFNα or IFNβ was found to be less effective (for a review, see Stadler et al. 1989). Although an IFNβ gel (Fiblaferon) eliminated the papillomas, it also prevented recurrent infections (Berthold et al. 1989; Brzoska et al. 1989). Some results according to literature data are summarized in Table 10.4.

Relatively few trials have been conducted with IFNγ, most of them through systemic injections (50 to 100 μg), which resulted in 50 to 60% reduction of the healing time (Gross et al. 1989a; Mahrle and Schulze 1990; Rockley and Tyring 1995), and also as adjuvant therapy in refractory cases (Zouboulis et al. 1992). Repeated therapy with interferons can be applied safely and successfully in cases of stubborn and recurrent condylomas

***Table 10.4*** Effect of different interferons on *C. acuminata*

| Route of application | IFN | Effect (%) |
|---|---|---|
| s.c. | γ (rec.) | 64 |
| i.m. | β | 82 |
| i.l. | β | 50 |
| i.l. | β | 67 |
| i.v. | β | 61 |
| i.v. | α (rec.) | 39 |
| i.v. | α | 48 |
| local | β (gel) | 69 |

*Note:* s.c. — subcutaneous; i.m. — intramuscular; i.l. — intralesional; i.v. — intravenous; rec. — recombinant.

(Reichel et al. 1992). Comparative trials showed a healing effect for all three types of IFN (Rockley and Tyring 1995), where the lowest number of recurrent infections was after treatment with IFNβ and IFNγ (Bonnez et al. 1995). The combination of IFNγ and IFNα did not show any advantages over monotherapy (Trizna et al. 1998). In general, treatment with interferons had given favorable effects in two thirds of all cases. However, with this treatment, recurrent infections remain a big problem due to the presence of latent reservoirs of HPV.

An important factor for papilloma regression is the expression of HLA class II antigens in the presence of CD4+ and CD8+ T lymphocytes. Biopsies comparing the local state in a positive response and in the absence of response showed some differences. A positive effect revealed increased involvement of macrophages, NK cells, and activated CD4+ T lymphocytes directed against infected keratinocytes, which corresponds to a positive Th1 response (production of IL-2 and IFNγ). All data show that the immunoreactivity in genital papillomas is associated with an increased Th1 or mixed Th1/Th2 mRNA expression (Grassegger et al. 1997).

In resistant cases a loss of Langerhans' cells in the lesion is observed leading to decreased expression of HLA class II antigens. It has been reported that these cases are neither connected with a disrupted IFNγ signaling nor with viral copy numbers, but the resistance correlates with differential expression of the early E7 and late L1 viral genes. The E7 gene is most expressed in patients who respond favorably, whereas in the resistant ones, it is the L1 gene that is most expressed (Arany et al. 1995a,b; Arany and Tyring 1996).

Our preliminary results show that topical application of Gammaferon gel has a healing effect in cases of condylomata, but the concentration of IFNγ has to be increased — in the order of about $10^6$ IU/g gel.

#### *10.1.5.2 Laryngeal papillomas*

These are caused by HPV types 6 and 11. The disease shows a bimodal distribution with regard to age — one group was affected between 6 months and 4 to 5 years of age and another was affected between the ages of 20 and 50 years (adult group). The common treatment is surgical with frequent recurrences. IFNα and β have been successfully used.

A correlation was found between frequent recurrences and low expression of TAP-1 antigen and MHC class I, which are associated with antigen presentation. IFNγ increases the expression of these two antigens (Vambutas et al. 2000). Bearing in mind these data and also the effect of IFNγ on other HPV, a curative effect on pharyngeal papillomas has to be also expected.

#### *10.1.5.3 Common warts*

They are benign formations induced mainly by HPV types 1, 2, 4, 7, and 11. They often cause cosmetic defects.

The treatment usually includes local application of drugs containing salicylic acid, podophylin, formaldehyde, cryotherapy, curretage, electrocauterizaton, 5-fluorouracil, iododeoxyuridin, and local injection with bleomycin. Different interferons have been used with varying success, mostly through local injection.

A case has been described of a patient who suffered for 26 years from warts resistant to different treatments. He was cured after immunotherapy with dinitrochlorobenzol after a single injection of IFNγ into the wart. This led to the disappearance of all warts after 1 to 2 days (Shiohara et al. 1989).

Our unofficial observations show that many common warts can be removed after daily topical treatment with Virogel G5 for 1 to 3 months (using leukoplast to cover the treated warts).

### *10.1.6 Human immunodeficiency virus infection*

The difficult and yet unsolved problem of healing HIV-infected people raises the question of the role of the immune system and more specifically the role of cytokines in the fight against HIV infection (Mathe et al. 1996; Valdez and Lederman 1997). Taking into consideration the important role of IFNγ in the defense against different pathogens (for a review, see Murray 1996a), this cytokine is of particular interest. Normally following infection, the synthesis of IL-12 is induced in lymphocytes, which stimulates these cells to synthesize IFNγ. IFNγ, on the other hand, stimulates the phagocytic activity of macrophages which kill pathogens by producing superoxide anions or NO (see p. 33). The role of oxygen metabolites produced by IFNγ-activated monocytes for suppressing the infectiousness of HIV particles has been shown in cell culture (Ennen and Kurth 1993).

A number of studies during the last 3 to 4 years show that disturbances of the immune system caused by HIV lead to an expressed disbalance in cytokine synthesis by T lymphocytes. The role of IFNγ in HIV infections can be demonstrated by two sets of data: (1) the changes in the cytokine profile which occur after infection with HIV and its progression to acquired immunodeficiency syndrome (AIDS) and (2) The effect of different cytokines on the course of this infection.

#### *10.1.6.1 Changes in cytokine profile*

As far as the first group of data is concerned, the large part of the studies show that during HIV infection and its progression to AIDS a gradual loss of T-cell function occurs with a domination of Th2 cytokines produced by CD4+ cells. When the number of these cells decreases or they are completely lost, Th2 cytokines are also produced by a subclass of CD8+ T lymphocytes (Paganellli et al. 1995; Rodriguez et al. 1997; Dalod et al. 1999; Westby et al. 1999). The following data show the shift in the cytokine profile from Th1 to Th2 type:

- CD4+ T cell clones isolated from seropositive (HIV+) individuals show a decreased secretion of Th1 cytokines and increased Th2 production (Meyaard et al. 1994; Agarwal and Marshall 1998). The same applies to vertically HIV-infected children, who produce considerably less Th1-type cytokines and more Th2 as compared to noninfected children (Leo et al. 1996; Than et al. 1997; Vigano et al. 1997). Decreased production of IFNγ and a decreased percentage of cells that synthesize IFNγ and IL-2 during HIV infection have also been reported in many other studies (Meyaard et al. 1996; Klein et al. 1997; Rodriguez et al. 1997; Agarwal and Marshall 1998; Empson et al. 1999). Changes in the cytokine profile were also found in the saliva. Whereas in the saliva of healthy individuals, types Th1 and Th0 are found; in HIV-infected individuals, the Th2 profile dominates (Leigh et al. 1998).
- Acute HIV infection of isolated T cells leads to a dramatic decrease in IFNγ mRNA expression (Fan et al. 1997).
- Th2 cytokines dominate in the genital tract of HIV+ women, which could be the reason for the decreased cytotoxic potential of the CD8+ T lymphocytes and could contribute to infection susceptibility and cervical neoplasia (Olaitan et al. 1998).
- The number of CD4+ T cells in HIV+ individuals positively correlates with the level of IFNγ and negatively with the IL-10 level (Salvaggio et al. 1996). In individuals with CD4+ T cells less than 400/μl, IFNγ mRNA in unstimulated lymphocytes is considerably decreased and IL-10 mRNA is increased as compared to patients with CD4+ T cells >400/μl (Diaz-Mitoma et al. 1995).
- In asymptomatic HIV+ adults normal cytokine secretion is observed during the course of 2 years (Canaris et al. 1998). In a different study involving asymptomatic HIV-infected it was found that 60% of them had an elevated number of lymphocytes capable of synthesizing large quantities of IFNγ (Caruso et al. 1996). Asymptomatic HIV-infected children also have an increased level of IFNγ in the serum (Bhatnagar et al. 1996).
- Lung lymphocytes of HIV-infected individuals secrete large quantities of IFNγ, but this ability is lost during the late stages of the disease (Twigg et al. 1999).
- During the progression of the disease in HIV+ patients, the production of IFNγ and IL-2 by mononuclear cells in the blood is decreased, whereas the production of IL-4 and IL-10 is increased as compared to healthy or infected but non-progressing individuals (Clerici et al. 1996, 1997a). In general, in agreement with the data presented above, the progression from HIV infection to AIDS symptoms is associated with a shift of the cytokine profile from Th1 to Th2 (Altfeld et al. 2000). The disturbed balance in the Th1/Th2 ratio leads to decreased synthesis of IFNα, also (Hober et al. 1998). Upon progression of the disease toward AIDS, a decrease of IFNγ along with a decrease in IL-13 also has been reported (Bailer et al. 1999).

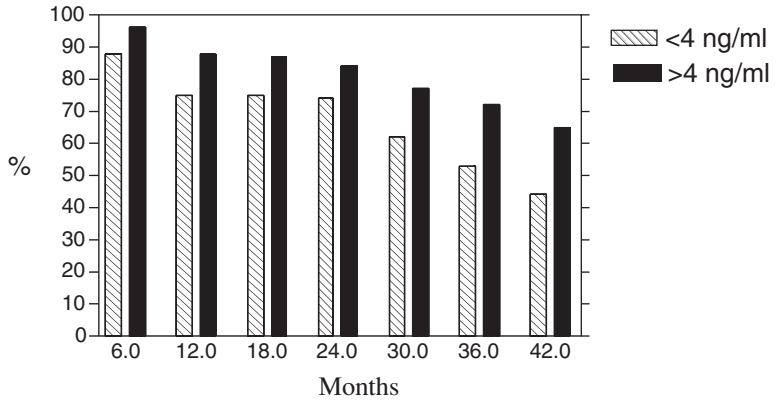


***Figure 10.6*** Survival (in %) of 347 HIV-infected individuals depending on the concentration of IFNγ in their blood. (According to data by Ullum et al. 1997.)

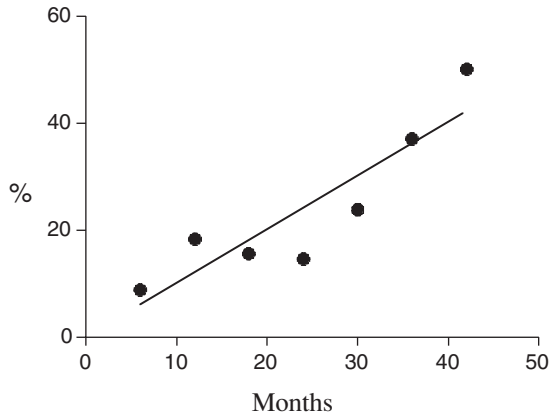


***Figure 10.7*** Increased survival rate (in %) of HIV-infected individuals with IFNγ concentration in the blood >4 ng/ml vs. those with an IFNγ concentration <4 ng/ml. (Calculated from the data in Figure 10.6.)

- The progression to AIDS leads to an increased serum level of IgE and to esinophilia. This is associated with an increased production of Th2 cytokines (Paganelli et al. 1995; Agarwal and Marshall 1998).
- A study of 347 HIV+ individuals shows a positive correlation between the level of IFNγ in the blood and the length of the asymptomatic period before clinical symptoms of AIDS appear (Ullum et al. 1997) (Figure 10.6). Based on these data, calculations show that when there is more than 4 ng/ml IFNγ in the blood, the life expectancy of HIV+ individuals is extended — 2.5 years after infection there are 50% more survivals (Figure 10.7).
- The IFNγ/IL-4 ratio is considerably lower in patients with cytopathic strains of HIV (Benyoucef et al. 1998). This ratio also decreases

concerning the cytokines of the CD4+ and CD8+ T cells (Meyaard et al. 1996).
- In individuals controlling the viremia without antiviral therapy a strong and constant proliferative response of the HIV-specific CD4+ T lymphocytes is observed, resulting in production of IFNγ and beta-chemokines (Rosenberg et al. 1997).
- In seronegative individuals the CD8+ T cells produce IFNγ but not IL-4. In seropositive patients a large number of CD8+ T cells produce both IFNγ and IL-4 (type Th0) or they become the Th2 type due to defective production of IL-12 by infected macrophages (Maggi et al. 1995, 1997).

In general, the data show that during HIV infection the stable condition without progression of the disease is related to the increased ability of T cells to synthesize IFNγ. This appears to be determined by the ratio between suppressor (CD8+CD28-) and cytotoxic (CD8+CD28+) T lymphocytes (Caruso et al. 1996; Mathe et al. 1996; Zanussi et al. 1996).

Special attention should be given to the relationship between the two cytokines, IFNγ and IL-12. Normally, IL-12 is induced shortly after HIV infection, and it strongly activates the synthesis of IFNγ by CD4+ and CD8+ T lymphocytes and macrophages. This is an important mechanism for stimulating the macrophages to phagocytic activity and for generation of Th1-type cells (Paganin et al. 1995; Seder et al. 1995; Lau et al. 1996; McDyer et al. 1997; Trinchieri et al. 1997). On the other hand, IFNγ stimulates the production of IL-12, which is heavily disturbed in HIV infection (Fantuzzi et al. 1996; Gately and Mulqueen 1996; Harrison and Levitz 1997; Trinchieri et al. 1997; Chougnet et al. 1998). There are also data showing that if monocytes/macrophages are pretreated with IFNγ, the glycoprotein gp120 of HIV induces IL-12 in these cells (Fantuzzi et al. 1996). Besides stimulating of IFNγ production, IL-12 can prevent T-cell receptor (TCR)–induced and Fas-induced apoptosis of CD4+ cells (Estauier et al. 1995).

The shift from Th1 to Th2 type cytokines is probably due to several factors:

- One of the mechanisms is the effect of gp120 from the coat of HIV. *In vitro* experiments show that this glycoprotein inhibits the synthesis of IFNγ and the cytotoxicity of NK cells (Peruzzi et al. 2000). It also inhibits the production of IFNa and IFNγ in leukocytes induced with the Sendai virus or with PMA (Nair et al. 1995).
- It also has been shown that another HIV transmembrane glycoprotein gp41 is a powerful modulator of cytokine secretion by peripheral mononuclear cells. It increases the secretion of IL-10, which suppresses the synthesis of IFNγ (Barcova et al. 1998).
- The product of the *nef* gene of HIV suppresses the synthesis of IFNγ and IL-2 without affecting the synthesis of IL-4, IL-8, IL-9, IL-13, and TNFα (Collette et al. 1996).

- A conserved sequence of the HIV core protein p24 is probably of importance. It has been shown that a peptide (Ch7) corresponding to this sequence specifically hampers the immune response induced by antigens in cultures of normal human lymphocytes (Luzzati et al. 1996).
- Stimulation of the TCR of the CD4+ Th1 lymphocytes leads to their apoptotic death (Estauier et al. 1995).
- In HIV+ patients the sensitivity of Th1 cells to induced apoptosis is increased (Ledru et al. 1998). According to this study, cells producing IL-2 are most susceptible to induced apoptosis followed by the IFNγ- and TNFα-producing cells. This is due to the fact that the CD4+ T cells of HIV patients express the Fas/Apo1 (CD95) cellular receptor and they become very sensitive to apoptosis induced by antibodies binding to Fas (Estauier et al. 1995). At the same time, the CD4+ and CD8+ T cells of HIV patients are less sensitive to the antiapoptotic activity of IL-7 and IL-2 (Vingerhoets et al. 1998).
- It is believed that TNFα is a mediator of CD4+ T-lymphocyte loss during HIV infection (Klein et al. 1996).
- One important and interesting mechanism is the induction by HIV of dimethyltransferase, which methylates and thus inactivates the promoter of the IFNγ gene (Mikovits et al. 1998).

Some data, which are not in complete agreement with the concept of a Th1 to Th2 shift during HIV infection (Hagiwara et al. 1996b; Breen et al. 1997; Fakoya et al. 1997; Kundu and Mrigan 1997; Rockstroh et al. 1998; Trumphfheller et al. 1998) could be explained by the complicated relationships in the cytokine network, which depend on many factors:

- First, the genetically based reactivity of the organism (Peterson et al. 1998) determines the initial reaction with an increased production of IFNγ as a defense mechanism against HIV infection.
- The balance between Th1 and Th2 functions depends on the nature of the antigen and the primary interaction between the T cells and the antigen-presenting cells (Peterson et al. 1998).
- There are data that the level of glutathion in the antigen-presenting cells determines a Th1 or a Th2 response (Peterson et al. 1998).
- The profile of induced cytokines may also depend on the antigen/antibody ratio. For example, the tetanus toxicoid antigen induces a typical Th1 response with high levels of IFNγ and IL-2, but when it is complexed with antibody in an equal amount or when the antibody is in excess, the response is secretion of IL-6, IL-10; that is, a Th2 response without synthesis of IFNγ and IL-2 (Berger et al. 1996).
- After immortalization of CD4+ T cells with herpes virus saymiri (HVS), IFNγ stimulates, rather than inhibits, HIV replication (Saha et al. 1997).

#### 10.1.6.2 *Effect of different cytokines*

The second group of data show the favorable effect of Th1 cytokines on the course of HIV infection:

- Treatment of monocytes with IFNγ completely inhibits their infection by HIV. Their invasiveness through the membrane matrix is also inhibited because of the suppression of the synthesis of a metalloproteinase (MMP-9) which degrades the basal membrane and is strongly expressed in HIV-infected monocytes (Dhawan et al. 1995a,b).
- The strongly hampered IL-12 production in HIV infection is stimulated by IFNγ (Harrison and Levitz 1997; Trinchieri et al. 1997; Chougnet et al. 1998). According to a study in children, IL-12 increases the HIV-1–specific cytotoxic activity independent of IL-2 and IFNγ (McFarland et al. 1998). On the other hand, it was shown that IL-12 suppressed the expression of the alpha chemokine repressor fusin (CXCR4), which is an important factor for the entry of lymphotropic strains of HIV into T lymphocytes. Fusin repression depends on the ability of IL-12 to induce synthesis of IFNγ (Galli et al. 1998). It has been found that IFNγ, as well as IFNα, suppress the expression of this chemokine receptor (Shirazi and Pitha 1998). We should mention here that IFNγ increases the expression of some other chemokine receptors too (CCR1, CCR3, and CCR5). These receptors play a role in the migration of immune cells toward the infection site, but at the same time it has been found that they increase HIV-1 entry into monocyte-like U937 cells (Zella et al. 1998). Thus, the end effect will probably depend on the balance between the suppression effect of fusin and the activation of these receptors. The role of the chemotactic protein IP-10 induced by IFNγ for the penetration of HIV-infected cells into the cerebrospinal fluid has been also pointed out (Kolb et al. 1999).
- Upon immunizaiton with HIV immunogens, the primary response is associated with production of IFNγ (Lekutis et al. 1997; Evans et al. 1998). The beta-chemokines and Th1 cytokines produced by blood mononuclear cells are also increased (Moss et al. 1997). After a DNA anti-HIV vaccine, the enhancing effect of manan-coated liposomes is strongly inhibited by antibodies against IFNγ. This shows the important role of this cytokine for obtaining an HIV-specific immune response (Toda et al. 1997). This is why liposomes containing IFNγ are recommended as adjuvants for anti-HIV vaccines (Lachman et al. 1995). In macaque monkeys, vaccination with a viral vector expressing HIV antigens and IFNγ increased the cytotoxicity against viral antigens due to the coexpression of IFNγ (Kent et al. 2000). In a different study it was found that, following HIV vaccination, the primary response is dominated by IFNγ, and after a few cycles of reimmunization, IL-4 also is increased, which is related to the production of antibodies (Evans et al. 1998).

- Treatment with IFNγ of human monocytes infected with HIV increases their ability to produce superoxide anions (Howel et al. 1997) — a function necessary for killing pathogens, which is hampered by HIV infection.
- Treatment of macrophages with IFNγ or with antibodies against IL-10 normalizes B7 molecule expression by macrophages, which are essential for the T-cell activation and whose expression is decreased in HIV infection (Orlikowsky et al. 1996).
- Treatment with IFNγ counteracts the negative effect of the Ch7 peptide, which inhibits the immune response (Luzzati et al. 1996).
- The antiviral drug AZT prevents the decreased expression of IFNγ and IL-2 caused by HIV (Fan et al. 1997). Also, a highly active antiretroviral therapy recovers the normal Th1/Th2 profile (Imami et al. 1999). Contrary to these data, in a different study it was found that this type of therapy did not change the cellular profile, at least during 1 year. The disturbed Th1/Th2 balance was more due to Th2 overexpression than to Th1 deficiency (Martinon et al. 1999).
- The peptide-nucleoide drug reticulose stimulates IFNγ synthesis and suppresses HIV replication (Hirschman and Chen 1996).
- *In vitro* treatment with IFNγ of blood mononuclear cells from asymptomatic HIV patients recovers the immune response of these cells (De Francesco et al. 1996).
- The introduction of HIV-induced interferon genes (α, β, or γ) into human target cells by using recombinant DNA approaches made the cells highly resistant to HIV infection (Leissner et al. 1998).

Analysis of all these data shows that the suppressed synthesis of Th1 cytokines is characteristic of HIV infection. The decreased production of IFNγ is a risk factor for AIDS development. This cytokine is a factor which prolongs the latent asymptomatic period and can be used for prolongaiton of this period and to fight opportunistic infections (Murray et al. 1985; Aboulafia and Mitsuyasu 1992; Thoma-Greber et al. 1993), and it can be used as an adjuvant in anti-HIV vaccines. The conduction of clinical trials with Th1 cytokines in this direction appears to be necessary and fully justified (Paganin et al. 1995; Mathe et al. 1996; Murray 1996a; De Paoli et al. 1997; Valdez and Lederman 1997; McFarland et al. 1998; Twigg et al. 1999).

### *10.1.7 Hepatitis*

Approximately 5% of the world's population is infected by the hepatitis B virus which causes necrotic inflammation with varying severity and duration. Patients with chronic inflammation run the risk of developing liver cirrosis and hepatocellular cancer. The humoral immunity against antigens of the viral coat contributes to the elimination of circulating viral particles, whereas cellular imminuty eliminates the infected cells (for a review, see Chisari and Ferrari 1995).

We have found few data dealing with hepatitis B and IFNγ, especially data on its clinical use. This is due in part to data on the inhibitory effect of IFNα on the replication of all three hepatitis viruses — A, B, and C, as opposed to IFNγ, which was found to be ineffective, and this was not due to the decreased number of its receptors (Caselmann et al. 1992; Lau et al. 1992). Application of a combination of IFNα and IFNγ did not improve results obtained by the use of IFNα alone (Carreno et al. 1993). Regardless of the considerable advantage of IFNα, for the sake of completeness, some data on IFNγ also will be presented.

Normal hepatocytes do not express MHC class I molecules, but they start to express them when infected by the hepatitis B virus (Montano et al. 1982; Pignatelli et al. 1986). IFNγ stimulates the expression of these antigens by liver cell lines more strongly than IFNα and TNFα (Skoskiewicz et al. 1985; Yoshioka et al. 1989).

In spontaneous recovery from hepatitis B infection, most T cells isolated during the acute phase of infection express Th1 profile cytokines dominated by production of IFNγ. This shows the role of this cytokine in controlling the infection and the recovery. Several years after recovery a large part of the cells were shown to produce Th0 profile (IFNγ, IL-4, and IL-5) cytokines (Penna et al 1997).

The replication of the duck hepatitis B virus is suppressed by IFNγ (Lavine and Ganem 1993).

In an adoptive transgenic mouse model of hepatitis B viral infection it was shown that introduction of cytotoxic T cells specific for this virus can suppress viral gene expression and replication in the liver without killing the hepatocytes. This effect is achieved through IFNγ and TNFα, which are secreted by cytotoxic T lymphocytes after antigen recognition (Guidotti and Chisari 1996).

Factors increasing the synthesis of IFNγ in whole peripheral blood are found in patients with acute hepatitis B (Osna et al. 1996). We should mention that 11 patients with chronic hepatitis B treated with IFNγ for a period of 6 months developed autoantibodies against different cellular components but did not show symptoms of autoimmune disease (Weber et al. 1994).

The same holds true for hepatitis C. In acute and chronic hepatitis C liver biopsies have shown increased production of IFNγ. The investigators conclude that IFNγ plays an important role in the cytokine network of the liver (Morshed et al. 1993). Others also mention the potential therapeutic role of IFNγ (Weber and Wright 1992), but currently there are no convincing data for its usefulness in hepatitis C. Clinical trials comparing IFNα to IFNγ led to the conclusion that IFNγ is not as effective as IFNα (Saez-Royuela et al. 1990).

However, short-term treatment with a combination of natural IFNβ and rIFNγ in chronic hepatitis B was well tolerated and as effective as long-term treatment with IFNα (Musch et al. 1998). Data were also obtained that the induction of Th1 cytokines by IL-12 plays a major role in eliminating the virus in chronic hepatitis B (Rossol et al. 1997).

The data are too scarce to determine the usefulness of IFNγ in hepatitis, especially when the effective treatment with IFNα is taken into consideration.

## 10.2 Nonviral intracellular pathogens

### 10.2.1 Bacterial infections

IFNγ has considerable antibacterial activity (Murray 1990, 1994; Degre 1996) associated with the induction of toxic nitrogen and oxygen derivatives (see p. 33). In laboratory experiments and in clinical practice the following more important bacterial species have shown sensitivity to IFNγ: *Listeria monocytogenes* (Kiderlen et al. 1984; Buchmeier and Schreiber 1985; Kurtz et al. 1989; Gregory and Wing 1993; Melissen et al. 1993; Metcalf and Campbell 1994); *Brucella* spp. (Stevens 1992; Jiang and Baldwin 1993; Zhan and Cheers 1993); *Salmonella* spp. (Kagaya et al. 1989; Matsumura et al. 1990); *Mycobacterium* spp. (see Sections 10.2.1.1.1 and 10.2.1.1.2); *Legionella pneumophila* (Blanchard et al. 1988; Horwitz 1992; Summersgill et al. 1992; Watanabe et al. 1993); *Chlamydia* spp. (see Section 10.2.1.2); *Yersinia* spp. (Nakajima and Brubaker 1993; Autenrieth et al. 1994; Bohn et al. 1994); and streptococci (Weigent et al. 1986). It should be mentioned that IFNγ did not show antibacterial activity against *Helicobacter pylori*, and it increased the inflammation of the stomach mucosa caused by it (Sawai et al. 1999; Smyth et al. 2000).

Only a few more important bacterial infections will be discussed here.

#### 10.2.1.1 Mycobacteria

Many experimental and some clinical data show that all members of the mycobacteria species such as *M. tuberculosis*, *M. leprae* (see below), and also some nontuberculosis mycobacteria are sensitive to IFNγ (e.g., see Holland et al. 1994; Levin et al. 1995).

*10.2.1.1.1 Tuberculosis.* We shall present data mostly on *M. tuberculosis*, which is one of the most dangerous intracellular pathogens in humans. Tuberculosis was a major health problem in developing countries, but lately its presence also has been observed in industrialized countries. A few years ago the World Health Organization (WHO) estimated that worldwide approximately 1.7 billion people were infected with *M. tuberculosis* (Kochi 1991), and this number is constantly increasing. Now it has been reported that one third of the world's population is infected with *M. tuberculosis* (Scanga et al. 2000), but the symptoms of the disease are limited by the activity of the immune system. Tuberculosis takes one of the highest places in morbidity and mortality rates. The appearance and increasing number of multi–drug-resistant (MDR) strains are of special concern.

Many experimental data show that IFNγ plays a central and unique role in defending the organism against mycobacterial infections (Wallis et al.

1992; Flynn et al. 1993; Murray 1994; Bermudez and Kaplan 1995; Bonecini-Almeida et al. 1998). This cytokine is induced by mycobacterial antigens and it activates effector cells — macrophages, lymphocytes, neutrophils — to kill bacteria at the sites of infection (see, e.g., Robinson et al. 1994; Hernandes-Pando et al. 1996). Cells that participate actively and produce IFNγ include CD4+ cells as well as one subpopulation of CD8+ cells (Lalvani et al. 1998; Tascon et al. 1998; Feng et al. 1999).

It is believed that this occurs through NO production (Anthony et al. 1994; Green et al. 1994; Hanano and Kaufmann 1995; Munk and Emoto 1995) and reactive oxygen radicals. Some cases of resistance to NO have been observed (Doi et al. 1993). However, the data concerning the role of reactive oxygen and nitrogen intermediate derivatives are contradictory. In experiments with mice it was found that in peritoneal macrophages IFNγ activated the synthesis of such derivatives and suppressed the growth of mycobacteria during the first days of infection. Later a progressive elimination of bacteria has been observed (Sato et al. 1998). Experiments were carried out with two types of knockout mice — one type was unable to produce reactive oxygen species due to nonfunctional alleles of the gp92phox subunit of cytochrome b, and the other did not produce NO due to the absence of an inducible NO synthase. It was found that alveolar and peritoneal macrophages that did not produce oxygen radicals but produced NO inhibited the growth of *M. tuberculosis* after stimulation with IFNγ. Knockout mice producing oxygen radicals but not NO could not suppress the bacteria in the spleen, but they were able to limit bacterial growth in the lung (Adams et al. 1997).

Contrary to these data, in mice with defective production of NO stimulation of the macrophages with IFNγ suppressed the growth of *M. avium*. It was concluded that NO did not play a role in the defense against these bacteria (Gomes et al. 1999). However, in a different experiment stimulation of NO production by IFNγ was necessary for growth inhibition of *M. bovis* by mouse macrophages, since the effect was abolished when NO synthase was inhibited. Human alveolar macrophages responded differently by showing a mycobacteriostatic activity independent of NO (Aston et al. 1998). From these contradictory data, it could be assumed that the role of NO is different depending on a number of factors, such as the animal species, the bacterial species, the localization of the macrophages, and so on.

Regardless of the conflicting results on the role of NO, all data support the opinion that active tuberculosis leads to a Th1-type immune response (Lai et al. 1997):

- In MDR tuberculosis the T-cell response is hampered when the number of CD4+ T cells is decreased (McDyer et al. 1997; Johnson et al. 1998).
- The effector cells start to produce IFNγ at the site of infection as a defense reaction. In 1988 it was found that pleural effusions caused by *M. tuberculosis* contain higher concentrations of IFNγ. This was confirmed many times by finding that no other nontuberculous effusions

led to increased concentration of this cytokine (Ribera et al. 1988, 1990; Shimokata et al. 1991; Barnes et al. 1993; Yanagawa 1997). This finding together with an increased adenosine deaminase is used for early diagnosis of tuberculous pleuritis (Valdes et al. 1993; Soderblom et al. 1996; Wongtim et al. 1999) and for differentiation of tuberculous exudate from other types of effusions (Soliman et al. 1994; Sathar et al. 1995). High concentrations of IFNγ were also found in cerebrospinal fluid in tuberculous meningitis (Donald et al. 1995).

- We should point out the different behavior of macrophages at the site of infection and of the mononuclear cells in peripheral blood. Compared to healthy individuals reacting to tuberculin, the peripheral mononuclear cells of tuberculosis patients show decreased production of Th1 cytokines (IFNγ and IL-2) after stimulation with the mycobacterium (Gergert et al. 1995; Zhang et al. 1995; Swaminathan et al. 1999), but after effective therapy IFNγ production is increased (Gergert et al. 1995).
- The same holds true for the bronchoalveolar T lymphocytes which in lung tuberculosis are activated to produce Th1 cytokines (Robinson et al. 1994). They react to purified protein derivative of *M. bovis* (PPD) in tuberculous exudates and produce much more IFNγ and IL-2 than peripheral lymphocytes (Shimokata 1996).
- Treatment with IFNγ increases the percentage of alveolar macrophages which phagocyte and kill mycobacteria in patients with lung tuberculosis (Shimokata 1996).
- Normal granulocytes rapidly engulf opsonized mycobacteria. However, they do not kill them effectively. The intracellular killing of bacteria is considerably stimulated if the granulocytes are previously incubated with IFNγ (Wadee 1990).
- The inhibiting effect of a 25-kDa fraction of *M. tuberculosis* on the bactericidal activity is blocked by IFNγ (Wadee 1990).
- Patients with active tuberculosis and low IFNγ production recover the ability to produce IFNγ several weeks after chemotherapy (Carlucci et al. 1993).
- Low IFNγ production is observed in patients with poor health and advanced tuberculosis (Huygen et al. 1988). Thus, in less severe forms of tuberculosis they have increased production of IFNγ as compared to IL-4; in moderately severe forms a mixed Th0 profile is exhibited (production of both IFNγ and IL-4), and in advanced forms the production of IL-4 and tumor growth factor β (TGFβ) is increased, as synthesis of IFNγ becomes hardly detectable; that is, a transition toward a Th2 profile occurs (Dlugovitzky et al. 1999). Mixed Th0 type cytokines also are observed in advanced tuberculosis with a heavy bacterial load (Somoskovi et al. 1999; Wilsher et al. 1999). A Th2-type response (with increased production of IL-10 and decreased IFNγ) also accompanies infection in anergic patients (Boussiotis et al. 2000). Long-term suppression of IFNγ production and increased production of IL-10

and TGFβ are also observed in HIV-infected tuberculous patients (Hirsch et al. 1999a). The negative role of IL-10 is demonstrated by the fact that IL-$10^{-/-}$ mice have increased resistance to mycobacteria and their macrophages produce more IFNγ and NO (Murray and Young 1999). The Th2-type response with increased production of IL-4 is related to tissue necrosis and cavitation (van Crevel et al. 2000).

- The reactivation of latent tuberculosis is associated with the transition from the Th1 to the Th2-type cytokines (Howard and Zwilling 1999).
- The early phase of acquiring immunity against *M. tuberculosis* is related to the appearance of protective CD4+ lymphocytes secreting IFNγ as a result of IL-12 induced by the mycobacteria. Due to treatment with this cytokine, resistance to bacterial infection increases (Cooper et al. 1995; Saunders et al. 1995). However, there are data that IL-12 is not the first cytokine induced by *M. bovis* BCG (bacille Calmette-Guérin) vaccine. Although IL-12 production is stimulated in this infection, experiments in mice with defective IFNγ and TNFα receptors show that IL-12 secretion is dependent on these two cytokines (Flesch et al. 1995; Kaufmann et al. 1995). On the other hand, however, IL-12 is of considerable importance for IFNγ induction and the control of the mycobacterial infection. Individuals with defective IL-12 receptors do not produce IFNγ and therefore suffer from severe mycobacterial infections (Altare et al. 1998; de Jong et al. 1998).
- Organisms with an inactivated IFNγ gene or the gene of its receptor (knockout mice) (Cooper et al. 1993; Dalton et al. 1993; Flynn et al. 1993; Kamijo et al. 1993, 1994) or people with mutations inactivating the corresponding gene (Jouanguy et al. 1996; Newport et al. 1996) cannot survive mycobacterial infections. It appears that IFNγ cannot be replaced by any other cytokine.
- During infection of mice with *M. avium*, a correlation was found between resistance and IFNγ expression (Appleberg 1994).
- Mice expressing BCG-resistant alleles respond with a higher secretion of IFNγ as compared to mice with BCG-sensitive alleles (Agrewala and Mishra 1995).
- BCG vaccination leads to a Th1-type response (Hoft et al. 1999).

Finally, we have to mention data showing mechanisms which suppress cellular immunity during infection with *M. tuberculosis*:

- Active infection can cause increased Fas expression and decreased expression of the antiapoptotic gene Bcl-2 in CD4+ T cells. Thus, when stimulated *in vitro*, the latter undergo increased apoptosis and decreased production of IL-2 and IFNγ but not of IL-4 (Das et al. 1999; Hirsch et al. 1999b).
- Virulent *M. tuberculosis* inhibit the activation of macrophages by IFNγ. This is due to interruption of the signaling metabolic pathway leading

to gene activation by IFNγ through the interaction of STAT-1 with the transctiptional apparatus (Ting et al. 1999).
- During the course of infection with *M. tuberculosis* antibodies against IFNγ are induced, especially at the site of infection (Madariaga et al. 1999).

Considering the role of Th1 cytokines in controlling mycobacterial infections (Bermudez and Kaplan 1995; Murray 1996a), clinical trials started recently. They show the favorable effect of IFNγ on atypical mycobacterial infections and in cases with MDR strains. A patient with acute lymphocytic leukemia and MDR tuberculosis of the brain and of the spinal cord was treated with IFNγ for 12 months, which led to the complete elimination of the damage caused by the tuberculosis (Raad et al. 1996). The state of seven patients with refractory disseminated nontuberculous mycobacterial infection treated subcutaneously with IFNγ was considerably improved with elimination of the fever and clearing of the damage caused by the disease (Holland et al. 1994). Five patients with MDR tuberculosis were successfully treated with an aerosolic form of IFNγ (Condos et al. 1997).

*10.2.1.1.2 Leprosy.* *M. leprae* also is sensitive to IFNγ (Kaplan et al. 1986; Krahenbuhl et al. 1990), and there are clinical data on its effect in patients with different forms of leprosy (see Nathan 1992 and references therein).

### *10.2.1.2 Chlamydia*

(For references, see Czarniecki et al. 1986; Rothermel et al. 1986; De la Maza 1987b; Byrne and Schachter 1992.)

The prokaryotic genus *Chlamydia* is represented by three obligate intracellular pathogens: *C. psittaci*, *C. pneumoniae*, and *C. trachomatis*.

The different biovars of *C. psittaci* affect different species of vertebrates with different clinical symptoms. People are usually infected by birds. In psittacosis different systems are affected, and it is most often manifested in the form of pneumonia. *C. pneumoniae* is widespread all over the world and causes atypical pneumonia.

Two biovars have been described for *C. trachomatis*. One of them is sexually transmitted, and affects the lymphatic system causing *lymphogranuloma venereum*. The other biovar affects the eyes and the urogenital system. When the eyes are affected, it causes chronic conjunctivitis known as *trachoma*, which is the number one cause of preventable blindness in the world. When the male urogenital system is affected, the result is urethral epididymitis. In women it leads to inflammatory processes that can have serious consequences such as infertility and ectopic pregnancy. It is reported that more than 4 million chlamydial infections of the genital tract occur in the United States each year. According to WHO, more than 600 million people live in trachoma-endemic areas. The sexually transmitted chlamydial infections are extremely common in developing as well as in industrialized

countries. In many parts of the world antibodies to *C. pneumoniae* are found in more than 35% of the adult population. It is believed that more than 1 billion people are affected by diseases caused by *Chlamydia* (Byrne and Schachter 1992). These data show the enormous importance of antichlamydial drugs for infections which are now treated long-term with antibiotics.

In the case of trachoma, the cellular immunity in response to chlamydial antigens is decreased. Increased Th2 activity facilitates the development of persistent eye infections leading to blindness. Evidence in the literature shows that during infection with *Chlamydia*, the organism produces interferons. In cell cultures all three types of interferon were found to inhibit the replication of *Chlamydia*, with the effect of IFNγ being most pronounced. The replication of *C. trachomatis* in primary cultures of human conjunctival epithelial cells is suppressed by IFNγ due to induction of IDO leading to tryptophan degradation (Rapoza et al. 1991). However, there are data showing that this effect neither depends on tryptophan (De la Maza et al. 1985), nor on oxygen (Rothermel et al. 1986).

After genital infection of mice with *C. trachomatis*, it has been shown that the clearance of *Chlamydia* depends mostly on IL-12 without the participation of IFNγ. However, protection from disseminated disease requires IFNγ (Perry et al. 1997).

A study showing the effect of IFNγ concentration on the growth of *C. trachomatis* should be considered. At 2 ng/ml, this cytokine completely suppressed the growth of *Chlamydia*. Lower concentrations, however (0.2 ng/ml), resulted in persistent infection, characterized by development of noninfectious atypical forms, from which infectious *Chlamydia* could develop, when IFNγ was removed (Beatty et al. 1993). The prophylactic effect of IFNγ against *chlamydial* infections has been shown in animal experiments, but a healing effect has not been found. In mice protective vaccination against *Chlamydia* is related to increased levels of IFNγ (Dong-Ji et al. 2000).

Increased stimulation of Th2 cytokines contributes to the development of a persistent chlamydial eye infection, leading to cicatrization and blindness. Peripheral blood mononuclear cells isolated from patients with cicatrization react to *Chlamydia* antigens with an increased number of IL-4–synthesizing cells, whereas in control individuals the reaction is an increased IFNγ level (Holland et al. 1996). In agreement with this are data that IL–10 knockout mice develop a stronger defense reaction against *C. trachomatis* — an increased production of IFNγ and increased delayed-type hypersensitivity (Yang et al. 1999). In contrast, IFNγ knockout mice show increased production of Th2 cytokines and are unable to suppress this infection, although, surprisingly, they still develop a delayed-type hypersensitivity (Wang et al. 1999).

### *10.2.2 Protozoa*

The following species of protozoa are sensitive to IFNγ: *Leishmania* spp. (see Section 10.2.2.1), *Trypanozoma cruzi* (Wirth et al. 1985; Reed and da Silva 1992; Silva et al. 1992), *Toxoplasma gondii* (Sethi et al. 1985; Suzuki et al. 1988;

Mellors et al. 1989; Subauste and Remington 1992), the malarial plasmodium in rodents (*Plasmodium berghii*, *P. chabaudi*) (Ferreira et al. 1986; Schofield et al. 1987; Vergara et al. 1987; Mellouk et al. 1991; Taylor-Robinson and Phillips 1998; Mohan et al. 1999; Yoneto et al. 1999) and in humans (*P. falciparum* and *P. malariae*) (Maheshwari 1990; Schofield 1992; Fell et al. 1994; Mellouk et al. 1994; Luty et al. 1999; Moore et al. 1999), and *Eimeria tenella* (Kogut and Lange 1989a,b).

#### *10.2.2.1 Leishmaniasis*

This disease is caused by different species and subspecies of the intracellular pathogen *Leishmania*. On a global scale, about 12 million people suffer from some form of this disease. The most severe forms of leishmaniasis are found in East Africa, Latin America, and India. The disease is also found in North America, eastern and southern Europe, and Asia. The visceral forms of the disease are fatal if not treated. The therapy of choice includes pentavalent antimonial drugs, but it is not successful in 25% of the cases. Amphoteracin B and pentamidine are alternative therapies, but they are associated with high toxicity.

Data in the literature and our experimental data show the killing effect of IFNγ on leishmanial protozoa. Several international clinical trials have shown the healing effect of the combination of IFNγ and pentavalent antimonial drugs (Kramer 1992; Badaro et al. 1990; Badaro and Johnson 1993; Harms et al. 1993; Squires et al. 1993). Liposomes were used to encapsulate these two components (Everlien and Hockertz 1999).

## *10.3 Fungi*

Activated macrophages play the main role in the fungistatic and fungicidal activity of the organism (Perfect et al. 1987).

We shall not discuss the specific diseases caused by fungi, and shall only mention that the following intracellular and extracellular fungi are sensitive to IFNγ: *Histoplasma capsulatum* (Brummer et al. 1988; Stevens 1992); *Cryptococcus neoformans* (Perfect et al. 1987); *Candida* spp. (Stevens 1992); *Coccidoides immitis* (Beaman 1987); *Paracoccidoides brasiliensis* (Brummer et al. 1988; Stevens 1992); *Blastomyces dermatitidis* (Brummer et al. 1988; Morrison et al. 1989; Stevens 1992).

## *10.4 Nonviral extracellular pathogens*

The following protozoa and helminths are sensitive to IFNγ: *Entamoeba histolytica* (Denis and Chadee 1989; Ghadirian and Salimi 1993) and *Schisostoma mansoni* (Pancre et al. 1990, 1994).

# *chapter eleven*

# *Noninfectious nonmalignant diseases*

## *11.1 Fibroproliferative diseases*

Fibroprolifeartive diseases are due to activated fibroblast proliferation and overproduction mainly of collagen leading to fibrosis. The basis of their pathogenesis is disturbances in the cytokine network. Some cytokines such as interleukin-4 (IL-4), tumor growth factor β (TGFβ), IL-1b stimulate collagen synthesis (Kahari et al. 1990; Kikuchi et al. 1995; Tiggelman et al. 1995; Sempowski et al. 1996). Interferon gamma (IFNγ) exerts an exactly opposite effect — a strong inhibition of collagen synthesis by fibroblasts (Duncan and Berman 1987; Melin et al. 1989; Elias et al. 1990; Granstein et al. 1990; Kahari et al. 1990; Gillery et al. 1992; Narayanan et al. 1992; Serpier et al. 1992; Nguyen et al. 1994; Bird and Tyler 1995; Harrop et al. 1995; Sempowski et al. 1996; Chizzolini et al. 1998; Jaffe et al. 1999; Yokozeki et al. 1999; Yuan et al. 1999; Yamabe et al. 2000), by synovial cells (Daireaux et al. 1990), by myofibroblasts (Granstein et al. 1990), by chondrocytes (Reginato et al. 1993; Goldring et al. 1994), and by osteoblasts (Smith et al. 1987; Hirose et al. 1989). An important enzyme which catalyzes one of the steps in the synthesis of procollagen and its production is propyl-4-hydroxylase, which is considerably increased in fibroblasts of systemic sclerosis (SSc) patients. IFNγ exerts a strong suppressing effect on this enzyme (Kawaguchi et al. 1992).

The combination of IFNγ and tumor necrosis factor α (TNFα) exerts a synergistic inhibitory effect on the synthesis of collagen (Scharffetter et al. 1989; Kahari et al. 1990). This effect occurs after pretreatment with IFNγ (Nanes et al. 1989). According to some investigators, IFNγ suppresses this synthesis at the level of transcription (Granstein et al. 1990), but according to others, its effect on transcription is minimal, as opposed to TNFα, which suppresses the promoter of the collagen gene (Kahari et al. 1990).

Dexamethasone suppresses the inhibitory effect of IFNγ (Bird and Tyler 1995), but it is interesting that this dexamethasone effect is expressed in dermal fibroblasts but not in colon fibroblasts (Martens et al. 1992). IFNγ also inhibits the synthesis of actin by myofibroblasts (Pittet et al. 1994).

There are data that the inhibition of collagen synthesis by IFNγ does not depend of tryptophan degradation (Yufit et al. 1995), but it is associated with nitric oxide (NO) induction (Trachtman et al. 1995; Owens et al. 1996).

A number of data show the potential role of IFNγ in the suppression of collagen overproduction, causing fibrosis of some tissues or in the whole organism. We shall discuss data concerning the skin, liver, and lung. There are also data on the inhibition of experimental fibrosis in rat kidney (Oldroyd et al. 1999).

### *11.1.1 Fibrosis of different tissues and organs*

#### *11.1.1.1 Keloids*

During wound healing, keloids develop as a result of abnormal overproduction of collagen, forming thick collagen bundles with different orientations, unlike the thin collagen fibrils oriented correctly in normal wound healing.

Keloid fibroblasts respond to platelet-derived growth factor (PDGF) and TGFβ with increased synthesis of collagen in the same manner as normal fibroblasts, but they show considerably higher stimulation of collagen synthesis by epidermal growth factor (EGF), and by histamine (Kikuchi et al. 1995). Due to these data, it is believed that these two factors — EGF and histamine — are involved in the pathogenesis of this process. Patients who develop keloids have a strongly decreased synthesis of IFNγ, TNFβ, and IFNα, and they produce more IL-6, TNFα, IFNβ (McCauley et al. 1992) and TGFβ (Tredget et al. 2000); that is, they show a cytokine shift toward a Th2 profile. They also exhibit an increased collagen metabolism (Berman and Flores 1998).

In preliminary experiments it was shown that injection of IFNγ decreases the size of keloids and improved the Dupuytren contractions (Pittet et al. 1994; Broker et al. 1996). IFNγ was also used for prevention of reccurrences after surgical removal of keloids (Broker et al. 1996; Berman and Flores 1998).

It was also used in fibrocontractive disorders of the skin (Leslie 1994). IFNγ has an additive antifibrosis effect with IFNα (Tredget et al. 2000).

It was proposed to use IFNγ after glaucoma filtering surgery to prevent the formation of a fibrous scar by proliferation of activated fibroblasts in the damaged Tenon's capsule (Latina et al. 1991; Nguyen et al. 1994). This favorable effect was also shown in experiments with rabbits (Lee et al. 1991; Leslie 1991). IFNγ also showed a suppressing effect on collagen synthesis during keloid development (see Berman and Duncan 1989).

#### *11.1.1.2 Fibrosis of the lung*

Fibrosis of the lung is the result of some lung and systemic diseases, but in many cases the cause remains unidentified — idiopathic pulmonary fibrosis (IPF). It has been reported that in Great Britain and in the United States the mortality from this disease is constantly increasing (Coker and Laurent 1998). The number of people affected by IPF in the USA and Canada is believed to be approximately 50,000 each year, and the potential market for its treatment is estimated as $2.5 billion (Biotechnology Report 2000).

Contrary to some reports that IFNγ increases the proliferation of human lung fibroblasts (Hunninghake et al. 1986), the possibility that it plays a pathogenic role in lung fibrosis is definitely disproved by a number of facts.

It was found that IL-6 inhibited collagen degradation in lung fibrosis, whereas IFNγ suppressed its synthesis (Bienkowski and Gotkin 1995). In cases of cryptogenic fibrotic alveolitis and progressing lung fibrosis, very few patients have an increased serum level of IFNγ. The conclusion was drawn that the disturbed production of IFNγ is a potentiating factor for a number of diseases leading to lung fibrosis (Pryor and Haslam 1992). In this fibrosis an inverse correlation was found between the level of procollagen III and the level of IFNγ. This confirmed the inhibitory effect of this cytokine on the development of lung fibrosis (Kuroki et al. 1995). In the lung interstitium of patients with cryptogenic fibrotic alveolitis and generally in lung fibrosis a domination of T helper 2 (Th2)–type cytokines (IL-4, IL-5) was found (Wallace et al. 1995; Sempowski et al. 1996), especially IL-4 expression by type II alveolar epithelial cells (Wallace and Howie 1999). There are data that the CD8+ T cells also participate in type II cytokine secretion (Atamas et al. 1999).

It is believed that in the development of fibrosis a critical role is played by the cytokines released by circulating cells and by local lung cells in response to epithelial and endothelial damage. Such fibrotic cytokines include TGFβ, TNFα, and endothelin - 1 (Coker and Laurent 1998), whereas IFNγ has an antifibrotic role. This role is associated on the one hand with the suppression of collagen synthesis and of the suppressed expression of type II fibrotic cytokines, and on the other hand with the suppression of angiogenesis, which is of importance for blood vessel remodeling. This is accomplished by the angiostatic CXC chemokine IP-10 induced by IFNγ (Keane et al. 1999). The balance between the angiogenic cytokine IL-8 and the angiostatic IP-10 plays an important role in IPF (Keane et al. 1997).

In a model of bleomycin-induced lung fibrosis in mice and rats, daily treatment with IFNγ led to decreased expression of TGFβ and reduced collagen content (Giri et al. 1986; Okada et al. 1993; Gurujeyalakshmi and Giri 1995). In a mouse model of silicosis the response was an increased IFNγ level and a decreased level of IL-4 (Davis et al. 1999).

Very few patients with cryptogenic fibrotic alveolitis and fibrotic alveolitis associated with SSc have increased IFNγ in the serum. Those with the highest IFNγ level respond to corticosteroids. This gave us reason to believe that the disturbed synthesis of this cytokine is a potentiating factor in the pathogenesis of fibrotic diseases of the lung.

All these data support as rational the use of IFNγ in lung fibrosis. A 12-month treatment of IPF patients with IFNγ-1b plus prednisolone resulted in a significant improvement (Ziesche et al. 1999; see also Du Bois 1999; Britton 2000; King 2000). However, the problem with the hematoalveolar barrier for this cytokine should be considered (see Chapter 8). Such a barrier may not even exist for the interstitial lung tissue, a problem that needs elucidation.

Phase II clinical trials with IFNγ (Actimmune) as a treatment for IPF are being conducted in the United States (Biotechnology Reports 2000).

#### 11.1.1.3 Fibrosis of the liver

IFNγ suppresses the activity of the hepatic stellate cells, which are the main producers of the extracellular matrix of this organ. Liver damage activates the stellate cells and also the liver lipocytes which are transformed into myofibroblast-like cells and begin to express smooth muscle α-actin. Thus, the amount of extracellular matrix is increased. On the other hand, IFNγ inhibits collagen synthesis, whereas it is stimulated by IL-1b, IL-4, and TGFβ (Wu and Danielson 1994; Tiggelman et al. 1995).

In a rat model of liver fibrosis induced with dimethylnitrosamine, IFNγ suppressed the accumulation of collagen, laminin, and fibronectin (Baroni et al. 1996). In liver fibrosis induced with tetrachloromethane IFNγ also suppressed the activation of lipocytes and the development of fibrosis (Rockey and Chung 1994).

Based on these data, IFNγ is believed to be a potential therapeutic agent for progressing liver fibrosis (Mezey 1991; Wu and Danielson 1994).

#### 11.1.1.4 Systemic sclerosis and scleroderma

SSc is an acquired disease associated with overproduction of collagen, which leads to fibrosis of the skin and the visceral organs. It is characterized by injury of the capillary endothelium, perivascular inflammatory reaction, and over-accumulation of collagen at the damaged sites. Scleroderma fibroblasts synthesize more intracellular adhesion molecule I (ICAM-I) (Xu et al. 1995) and release more soluble intercellular adhesion molecule I (sICAM-I). Stimulation with phytohemaglutinin reveals inhibition of IFNγ synthesis. Scleroderma fibroblasts show an increased collagen, fibronectin, and laminin adhesion, which is suppressed by IFNγ (Majewski et al. 1992). The serum of SSc patients exerts an increased mitogenic activity on skin fibroblasts (Bryckaert et al. 1994).

A number of experimental data show that IFNγ suppresses the synthesis of collagen stimulated by IL-4 in normal as well as in scleroderma fibroblasts (see references in Chapter 3, Section 3.4). This can be explained by the fact that IFNγ suppresses the expression of the IL-4 receptor (Byron et al. 1991). Scleroderma fibroblasts were found to be especially sensitive to the inhibition of collagen synthesis by IFNγ (Serpier et al. 1992). It also has been observed that IFNγ increases fibrinolysis in SSc (Gluszko et al. 1998).

Literature data on clinical trials (Kahan et al. 1989; Torres and Furst 1990; Pope 1993; Vlachoyiannopoulos et al. 1996) show the possibility of treating this fatal disease by parenteral administration of IFNγ. The treatment is long-term with 50 to 60 $\mu g/m^2$ (even up to 150 $\mu g/m^2$) administered subcutaneously three times a week for 6 months. Considerable improvement was also obtained in lung sclerosis. Side effects and unfavorable results were observed with higher doses, which is not surprising considering that IFNγ has a favorable effect at optimal concentrations.

In progressing scleroderma improvement was observed after subcutaneous injection of 30 to 150 $\mu g/m^2$ IFNγ (Fierlbeck and Rassner 1988; Vlachoyiannopoulos et al. 1996). Improvement after treatment with IFNγ

***Table 11.1*** Effect of Virogel G5 on scleroderma

| Patient | Age | Diagnosis | Duration of treatment | Skin fold (mm) Before | Skin fold (mm) After | Subjective feeling |
|---|---|---|---|---|---|---|
| 1 | 30 | SSc 2nd stage | 2 months (twice daily) | 0 | 3 | Considerable softening |
| 2 | 18 | SSc 2nd stage | 2 months (once a day) | 0 | 2–1 | Softening |
| 3 | 56 | SSc 2nd stage | 1 month (twice daily) | 0 | 1 | Softening |
| 4 | 50 | SSc 2nd stage | 1 month (once a day) | 0 | 3 | Slight softening |
| 5 | 61 | Morphea (2 plaques) | 2 months (twice daily) | 6/2.5 (cm) | 1.5/1 | Considerable softening |

was observed in 8 of 20 patients with diffuse systemic scleroderma (Bletry et al. 1993). It is interesting to mention the skin fibrosis induced by ionizing radiation observed in survivors of the Chernobyl nuclear plant disaster, which was influenced favorably by IFNγ (Peter et al. 1999).

Favorable results were obtained after topical treatment of the dermal form of systemic sclerosis with IFNγ in the form of a gel (Virogel G5) in the Clinic of Rheumatology (Sofia, Bulgaria). The hard skin areas were treated every day during two months. As a result, the normal softness and elasticity of the skin were recovered (Arnaudova et al. 1993). The results are summarized in Table 11.1.

## *11.2 Atherosclerosis*

There is no doubt about the close relationship between the pathological changes that occur in atherosclerosis and the immune system (Hansson 1994). Inflammatory changes in the vascular endothelium lead to increased leukocyte adhesion, increased proliferation of smooth muscle cells (SMC), cholesterol deposition in atherosclerotic plaques, and development of fibrosis. The mononuclear cells that interact with the endothelium play a key role in the development of lesions. Their adhesion to the endothelium is accomplished through immune complexes (CD11/CD18) between the leukocyte adhesion molecules ELAM and ICAM localized on the endothelial surface. A number of monocytic products such as free radicals, oxygen peroxides, hydrolases, and lipases contribute to endothelial damage (Beilke 1989; Wautier 1989; Hansson 1994). T lymphocytes and macrophages in the atherosclerotic lesion produce a number of cytokines which participate in the control and development of the pathological process (Kosaka et al. 1992; Filonzi et al. 1993; Kishikawa et al. 1993; Nagashima et al. 1994; Ramshaw et al. 1994; Stemme et al. 1995).

A number of processes associated with the pathogenesis of atherosclerotic lesions show the role of the cytokines and more specifically of IFNγ

in the control of atherogenesis. Such processes include: (1) increased SMC proliferation; (2) induction of the 15-lipooxygenase enzyme; (3) deposition of intracellular lipids in SMC and macrophages with the formation of characteristic foam cells under the effect of phospholipase A(2) (Menschikowski et al. 2000); (4) cholesterol deposition and formation of an ateromic nucleus; (5) fibrosis development; and (6) induction of cellular oncogenes.

SMC proliferation is controlled by specific growth factors and cytokines (for review, see Casscells 1991). PDGF and IL-1 are secreted in the arterial wall after its atherosclerotic damage, and they stimulate SMC proliferation. IFNγ suppresses the mitogenic effect of PDGF (Kosaka et al. 1992; Nagashima et al. 1994) and inhibits SMC proliferation and α-actin expression (Hansson et al. 1989). On the other hand, IFNγ induces the IP-10 chemokine which according to some investigators has a mitogenic effect on SMC (Wang et al. 1996), although many experiments show that it is an angiostatic chemokine (Angiolillo et al. 1996; Arenberg et al. 1996 and others, see p. 36). However, it appears that the proliferation-inhibiting effect of IFNγ prevails over the effect of the IFNγ-induced chemokine. This has been shown in experiments involving arterial wall damage inflicted by a ballooning catheter. The animals injected with IFNγ have developed smaller lesions than control animals (Hansson et al. 1991). Other data showing that IFNγ suppresses the proliferation of dividing SMC but activates proliferation of resting SMC cells should also be considered (Yokota et al. 1992).

15-Lipooxygenase is an enzyme which peroxidases lipids and is associated with the specific inflammatory cells involved in asthma and atherosclerosis. A study of numerous factors has shown that IL-4 and IL-13 only (Th2 cytokines) induce this enzyme, whereas IFNγ, as a Th1 cytokine, suppresses their activity and therefore the lipooxygenase induced by them (Sigal et al. 1993; Nassar et al. 1994).

Cholesterol deposition in atherosclerotic plaques and the development of fibrosis is also suppressed by IFNγ (Hansson 1994). SMC proliferation is associated with the activation of the c-myc oncogene. IFNγ suppresses the activity of this gene whose disturbed regulation appears to be important for atherosclerosis pathogenesis (Bennett et al. 1994a,b). In addition, during increased SMC proliferation, an overexpression of the c-fos and N-ras cellular oncogenes is observed. These genes are partly suppressed by IFNγ (Dong and Wang 1994). Expression of the cellular oncogene c-fms, which is also expressed by SMC in atherosclerosis, is activated by PDGF, EGF, and fibroblast growth factor (FGF), and also is suppressed by IFNγ (Inaba et al. 1995).

It has long been suspected that atherosclerosis development may be associated with an inflammatory process caused by viruses (Capron 1990; Bayad et al. 1993; Melnick et al. 1993; Shih and Kelemen 1995; Buja 1996). Recently, several reports connect this process with infection by cytomegalovirus (CMV), a member of the herpes virus family (Epstein et al. 1996; Melnick et al. 1996; Menschikowski et al. 2000). A number of facts support this conclusion.

In a large percentage of atherosclerosis patients, antibodies to CMV antigens were found (Musiani et al. 1990). CMV DNA was found in atheroclerosis

in the arterial walls of atheromatous plaques (Hendrix et al. 1990; Schonian and Maisch 1992). It has been shown that the early gene of CMV stimulates SMC proliferation in blood vessels (Yonemitsu et al. 1997). The deposition of intracellular lipids depends on the intake of oxidated low-density lipids (LDL), a process stimulated by CMV infection (Zhou et al. 1996). There are data showing that in young individuals atherosclerosis development may be connected with *Chlamydia pneumoniae* infection (Kuo et al 1995; Menschikowski et al. 2000). Taking into consideration the antiviral and antichlamydial properties of IFNγ, it is logical to assume that this cytokine can have a favorable effect. A number of data show the role of IFNγ in the immune defense against CMV (Gehrz 1992; Lucin et al. 1994; Schut et al. 1994; Heise and Virgin 1995; Orange et al. 1995; Davignon et al. 1996).

The combination of these effects of IFNγ suggests that this cytokine could be used in atherosclerosis prophylaxis.

However, it appears that more studies on the role of IFNγ in this process are needed. Contrary to the data described above, a recent study provides evidence that in the absence of leukocytes, IFNγ can induce atherosclerotic changes (Tellides et al. 2000). It should be taken into consideration, however, that the experimental design in this case is very different — the effect of IFNγ on a human and on a pig artery transplanted into immunodefective SCID mice which do not produce T and B lymphocytes or natural killer (NK) cells. IFNγ stimulates the proliferation of SMC in resting cells (Yokota et al. 1992), as are the cells in these experiments, but it inhibits the proliferation of SMC already induced to divide. Apart from this, there is no doubt that the development of fibrosis, which is an important element of atherosclerosis, is suppressed by IFNγ.

Recent data about the role of IL-10 are also of interest. In atherosclerotic plaques of mice with defective production of this Th2 cytokie increased infiltration of T cells, abundant secretion of IFNγ, and decreased collagen content were found. Administration of IL-10 led to a 60% decrease of the atherosclerotic lesion (Mallat et al. 1999).

On the other hand, the negative role which IFNγ may play in already existing atherosclerotic plaques has to be taken into consideration. The stability of the plaque and its resistance to rupture depend on the size of the atherotic nucleus and the strength of the extracellular matrix coat composed mainly of collagen, elastin, and proteoglycans. IFNγ suppresses the synthesis of these components and on the other hand increases the expression of enzymes that degrade the extracellular matrix (Libby and Aikawa 1998; Libby et al. 1998; Weitkamp et al. 1999). The enzyme lysyloxidase responsible for cross-linking the extracellular collagen and elastin is also suppressed (Song et al. 2000). All this weakens the protective coat and may lead to wall rupture and thrombosis.

Thus, it could be speculated that the role of IFNγ may be two sided — preventive against the development of atherosclerosis and decreasing plaque stability in already formed plaques.

All these data indicate the need of more studies on this problem.

## 11.3 Multiple sclerosis

A number of data show that multiple sclerosis (MS), in which the myelin coat of the nerve fibres is attacked, is due to endogenously produced IFNγ. For example, an increased number of IFNγ-secreting cells (Lu et al. 1993) and an increased level of IFNγ (Beck et al. 1988) are found in MS patients prior to or during attacks. The strongest argument is the fact that injection of IFNγ in MS patients leads to exacerbation of the disease (Panitch et al. 1987), whereas IFNβ inhibits IFNγ secretion and suppresses the symptoms of MS (Rep et al. 1996). The conclusion is that IFNγ is contraindicated in MS and compounds inhibiting its effect have to be used. Besides IFNβ, the soluble receptor of IFNγ (its extracellular domain), which is believed to be not immunogenic (Michiels et al. 1998), may be useful. Peptides imitating the N-terminal end of IFNγ that would block its receptor also may be used. Another possibility could be the inhibition of the electrostatic interactions of IFNγ with the membrane glucosoaminoglycans, which store IFNγ prior to its binding to the high specific receptor. This interaction is inhibited by heparin and by the negatively charged peptide in the C-terminal domain of IFNγ (Douglas et al. 1997).

## 11.4 Psoriasis

There are conflicting data on the involvement of IFNγ in the pathogenesis of psoriasis. This chronic papulosquamous skin disease, which affects about 2% of the U.S. population (Morhenn et al. 1987), is associated with cellular immune mechanisms and is characterized by increased keratinocyte proliferation and intensive mononuclear cell infiltration into the epidermis (mainly T cells and neutrophils) (Baker et al. 1988; Schlaak et al. 1994; Bruch-Gerharz et al. 1996).

Unlike normal keratinocytes whose proliferation is suppressed by IFNγ, psoriatic keratinocytes are less sensitive to this cytokine (Morhenn 1988; Nickoloff et al. 1988, 1989), and it does not affect DNA synthesis in these cells (Schulze and Mahrle 1986). In addition, overexpression of the anti-apoptotic oncogene Bcl-x in psoriatic cells blocks normal apoptotic death and prolongs their life (Wrone-Smith et al. 1995).

The psoriatic plaque lacks two proteins — catepsin D and zinc-alpha(2)-glycoprotein, which in normal epidermis are associated with apoptosis and desquamation. Their expression is increased by IFNγ, but in the psoriatic epidermis it is suppressed (Chen et al. 2000). Because of all these data, the early opinion seems justified that an impaired response to IFNγ is at the basis of this disease (Nickoloff et al. 1989).

There are conflicting data whether the decreased sensitivity of psoriatic keratinocytes to the antiproliferative activity of IFNγ is due to a decreased expression of its receptor. According to some data, in psoriatic lesions these receptors are missing in the upper layers of the epidermis (Scheynius et al. 1992). According to other reports, the receptors are expressed equally well in the normal and in the psoriatic epidermis (Fransson et al. 1995).

However, there are data that the psoriatic keratinocytes are as sensitive to IFNγ as are the normal keratinocytes (Morhenn et al. 1987). It should be also considered that keratinocytes from different anatomical regions respond differently to IFNγ (Brysk et al. 1983; Nickoloff et al. 1984, 1985; Smith and Higgins 1993). There are also data that IFNγ in certain doses suppresses proliferation of keratinocytes and induces class II major histocompatibility complex (MHC) and β2-microglobulins, but in high doses it suppresses the expression of these molecules (Symington 1989).

Normal keratinocytes do not express ICAM-I and HLA-DR, but IFNγ induces these molecules in cell cultures (Basham et al. 1983; Aubock et al. 1985). This leads to increased migration and adhesion of leukocytes in the epidermis (Griffiths et al. 1989a,b; Groves et al. 1993).

An important factor in psoriasis development is the activation of the c-erbB oncogene (the receptor for EGF), which occurs under the influence of TGFα. IFNγ increases its mRNA expression in keratinocytes (Elder et al. 1990). On the other hand, it was found that all cells in the psoriatic lesion produced IFNγ (Barker et al. 1991; Schlaak et al. 1994). The number of IFNγ-producing cells — CD4+ and CD8+ T cells (Szabo et al. 1998), mastocytes (Ackermann et al. 1999), and circulating blood T lymphocytes (Austin et al. 1999) — is also increased. The amount of IFNγ in psoriatic vesicles and in the serum/plasma of psoriatic patients is also considerably increased (Bjerke et al. 1983; Diezel et al. 1983; Gomi et al. 1991; Chodorowska et al. 1998). IL-12, which induces IFNγ, is also increased (Yawalkar et al. 1998).

Attempts to treat psoriasis with IFNγ gave conflicting results. In patients with psoriasis and psoriatic arthritis IFNγ, after initially improving the state of some patients, led to its subsequent exacerbation (Fierlbeck and Rassner 1988, 1990). Improvement of psoriatic arthritis was not observed with daily doses lower than 200 μg (Fierlbeck and Rassner 1988). Injection of IFNγ under the psoriatic plaque led to the induction of HLA-DR, but DNA synthesis was not suppressed and the psoriatic lesion was not eliminated (Schulze and Mahrle 1986). Following an intramuscular administration of IFNγ, improvement was reported in some patients when higher doses were used — 0.25 mg/m$^2$ (Morhenn et al. 1987).

All these data show that IFNγ together with other cytokines plays a role in psoriasis pathogenesis and its use in psoriasis is contraindicated, although there are conflicting data. This also is confirmed by the favorable effect of IL-10 and of the T peptide (an octapeptide from the V2 region of gp10 of HIV), which induces IL-10 and suppresses IFNγ expression (Raychaudhuri et al. 1999).

## *11.5 Chronic granulomatous disease*

Chronic granulomatous disease (CGD) is a rare disease due to an inherited defect of the immune system. The leukocytes (neutrophils, monocytes, macrophages, eosinophils), although retaining their phagocytic ability, are not able to kill the ingested bacteria due to a defect in their NADPH oxidase

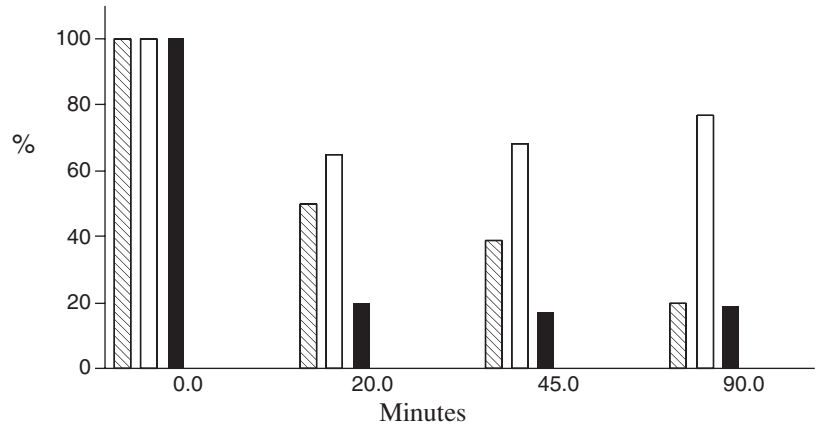


***Figure 11.1*** Effect of IFNγ (0.01 mg/m$^2$ injected subcutaneously for 3 days) on the bacteriocidal activity of monocytes from patients with CGD. (According to data by Sechler et al. 1988.) Healthy controls (▨); CGD patients before (□) and after IFNγ (■). Ordinates show the percentage of surviving bacteria.

system, which normally produces reactive oxygen radicals (superoxide, hydrogen peroxide, and other toxic oxygen metabolites). This system includes the membrane-bound cytochrome b558 and other cytosolic components. The suppressed production of superoxide anions is due to the inhibited activity of genes coding for the subunits of NADPH oxidase (Kuga et al. 1996). The effect of IFNγ may be due to the antagonism with IL-10.

In most affected people (up to 70%) the disease is linked to the X chromosome, and in most cases the defect is within or due to a lack of the heavy chain (91 kDa) of cytochrome b (Royer-Pekora et al. 1986; Dinauer et al. 1987). In the remaining 30 to 40% of the cases the disease is manifested as an autosomal recessive inheritance and is due to the lack of a cytosolic 47-kDa protein which activates the oxidative metabolism of phagocytes. Less often the disease is associated with a defect in the light chain of cytochrome b (Dinauer et al. 1990) or in the cytosolic proteins 47 kDa and 67 kDa (Izu et al. 1992). Due to these defects, affected people are very susceptible to recurrent pyogenic infections — chronic granulomatous inflammation, abscesses in the liver caused by *Streptococcus aureus*, osteomyelitis, invasive infections of *Candida albicans* and others. The infections are manifested even in early childhood, and in small children they may be fatal.

Experimental data show that IFNγ induces the expression of the heavy chain of cytochrome b, increases manyfold the production of superoxides by phagocytes, and leads to the death of the phagocyted bacteria (Figure 11.1).

Multicenter clinical trials show that this cytokine has a good healing effect in this disease (Sechler et al. 1988; Gallin et al. 1991, 1995; Izu et al. 1992; Curnutte 1993; Malmvall and Follin 1993; Lekstrome-Himes et al. 1994) and is used for prevention of infections (Ezekowitz et al. 1991). The treatment is long — about 1 year — with subcutaneous injections of IFNγ three times

a week in doses of 50 μg/m$^2$ when the body surface is larger than 0.5 m$^2$ and 1.5 μg/kg when the surface is smaller than 0.5 m$^2$. This was confirmed by a CGD-affected child treated in Bulgaria for 1 year with subcuataneous injections of Gammaferon three times a week with $10^6$ IU (about 20 μg). The IFNγ therapy leads to increased chances for survival in this disease (Kolbeck 1993). The potential market for treatment of CGD in the United States is estimated to be $10 million (Biotechnology Report 2000).

## *11.6 Skin ulcers in autoimmune vasculitis*

We have not found any experimental or clinical data dealing with the effect of interferons on these skin ulcers in autoimmune vasculitis. It is known that IFNγ suppresses wound healing, possibly because of the suppressed fibroblast proliferation and collagen synthesis (Miles et al. 1994; Fu et al. 1995). However, it was reported that IFNγ blocks mRNA synthesis of the c-cis oncogene, which is the β-chain of PDGF (Suzuki et al. 1989). This oncogene may play a role in vasculitis pathogenesis, which suggests that IFNγ can exert a favorable effect in vasculitis ulcers. Clinical trials in Bulgaria with Virogel G5 confirm this expectation. Several patients with nonhealing ulcers (some multiple) resulting from autoimmune vasculitis were treated. Daily application of the gel on the ulcers was performed until the beginning of epithelization. The results shown in Table 11.2 (Arnaudova et al. 1993) illustrate the favorable effect leading most often to complete healing.

## *11.7 Rheumatoid arthritis*

Rheumatoid arthritis (RA), a chronic disease of not well-elucidated etiology, affects 1 to 2% of the European population, and there is no effective treatment for it. The data on the role of IFNγ in the pathogenesis of RA and the possible use for its treatment are most controversial.

Some properties of IFNγ led to the conclusion that this cytokine plays a pathogenic role in the induction and maintenance of the inflammatory process in chronic diseases such as RA (Obert and Brzoska 1986; Talal and Flescher 1988; Weyand and Goronzy 1999). This idea is based on the fact that IFNγ induces the MHC class II antigens in various types of cells and stimulates the macrophages to produce IL-1, an important mediator of the inflammatory process (see discussion and references in Brzoska and Obert 1987; Lemmel et al. 1987, 1988a,b; Meske et al. 1989; Nakajima et el. 1990; Sprekeler et al. 1990; Duff 1993). It is also reported that IFNγ stimulates a chondrocyte subpopulation to secrete increased amounts of stromelysine (Quintavalla et al. 1993) — a metalloproteinase involved in cartilage degradation in joints affected by RA. It was also found that IFNγ stimulated the proliferation of the synovial fibroblasts (Brinckerhoff and Guyre 1985).

Such data would suggest an increased level of IFNγ in the synovial fluid and in the affected tissues as well as an increased number of T cells synthesizing this cytokine, as reported by a number of investigators (Hooks et al.

***Table 11.2*** Effect of Virogel G5 on ulcers in autoimmune vasculitis

| Patient | Age | Diagnosis | Localization | Number (size, cm) | Duration | Duration of treatment | Degree of healing |
|---|---|---|---|---|---|---|---|
| 1 | 52 | Granulomatosis Wegeneri | Lower limb | (2.5 × 2) | 3 months | 25 days | Complete |
| 2 | 44 | Systemic lupus erythematosus | Head | 3 (1–2) | 40 days | 14 days | Complete |
| 3 | 18 | Systemic sclerosis | Hand (periungual) | Several (0.5) | 2 months | 11–30 days | Partial<br>Complete |
| 4 | 47 | Systemic lupus erythematosus | Lower limb | 1 (3 × 1) | 3 months | 21 days | Complete |
| 5 | 36 | Systemic sclerosis | Hand (periungual) | Several (0.5–1) | 2 months | 20–15 days | Complete<br>Partial |
| 6 | 28 | Diabetes mellitus | Hand, gluteus, back | Several (0.5–2) | 3–4 months | 14, 20, 30 days | Partial<br>Partial complete |
| 7 | 20 | Systemic sclerosis | Hand (periungual) | Several (0.5) | 4 months | 30 days | Complete |

1979; Cesario et al. 1983; Degre et al. 1983; Hopkins and Meager 1988; Saxne et al. 1988; Yocum et al. 1989; Kusaba et al. 1998; Isomaki et al. 1999; Ronnelid et al. 1999; van der Graaf et al. 1999; Yin et al. 1999; Berner et al. 2000; Canete et al. 2000; Yudoh et al. 2000).

Also, there are data describing a positive correlation between the number of IFNγ-producing cells and the severity of the disease (Schuerwegh et al. 1999). The increased level of IFNγ in RA is explained by the increased expression of IL-12 in synovial tissues (Morita et al. 1998; Kim et al. 2000a). IL-18, which is responsible for a Th1 response, is believed to have the same effect (Gracie et al. 1999). The same holds true for the IFNγ-stimulating IL-12 that has caused a strong exacerbation of RA after its administration in a case of a metastatic cervical cancer (Peeva et al. 2000). The conclusion was also drawn that the *in vitro* differentiation of peripheral T cells toward a Th2 profile is disturbed in RA (Asselin et al. 1998).

In agreement with the above data are the results of different approaches for the treatment of RA. Thus, the favorable results after treatment with dexamethasone are associated with increased IL-10 and decreased IFNγ levels (Verhoef et al. 1999). The fact that IL-4 was found more often when single joints were affected than in polyarthritis and frequent combination of IL-4 and IL-10 in nonerosive than in erosive RA led to the conclusion that these two Th2 cytokines played an anti-inflammatory and disease-limiting role (Murray et al. 1998). There are also data showing that the anti-inflammatory role of methotrexate in the treatment of RA leads to increased IL-4 and IL-10 levels and decreased IFNγ; that is, to a shift from Th1 to Th2 cytokines (Constantin et al. 1998). Such a shift is also observed during treatment of RA with cyclosporin (Kim et al. 2000b). Russian investigators even report treatment of RA with anti-IFNγ antibodies (Lukina et al. 1998).

In contrast to the above data, other studies show that IFNγ is missing or is present in lower concentration in RA joints, its production is also strongly decreased, and a Th2-type profile prevails (Hasler et al. 1983; Husby et al. 1985; Lotz et al. 1986; Ridley et al. 1986; Firestein and Zvaifler 1987; Malaise and Franchimont 1987; Seitz et al. 1987; Herzog et al. 1988; Stolzenburg et al. 1988; Zangerle et al. 1992; Chen et al. 1993a; Kanik et al. 1998). The favorable effect of the application of anti-TNFα antibodies (Elliot et al. 1993) is associated with an increased number of IFNγ-synthesizing cells and a considerable increase in the Th1/Th2 ratio (Maurice et al. 1999).

A predominating Th2 profile was found in erosive RA, which subsequently turned into Th0 and finally into Th1 (Aarvak et al. 2000). IL-11 expression (a type 2 cytokine) was found associated with osteoclast differentiation and destruction of the joints in RA. Its synthesis was partially dependent on prostaglandin E2 (PgE2). It is interesting that IFNγ inhibits IL-11 production in cultures of rheumatoid synovial fibroblasts stimulated with IL-1a but not in freshly isolated cells of the same type (Taki et al. 1998); a fact that shows the complexity of the cytokine/synovial cell relationship in RA.

In another study, in early synovitis, increased IL-2 and IFNγ (Th1) levels were found in the blood, whereas in chronic RA IL-6 and IL-10 were

increased (Th2) (Kanik et al. 1998). These data are in disagreement with the report that the cytokine profile does not depend on the stage of the disease.

Concerning the concentration of IFNγ in the blood, the data also are contradictory. According to some data, the concentration of IL-4 in the blood is higher and that of IFNγ is lower (van Roon et al. 1997; Swaak et al. 1997; Haddad et al. 1998; Raziuddin et al. 1998). Other investigators find a dominant Th1 profile (Aarvak et al. 2000; Yudoh et al. 2000) or do not find any changes in the Th1/Th2 ratio (van der Graaf et al. 1999; Berner et al. 2000).

It has been reported that chondrocytes stimulated with IFNγ to produce class II MHC are more resistant to lysis (Yamaga et al. 1993). It has been found that IFNγ completely eliminates the bone resorption caused by IL-1 and TNF (Gowen et al. 1986).

The paradox of data showing both a stimulatory and an inhibitory effect of IFNγ on the inflammatory reaction in RA is hard to explain, but the following possibilities or a combination of them could be proposed:

- The complex cytokine network (Kawade 1990), including several negative and positive feedback loops, leads to many interactions, the results of which depend on a number of exogenous and endogenous hard-to-control factors.
- The hypothesis has been proposed that IFNγ applied locally stimulates the rheumatoid process, whereas its systemic application would inhibit it (Billiau 1987; Heremans and Billiau 1989). This, however, does not agree with data on its healing effect (see below) obtained in Germany after systemic application and in Bulgaria following its topical application.
- Another possible hypothesis is that the effect of IFNγ depends on the activity of the macrophages. Normal and slightly activated macrophages would be induced by the IFNγ to produce IL-1 causing inflammation, whereas IFNγ would suppress its production in strongly activated macrophages, as is the case of RA (Brzoska and Obert 1987; Klein 1988).
- It is especially important to consider the fact that the effect of IFNγ is dependent on its concentration, which has an optimum for activity (Kleinerman et al. 1986). As seen from literature data (Lemmel et al. 1988a,b; Obert et al. 1989a,b; Obert and Brzoska 1989b), and from clinical trials in Bulgaria (see below), it appears that the lower doses are more effective in this disease.
- Another very important fact is the finding of a high level of soluble extracellular IFNγ receptor (sIFNγRα) in the plasma of RA patients (Bello et al. 1998). The role of sIFNγR as a blocker of IFNγ leaves open the question of the actual concentration of this cytokine as a free, active ligand that can bind to the cellular membrane receptors.
- We shall also draw our attention to the role of the two complement-regulating factors — factor H and FHL-1 (reconectin) — the result of

alternative processing of the same gene transcript. This gene is induced by the proinflammatory IFNγ as well as by the anti-inflammatory dexamethasone. The gene is expressed in the synovial fibroblasts and its products are found in the synovial fluid of RA patients, where they play a regulatory and a protective role (Friese et al. 2000). This could explain the seemingly contradictory fact of the same effect of dexamethasone and of IFNγ.

- The level of a cytokine in the blood and at the site of the inflamed joint is often different, which appears to be due to its differential distribution between these two compartments.
- Finally, we should also mention the effect of many factors on the determination of cytokine level, such as the standards of different manufacturers, the different methods used, and so on (Aziz et al. 1999).

What are the results from the clinical application of IFNγ?

In Germany this cytokine is used for treatment of RA by parenteral administration (Obert and Hofschneider 1985; Obert and Brzoska 1986; Brzoska and Obert 1987; Lemmel et al. 1987, 1988a,b; Klein 1988; Veys et al. 1988; Sprekeler 1988; Sprekeler et al. 1988, 1990; Meske et al. 1989; Obert and Brzoska 1989a,b; Obert et al. 1989a,b). The results obtained show a favorable effect in about 60% of all cases, and the known side effects have been reported (Obert and Brzoska 1989a,b). The most serious adverse effect was the induction of anti-DNA antibodies in single cases (Seitz et al. 1988a; Sprekeler 1988). In a review, however, the opinion was expressed that the clinical trials of RA treatment with IFNγ were variable and "the use of IFNγ in rheumatoid arthritis has not yet been established as being of high value" (Duff 1993).

Taking into consideration the conflicting data and the side effects upon parenteral administration, trials have been conducted in Bulgaria by local treatment of the affected joint with IFNγ-containing gel (Virogel G5, with 2 μg IFNγ/g gel).* After gel application, Gammaferon was helped to penetrate through the skin pores by treatment of the joint with ultrasound (sonophoresis, see Chapter 8, Section 8.2) at a therapeutic frequency of 1 Mhz, 0.6 W/cm$^2$ for a period of 6 to 8 min every day until improvement was recorded. The treatment was performed on 14 patients with rheumatoid gonitis. One knee was used as a placebo control. The results in Table 11.3 show the rapid subjective and objective improvement without any side effects.

These preliminary clinical data are in agreement with the results obtained in Germany after systemic adiministration of IFNγ. They justify the performance of more extensive clinical trials with local application of IFNγ as Virogel G5 and sonophoresis. Studies for elucidation of the reasons for the conflicting results should also continue.

We should also mention the positive results with IFNγ in chronic juvenile arthritis resistant to other treatment (Coto et al. 1998).

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***Table 11.3*** Effect of IFNγ (Virogel G5 applied with sonophoresis) on different symptoms of RA (average data from 14 patients)

| Symptom | IFNγ | $p^c$ | Placebo | $p^a$ |
|---|---|---|---|---|
| Swelling[a] decrease (cm) | 1.57 ± 0.22 | $p < .0001$ | 0.41 ± 0.37 | Not significant |
| Swelling[b] decrease (cm) | 1.23 ± 0.33 | $p < .003$ | 0.05 ± 0.33 | Not significant |
| Flexion % increase | 21.8 ± 2.5 | $p < .0001$ | 12 ± 5 | Not significant |
| Leukocytes in synovial fluid % decrease | 66.2 ± 7.3 | $p < .0001$ | ND | — |
| Ragocytes in synovial fluid % decrease | 77.5 ± 9.4 | $p < .001$ | ND | — |

[a] Circumference over the patella.

[b] Circumference above the patella.

[c] *p*: statistical significance according to Student; ND – not determined.

## *11.8 Osteopetrosis*

Osteopetrosis is a rare inherited disorder caused by a disturbed balance between the activity of osteoblasts building the bone and that of osteoclasts responsible for bone resorption. The ineffective function of osteoclasts results in bones that are abnormally dense and heavy, yet easily broken. The increased bone mass can limit the available bone marrow space, which leads to anemia (reduced production of erythrocytes), bleeding (reduced platelets number), and infections (reduced leukocyte number). When the bones are dense enough to prevent blood vessels and nerves to pass through them, blindness, deafness, and stroke can occur.

It is manifested as an autosomal disease in three forms: (1) early, infantile ("malignant") form, exhibited in the first months after birth, and the affected children die during the first 10 years of life; (2) late form affecting adults with a good prognosis, since 50% of affected people may be asymptomatic; (3) intermediate form affecting children but with a better prognosis than the early, infantile form.

Bone marrow transplantation which replaces the abnormal osteoclasts with healthy ones has been used with some success to treat the most severe infantile form. Calcitrol and glucocorticoids are used in the late form (for reviews, see Carolino et al. 1998).

We mention this disease since a good healing effect with IFNγ was found (Key et al. 1995), and successful clinical trials have been conducted in the United States with Actimmune (IFNγ-1b), supporting the Food and Drug Administration (FDA) approval of such treatment. The potential market for Actimmune to treat osteopetrosis in the United States is estimated to $10 million annually.

## *11.9 Allergic disorders*

Allergic states, such as atopic dermatitis, asthma, and other various allergic reactions, undoubtedly are genetically determined, but do not follow the classic Mendel's laws of inheritance (for review, see Borish 1999), probably due to their multigenic character.

All allergic disorders are associated with hyperimmunoglobulinemia E (HIE syndrome). The IgE molecules play a central role in the pathogenesis of the primary hypersensitivity reaction by binding the high-affinity IgE receptors of the mastocytes and causing secretion of mediators and pro-inflammatory cytokines after exposure to allergens (for review, see Leuing 1999). These cytokines (IL-4, IL-5, IL-10, IL-13) are produced by the Th2 cells (Haczku et al. 1996; Till et al. 1997; Haas et al. 1999). Domination of Th2 cytokines over the Th1 type is a major characteristic of allergic reactions. The disturbed balance between Th1 and Th2 lymphocytes leads to domination of the humoral immunity components over those of the cellular immunity (see Chapter 1, Figure 1.1) (Hilkens et al. 1996b; Kapsenberg et al. 1996; Haas et al. 1999; Leung 1999). It has been observed that some lectins may cause a Th2 response (Haas et al. 1999).

In some cases, the Th1 subpopulation is affected, and in others the Th2, but the Th1/Th2 ratio always decreases. This leads to esinophilia and increased IgE content. It was found that in allergic patients the number of IL-4–producing CD4+ cells increased more than twofold and that of CD8+ sixfold as compared to nonallergic patients, which leads to an increased IFNγ level (Meissner et al. 1997). It was shown that IFNγ regulated IgE production in HIE syndrome (King et al. 1989). In a mouse model it was found that only aerosolic IFNγ was active in the allergic sensitization of airways, but it was not active if administered parenterally (Lack et al. 1994). In the same model aerosolic IFNγ suppressed the overreactivity of airways, whereas parenterally administered IFNγ suppressed the IgE level and the cellular infiltration (Hofstra et al. 1998).

The production of other Th2 cytokines — IL-5, IL-10, and IL-13 — also is increased (see above). Mice lacking an IFNγ receptor maintain a Th2 cytokine profile and have a disturbed ability to deal with esinophilic lung infiltrates (Coyle et al. 1996). In antigen-sensitized mice esinophilic infiltration occurs in the trachea, which is prevented by intraperitoneal injection of IFNγ (Iwamoto et al. 1993) or of IL-12 (Iwamoto et al. 1996) and by its application as an aerosol (Nakajima et al. 1993).

These data suggest the use of IFNγ in allergic states, where its effect is different in atopic and nonatopic conditions (Reinhold et al. 1993). It is important to point out the effect of corticosteroids on allergic states. The latest data show that treatment of macrophages with dexamethasone inhibits the synthesis of IL-12 and subsequently of IFNγ. This increases the synthesis of IL-4 by CD4+ T cells. Thus, the corticosteroids, although they directly inhibit the induction of Th1 and of Th2 cytokines (Braun et al. 1997; De Kruyff

et al. 1998), actually indirectly stimulate the production of Th2 cytokines through the inhibited synthesis of IL-12. Therefore, treatment of allergic diseases with corticosteroids runs the potential risk of exacerbating the allergic symptoms due to overproduction of Th2 cytokines (De Kruyff et al. 1998).

### *11.9.1 Atopic dermatitis*

Atopic dermatitis is manifested as a chronic recurrent inflammation of the skin. Its incidence increased from 3% in 1960 to 10% during the last few years. The disease starts in early childhood. In 60% of the patients the symptoms of the disease are manifested during the first year of life and in 90% until the fifth year. In the most severe cases there is a 50% chance of developing a permanent disease. The patients have an increased susceptibility to skin infections caused by bacteria, viruses, and fungi. They develop pustules and impetigo from *Streptococcus aureus*, herpes simplex, herpes zoster, and other viruses with complications such as herpes keratitis, darkening of the cornea, and even blindness.

Much data, mostly from the last few years, show that atopic dermatitis is associated with a defect of the immune system manifested as an imbalance between the Th1 and Th2 helper T lymphocytes, with domination of the Th2 and inhibition of the Th1 phenotype (Gruner et al. 1990; Boguniewicz and Leung 1992; Bohm and Bauer 1997; Koning et al. 1997; Leonard et al. 1997; Motoki et al. 1997; Nakazawa et al. 1997; Campbell et al. 1998; Nakagawa et al. 1998; Teramoto et al. 1998; Jung et al. 1999a) (see Chapter 1). In children with atopic dermatitis it was found that not only the number of cells synthesizing IFNγ decreased, but also the amount produced by each individual cell (Campbell et al. 1999). The disturbed ratio between IFNγ on the one hand and IL-4, IL-10, and IL-13 on the other leads to overproduction of IgE by B lymphocytes, to esinophilia, and to hyperstimulation of the Langerhans' cells in the skin which contain IgE receptors on their surface. In patients with atopic dermatitis a considerable decrease is observed in the percentage of CD3+/CD8+ peripheral T cells expressing IFNγ and an increased percentage of CD3+/CD8- T cells expressing IL-4 (Ferry et al. 1997).

It should be mentioned that in children with atopic dermatitis, increased IFNγ mRNA was found but a decreased content of IFNγ. This means that the defect in its production may be at the level of translation (Koning et al. 1997). There are data that the IFNγ deficit is not due to a lack of cytokines which induce it (such as IL-12, IL-2, IL-18) (Jung et al. 1999b).

All of these data show that IFNγ should exert a favorable effect by recovering the immune balance. Some data show that the atopic keratinocytes are extremely sensitive to IFNγ (Pastore et al. 1998). This is confirmed by clinical data where IFNγ was administered subcutaneously usually in doses of 50 μg/m$^2$ daily or every other day for 4 weeks and even up to 22 to 24 months (Hanifin et al. 1993; Reinhold et al. 1993; Musial et al. 1998; Noh and Lee 1998; Schneider et al. 1998; Stevens et al. 1998; Ellis et al. 1999; Jang et al. 2000). Improvement of the pathological symptoms was observed

and the interferon was well tolerated. In one clinical study it was found that favorable results were obtained with IFNγ. This therapy was recommended when esinophils were increased by less than 9% and the level of IgE was lower than 1500 IU/ml (Noh and Lee 1998).

When "rash" immunotherapy was used against sensitizing allergens, it was shown that IFNγ production by CD4+ T cells activated by the allergen was increased (Lack et al. 1997).

### *11.9.2 Asthma*

Asthma attacks are associated with a mechanism which is induced by an increased IgE level due to a disturbed balance between IFNγ and IL-4. In asthma the peripheral blood mononuclear cells spontaneously synthesize more IgE than those of normal individuals. IFNγ suppresses the synthesis of IgE induced by IL-4 in asthmatic individuals (Lui and Xing 1997). The continuous treatment with pollen of peripheral mononuclear cells isolated from asthmatic individuals leads to a decrease in the IFNγ/IL-5 ratio (Lagging et al. 1998). This also has been found for bronchoalveolar asthma (Kimura et al. 1999). An increased IL-4/IFNγ ratio is also found in children with moderate and severe asthma (Hoekstra et al. 1997; Nurse et al. 1997). In allergic asthma it was found that the production of IL-12 and of IL-12–dependent IL-8 secretion was also decreased (van der Pouw-Kraan et al. 1997).

A decreased IFNγ production in children with bronchiolitis is an indication of decreased pulmonary function and increased sensitivity to histamine after recovery of the disease and is associated with the subsequent development of asthma (Renzi et al. 1999).

In experiments with mice it was shown that IL-12 suppressed the allergic inflammation of the airways by inducing the synthesis of IFNγ (Bruselle et al. 1997). Decreased production of IFNγ due to decreased synthesis of IL-12 was also found in people suffering from allergic asthma (Van der Pouw Kraan et al. 1997). The CD8+ T cells are also an important source of IL-4 in asthmatic individuals (Stanciu et al. 1997). It has been shown that the favorable antiasthmatic effect of theophylline is due to the suppression of IL-4 synthesis (Tohda et al. 1998). Asthma attacks are also associated with increased levels of IL-6 and TNFα (Subratty and Hooloman 1998).

The reason for the altered relationship between Th1 and Th2 cells in asthma has not yet been elucidated. In any case, it is not due to a damaged IFNγ gene. No mutations in this gene were found in 265 asthmatic patients (Hayden et al. 1997).

It is of interest to mention the role of the smooth muscle cells of the airways concerning their reaction to the production of Th1 and Th2 cytokines. Such atopic cells from asthmatic individuals initially synthesize Th1-type cytokine mRNA (of IFNγ, IL-2, and IL-12) upon allergen sensitization, which is followed by an increased expression of Th2 cytokines (IL-5 and granulocyte-macrophage colony-stimulating factor [GM-CSF]). In isolated allergy-affected smooth muscle cells from the airways of asthmatic individuals, treatment

with IFNγ or with IL-2 leads to the elimination of their increased constrictive response to acetylcholine and their decreased relaxation response to isoproterenol (Hakonarson et al. 1999).

In contrast to the data presented above there are reports of an increased level not only of IL-4 and IL-5, but also of IFNγ in the serum of asthmatic patients, in whom a correlation is found between the asthmatic attacks and the increased IFNγ level but not with the level of IL-4 and IL-5 (Ten Hacken et al. 1998). There are data that along with the presence of the Th2 type in asthma, the increased level of IFNγ in the blood is due to the CD8+ T cells (Magnan et al. 2000). In contrast to the main mechanism of the allergic state are data that alveolar macrophages secrete more IFNγ and less IL-10; that is, Th1 cytokines predominantly play a pathogenic role in asthma (Cembrzinska-Nowak et al. 1993). This has justified the idea of steroid inhalation, which would shift the T-cell ratio in favor of the Th2 profile (John et al. 1998).

The reason for these contradictory data is not clear given the fact that the majority of data support a therapy that shifts the cytokine profile from Th2 to Th1 (Barnes 1996). It could be hypothesized that in some cases this is associated with increased NO production by the epithelial cells of the airways. The increase in NO is of considerable importance for the development of asthma, and these cells express NO synthase II (Guo et al. 2000), whose expression is suppressed by corticosteroids. It is possible that IFNγ, which can induce the synthesis of NO (see p. 33), may have a negative effect. The balance between its concentration and that of the other cytokines is probably of importance. There also are data that IFNγ is not effective for the treatment of steroid-dependent asthma (Boguniewicz et al. 1993).

### *11.9.3 Other allergic reactions*

IgE plays a major role in allergic reactions and in the pathogenesis of the fast hypersensitivity reaction due to its ability to specifically bind the high-affinity receptors on the mastocytes and to stimulate in this way the production of mediators and proinflammatory cytokines after exposure to allergens. Eosinophils and T lymphocytes of the Th2 type producing IL-4, IL-5, IL-10, and IL-13, but not IFNγ, dominate during the late response (for review, see Leung 1997, 1998). The number of cells synthesizing IL-4 is increased in atopic organisms in response to allergens (Gabrielsson et al. 1997).

For example, food allergies are associated with the stimulation of IL-4 (without IFNγ suppression, unlike atopic dermatitis) leading to the production of IgE (de Jong et al. 1996; Campbell et al. 1998).

In allergic rhinitis the immune response also is of the Th2 type (Benson et al. 2000; Moverare et al. 2000). In some cases, no changes in IFNγ and IgE were found, but the synthesis of IL-5 was induced (Ohashi et al. 1998). In other studies of allergic rhinitis increased IL-4 and IL-5 mRNA expression was found in the nasal mucosa of patients following exposure to the allergen (Lee et al. 1997a), and an increased IL-5/IFNγ ratio and elevated IL-10 were

found in the nasal lavage at the beginning of the pollen season (Benson et al. 1997). Spontaneous expression of IL-4 mRNA is observed in cases of pollen allergies, unlike in healthy individuals. Decreased IFNγ with increased IL-4 and IL-5 synthesis is found only during the pollen season (Munoz-Bellido et al. 1998). Specific immunotherapy led to a decrease in IL-4 expression during the pollen season (Soderlund et al. 1997). According to a different study of pollen rhinitis, IL-4 was also increased and IFNγ decreased, but the favorable effect of the immunotherapy was mostly due to modulation of the Th2 cells rather than of the Th1 cells (Ohashi et al. 1997).

A study of allergies to house dust containing the *Dermatophagoides pteronyssinus* allergen also showed a considerably increased IL-4 mRNA expression as compared to that in nonallergic individuals, whereas no differences in the IFNγ level were found (Laan et al. 1998).

Decreased production of IFNγ by peripheral mononuclear cells stimulated with a milk allergen was found in children allergic to cow milk (Sutas et al. 1997). Contrary to this study, increased secretion of both Th1 and Th2 cytokines in the blood was reported in children allergic to cow milk (Hauer et al. 1997). Increased IL-4 and IL-13 transcripts and the absence of IL-2 and IFNγ were found in allergic conjunctivitis (Fujishima et al. 1997). After chronic sensitization of the skin with trinitrochlorobenzene, a shift of the cytokines from a Th1 to a Th2 profile also was observed (Kitagaki et al. 1997).

Decreased IFNγ secretion was found in newborns, who exhibited susceptibility to allergies later in life, and it was due to the disturbed function of the costimulatory mechanisms (Pohl et al. 1997).

## *11.10 Lupus erythematosus*

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by a hyperactivation of the B cells, production of autoantibodies, and deposition of immune complexes in vitally important organs. SLE patients have a defect in the antigen presentation system, which is associated with a decreased expression of the B7-1 costimulatory molecules induced by IFNγ (Tsokos 1996; Tsokos et al. 1996).

It is generally accepted that the pathogenesis of SLE is associated with a disturbed balance in the cytokine network (in the Th1/Th2 phenotype ratio), although to a certain extent the data are contradictory. In autoimmune mice IFNγ suppressed the sensitivity of B lymphocytes to the proliferation-stimulating effect of IL-4 (Minoda et al. 1991). In a mouse model of SLE IFNγ also suppressed B-cell proliferation. Low doses of IFNγ in combination with the immunosuppressant FK506 is recommended for suppression of a polyclonal B-cell proliferation (Minoda et al. 1992). After improvement of the disorder by treatment with methotrexate, the cytokine profile was normalized, which showed the role of the Th1/Th2 ratio for the progression of the disease (Segal et al. 1995). A mouse model of lupus nephritis antibodies

against IFNγ did not influence the progression of the disease, which disproves the role of this cytokine in its pathogenesis (Nicoletti et al. 1992).

In contradiction with these data are several experiments in mice which develop an autoimmune disease similar to SLE in humans and die from glomerulonephritis. In these experiments soluble IFNγ receptors and monoclonal antibodies against IFNγ increased the survival rate of the mice, whereas application of IFNγ reduced it. This has supported the idea of using IFNγ inhibitors to treat the early stages of the disease (Ozmen et al. 1995). In other experiments also with mice susceptible to lupus, no clear Th1 or Th2 cytokine profile was found — there was an overexpression of transcripts of both types of cytokines (IL-β1, IL-10, and IFNγ) in their lymph nodes (Prud'homme et al. 1995). In another experiment with such mice the IFNγ/IL-4 ratio was increased as compared to the normal level, and as the animals aged and the disease progressed, the ratio increased, whereas in normal animals it decreased (Shirai et al. 1995).

In humans, however, most data from SLE patients support the role of the Th2 cytokines in the pathogenesis of the disease, although some controversies still remain. It was found that IFNγ restores HLA class II expression on T cells and activates the T helper cells without influencing their proliferation (Volk and Diamantstein 1986). In another study of SLE patients an increased IFNγ and a decreased IL-4 levels were found, and the conclusion was drawn on the role of this shift in the pathogenesis of the disease (Funauchi et al. 1991a,b). Other investigators report highly increased IFNγ and IL-6 in SLE patients with lymphadenopathy and nephrotic syndrome (al-Janadi et al. 1993). Some SLE patients had an increased IL-10 serum level, which supports treatment with antagonists of IL-10, such as its soluble receptors, or Th1 cytokines (Ishida 1994). And in fact the administration of anti–IL-10 antibodies delayed the development of the autoimmune reaction (Ishida et al. 1994). This is also in agreement with other observations of increased Th2 (IL-4, IL-6, IL-10) expression and low or undetectable expression of Th1 cytokines (IL-2, IFNγ) in SLE patients (Richaud-Patin et al. 1995; Hagiwara et al. 1996a). Such a profile explains the strong effect on B-lymphocyte proliferation and differentiation associated with the overproduction of autoantibodies. In a different study, however, no change in the Th1/Th2 ratio was found except in lupus-induced glomerulonephritis where the Th1 type was dominant (Akahoshi et al. 1999). Others also found a decreased NK activity associated with a defect in IFNγ and IL-2 production in SLE patients (Gaspar et al. 1988). However, incubation of the mononuclear cells with IFNγ or IL-2 did not improve their cytotoxicity.

It is hard to speculate about the reasons for these contradictory data after taking into consideration the complex concentration-dependencies of the cytokine activities, the differences in the reactivity of the organism, the differences in cytokine expression at the level of transcription and translation, and so on. The prevalent conclusion from studies on humans is that a dominant Th2 cytokine profile is of importance for the development of the

disease. In agreement with this conclusion is one clinical trial where a good effect has been observed after treatment with IFNγ (Gammaferon) in combination with cyclophosphamide (Egorova and Balabanova 1994).

Our experience with ulcers associated with SLE shows that local application of IFNγ (Virogel G5) stimulates their healing (see Table 11.2).

## *11.11 Severe traumas, burns, and surgical operations*

The most common deviation in immune response that occurs after heavy trauma is the decreased ability of the monocytes to express MHC class II antigens (HLA-DR) on their surface. Their normal expression is restored toward the end of the healing process, but the expression did not return to normal levels if the outcome was fatal (Hershman et al. 1988, 1989). This decreased function is considered to be critical in cases of infected severe traumas (Polk et al. 1992). It has been shown that although IFNγ does not affect HLA-DR expression in healthy organisms, it considerably increases this expression after severe traumas (Hershman et al. 1989). The same also was found in experiments on mice with artificially infected wounds, where IFNγ induced the expression of the Ia antigen (MHC class II in mice) and favorably influenced their survival (see Polk et al. 1992 and references therein). Defective Ia expression in mice also was found after hemorrhagic shock (Ayala et al. 1993). The decreased immune resistance after hemorrhagic shock in rats was restored following treatment with IFNγ (Livingston et al. 1988).

Therefore, severe traumas dramatically decrease the function of the Th1 cells *in vivo*, and the recovery of this function should have a favorable effect (Kelly et al. 1999). There are data that this functional defect is not due to an increase in Th2 activity but to an overall T helper cell dysfunction (annergy) leading to suppression of cytokine production (Meert et al. 1998; Puyana et al. 1998; De et al. 2000). The application of IFNγ (100 μg daily, subcutanously for 21 days) did not lead to coagulopathy and fibrinolysis as supposed (Dries et al. 1998).

Severe burns also lead to an immune deficit. It has been found that after a severe burn the NK activity of the peripheral lymphocytes is only 30% of that of controls. There are data for a shift of the cytokine profile from Th1 to Th2 type (Takagi et al. 1998) occurring with the participation of CD8+ T cells (Zedler et al. 1999). In mice the Th2 profile with decreased production of IL-2 and IFNγ, which occurs after burns, continues for up to 14 days until the normal Th1 profile is restored (Hunt et al. 1999). For the shift of the cytokine profile after burns, it is proposed that a role is played by the production of nitrogen derivatives by the macrophages (Schwacha and Somers 1998).

The data on the role of the cytokine IL-10 are controversial. According to a study in mice, this cytokine does not play an essential role for survival and for the modified immune function after burns (Kavanagh et al. 1999). According to other data, again from experiments with mice (Lyons et al. 1999), IL-10 plays a role in the immune suppression immediately after

burns. The investigators believe that inhibition of this cytokine from the first day after a burn would be useful for the reestablishment of the disturbed immune function.

Despite the expectations, clinical trials did not find IFNγ and IFNα to be useful (Stein et al. 1984). Also, trials with IFNγ-1b conducted in 23 clinical centers for burn victims did not find any differences in the progression and outcome of burns upon the application of this interferon (Wasserman et al. 1998).

In a model of corrosive burns of the oesophagus in rats, consecutive treatment with EGF and IFNγ considerably decreased residual stenosis (Berthet et al. 1994a,b).

The formation of hypertrophic scars following burns is associated with collagen deposition and is due (at least in part) to the increased expression of IL-6 by fibroblasts isolated from such scars (Xue et al. 2000). As we mentioned before, this cytokine is suppressed by IFNγ.

Regardless of the unsatisfactory clinical results, the experiments and clinical trials for the role of IFNγ in all types of severe traumatic damage should be continued (Polk et al. 1992).

# *chapter twelve*

# *Malignant diseases*

## *12.1 Background for using IFNγ in malignancies*

Several activities of IFNγ justify its using for treatment of malignant diseases. They include its antiproliferative, immunomodulatory, and antitumor activities, its activating effect on cellular immunity as a member of the Th1 cytokines (see Chapter 3), and also its possible protective role in malignant transformation.

Decreased production of Th1 cytokines by stimulated peripheral leukocytes is found in malignant tumors. In patients with cancer of the kidney, prostate, and bladder the level of Th1 cytokines (IFNγ and IL-2) was decreased and the level of Th2 cytokines (IL-4, IL-6, IL-10) was not affected. These data led to the conclusion that it is the function of the Th1 cytokines which is disturbed, but there is no shift from a Th1 to a Th2 profile (Elsasser-Beile et al. 1998, 1999). The serum of cancer patients (breast cancer, colorectal cancer, ovarian cancer) contains substances which suppress the synthesis of IFNγ by phytohemaglutinin (PHA)-stimulated peripheral blood mononuclear cells (Muster et al. 1996).

In experiments with mice it has been shown that the endogenous IFNγ is a factor controlling carcinogenesis, both chemical and spontaneous. Mice with a defective IFNγ receptor, as well as with a defect in the STAT-1 metabolic pathway (see Chapter 4), develop more tumors and in a shorter period than normal mice following treatment with methylcholantrene. Also, such mice are not sensitive to IFNγ and after additional inactivation of the p53 tumor suppressor gene ($p53^{-/-}$ mice) develop a wide spectrum of tumors as compared to only p53 knockout mice (Kaplan et al. 1998).

IFNγ increases the activity of lymphokine-activated killer cells (LAK), tumor-infiltrating leukocytes (TIL), and macrophages in pleural exudates of melanoma and breast and kidney cancer patients. Thus, the combination of IFNγ and low doses of IL-2 appears to be useful in immunotherapy (Papamichail and Baxevanis 1992).

The TIL, which play a major role in tumor cell elimination, cannot exert their tumorocidal activity in the absence of IFNγ (Tuttle et al. 1993). The

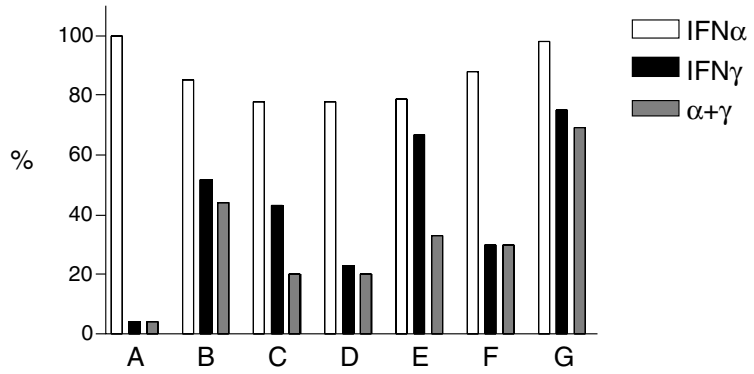


***Figure 12.1*** Growth (in %) of different human tumor types treated with IFNα, IFNγ, or with their combination (500 IU/ml). (According to data by Denz et al. 1985.)
A. Colon adenocarcinoma (CCL218)
B. Lung adenocarcinoma (CCL185)
C. Breast cancer (BT20)
D. Laryngeal cancer (KB)
E. Histiocytic lymphoma (U937)
F. Myeloma (U266)
G. Acute T-cell leukemia (MOLT4)

ability of TIL to lyse autologous tumor cells is strongly increased after their incubation with IFNγ (Wiebke et al. 1990). The intensity of tumor infiltration by TIL positively correlates with the expression of IFNγ, tumor necrosis factor (TNF), and IL-2. It was found, however, that in some tumors (breast and ovarian) TIL rarely express these cytokines (Vitolo et al. 1992).

In malignant tumors, as in allergic diseases, an important role is played by the Th1/Th2 cytokine ratio. In a number of malignant tumors (melanoma, colon cancer, lung cancer, ovarian and kidney cancer, and sarcomas) local immunosuppression was found in the tumor bed with a predomination of Th2 (IL-10) cytokines (Spellman et al. 1996). Gradual loss of the Th1 population in the spleen was found in mice upon progression of the tumor growth (Ghosh et al. 1995). All these data indicate the rationale of using factors (such as IFNγ) that change this profile in the direction of the Th1 cytokines. An example of the growth suppression of different malignant cells by IFNγ, IFNα, and their combination is shown in Figure 12.1.

Results which contradict this concept also have been reported. The report that IL-4 (a Th2 cytokine) inhibits the growth of a number of tumors which express its receptor (IL-4R) was unexpected (Morisaki et al. 1992; Obiri et al. 1994). The effect was synergistically increased in combination with IFNγ and TNFα (Morisaki et al. 1992). On the other hand, however, it was found that incubation of TIL with IL-4 did not lead to an increase of their antitumor cytotoxicity (Shimizu et al. 1991). Unusual was also the report that IFNγ inhibited the growth of the OVCA cell line by increasing IL-6 expression (Offner et al. 1995).

The use of IFNγ for immunotherapy of malignant tumors may be applied in seven purposes:

**For a direct stimulation of the cellular immunity.** IFNγ stimulates the tumorocidal activity mainly of the macrophages by inducing nitric oxide (NO) production (see Section 3.6). The role of IFNγ-stimulated macrophages can be seen from experiments with genetically defective mice (CBA/HeJ, A/J, and P/J) whose macrophages are unable to develop antitumor activity after stimulation with IFNγ (Boraschi et al. 1984).

It should be taken into consideration that macrophages from different anatomical regions of cancer patients exhibit different sensitivities to cytokines. Thus, IL-6 inhibits the tumorocidal activity of alveolar macrophages in lung cancer patients, but this effect is not observed with peritoneal macrophages of ovarian cancer patients and with peripheral monocytes in both types of cancer. However, in both cases IFNγ in combination with lipopolysaccharide (LPS) stimulates the tumorocidal activity of peritoneal macrophages and peripheral monocytes (An et al. 1996).

There are different data regarding the effect of IFNγ on the LAK and natural killer (NK) cells (Platsoucas 1986; Wiebke et al. 1990; Yanagawa et al. 1990). According to some investigators, IFNγ decreases LAK activity in healthy individuals (Yanagawa et al. 1990). LAK cell cytotoxicity is stimulated by their contact with target tumor cells and is due to the synergistic effect of IFNγ and TNFα secretion (Chong et al. 1989). The appearance of LAK cells under the effect of IL-2 occurs with the aid of IFNγ (Itoh et al. 1985). IFNγ-lacking patients with cancer of the digestive tract and of the breast also lacked or had an extremely low production of LAK activity by peripheral mononuclear cells in response to IL-2. Antibodies against IFNγ also completely inhibited LAK activity, and the addition of exogenous IFNγ restored it (Shiiba et al. 1986).

The NK cells produce *perforin*, which participates in the membranolytic activity responsible for lysing tumor cells. However, to this end, the perforin has to bind the cholinphosphate-containing lipid PAF (platelet-activating factor) released by NK cells, which is incorporated into the membrane via its specific receptor. IFNγ stimulates the expression of this receptor and the killing of tumor cells via the perforin metabolic pathway. Tumor cells where IFNγ cannot induce the PAF receptors are resistant to perforin lysis (Berthou et al. 2000).

The cooperation between T lymphocytes and macrophages is also IFNγ dependent. It is believed that T cytotoxic cells recognize foreign antigens in association with cellular surface markers, such as the major histocompatibility complex (MHC) class I molecules. Activation of macrophages with IFNγ increases the expression of the MHC antigens and makes the tumor cells more susceptible to the cytotoxic lymphocytes.

Here we have to point out a special feature of the IFNγ effect. A large number of studies show that IFNγ independently of its stimulatory effect on the NK and LAK cells makes the malignant target cells more resistant to the lytic activity of NK and LAK (Wallach 1983; Gronberg et al 1985, 1989a,b;

De Fries and Golub 1988; Zoller et al. 1988; Toledano et al. 1989; Garbe et al. 1990; Jabrane-Ferrat et al. 1990a; Lollini et al. 1990; Maziarsz et al. 1990; Naganuma et al. 1992; Cesano et al. 1993; Garbe and Krasagakis 1993; Nishimura et al. 1994; Ishigami et al. 1996). The protective effect of IFNγ is expressed in homologous but not in heterologous effector cells (Laskay and Kiessling 1986).

One of the explanations for the protective effect is that the NK cells recognize "self" and "foreign" due to the expression of MHC class I antigens, which are usually decreased in malignant tumors. IFNγ, by stimulating class I MHC expression on tumor cells, makes them self and unrecognizable for the NK/LAK cell immunological control. The conclusion was even drawn that the sensitivity of target cells to lysis by NK/LAK is inversely proportional to MHC class I expression (e.g., see Storkus et al. 1987).

This, however, does not always appear to hold true. For example, in neuroblastoma cells, which show a low expression of MHC class I antigens, IFNγ induces these antigens and increases the lysis of the tumor cells by NK (Handgretinger et al. 1989). In addition, a constant presence of IFNγ has been found to be necessary for the increased resistance, which is an indirect argument against the role of MHC class I antigen expression (De Fries and Golub 1988).

Other experiments also show that the mechanism of the IFNγ-increased tumor cell resistance is not related to the expression of MHC class I antigens. Thus, K562 cells, which do not express these antigens, begin their expression after treatment with IFNγ and become resistant to lysis by NK cells. However, if they are treated with anti–MHC class I antibodies, their sensitivity to lysis by NK cells is not recovered. Also, spontaneously arisen subclones of K562 that cannot express MHC class I under the effect of IFNγ still become resistant to NK lysis after treatment with this cytokine (Nishimura et al. 1994). Resistance to LAK lysis is not due to disturbed binding between effector and target cells; in the TALL-01 cell line (acute T-cell lymphatic leukemia) neither IFNγ (which suppresses lysis) nor granulocyte-macrophage colony-stimulating factor (GM-CSF) (which stimulates lysis) change the expression of surface adhesion molecules (Cesano et al. 1993).

A strong argument against the role of MHC class I in this process are experiments with transformed K562 cells containing a DNA vector expressing these antigens to the same extent as after treatment with IFNγ. However, the transformed cells did not become resistant to NK lysis (Maziarsz et al. 1990). Some investigators believe that the induction of soluble intercellular adhesion molecules by IFNγ could explain their increased resistance.

An attempt was made to explain the increased resistance by the reduced $Ca^{2+}$ influx into the target cells treated with IFNγ (Gronberb et al. 1989b). Artificial activation of protein kinase C (PKC) with phorbol myristate acetate (PMA) in the presence of $Ca^{++}$ recovers the defective sensitivity of the IFNγ-treated cells. The investigators believe that the $Ca^{2+}$/calmodulin metabolic pathway is involved in the induction of MHC class II by IFNγ. In the HL-60 cell line calcium deprivation led to the lack of MHC class II induction by

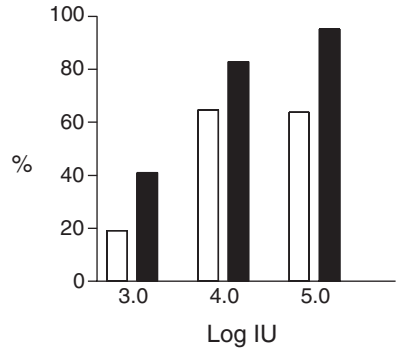


***Figure 12.2*** Effect of murine IFNγ (■) and of TNFα (□) on experimental lung metastases of carcinoma M109 injected intraperitoneally with the corresponding cytokine 2 days before intravenous introduction of $5 \times 10^4$ tumor cells. Ordinates — % of metastases inhibition. (According to data by Schultz and Altom 1990.)

IFNγ (Koide et al. 1988). However, we have to admit that the mechanism behind this process still remains unclear.

On the other hand, IFNγ, by inducing the expression of MHC class I and class II surface antigens, makes the tumor cells susceptible to the cytotoxic activity of a number of other effector cells. To this effect, a role is also played by the IFNγ-induced expression of the intercellular adhesion molecule-1 (ICAM–I) necessary for the attack of the target tumor cells by the cytotoxic effector cells.

IFNγ also suppresses the abnormally high level of prostaglandin E2 (PgE2), which has a negative effect on NK/LAK cell activity by inhibiting IFNγ secretion by these cells and by other T lymphocytes (Ybarrondo et al. 1990). Therefore, the final result depends on the balance between the decreased sensitivity of the tumor cells to the nonspecific NK lysis and all other specific tumorocidal effects of the cytotoxic T lymphocytes due to the MHC antigen expression. In *in vivo* experiments the balance was in favor of the antitumor effect.

A cytostatic antitumor activity of the neutrophil leukocytes after their treatment with IFNγ has been also reported (Shalaby et al. 1985; Steinbeck et al. 1986, 1989; Miyake et al. 1988; Livingston et al. 1989; Morrison et al. 1989; Cemerlic et al. 1991).

We should also draw attention to one more fact. Data from experiments with mice show that the preoperative (neoadjuvant) application of IFNγ is more effective than its postoperative (adjuvant) application (Schultz and Altom 1990) (Figure 12.2). Most probably this is due to the preliminary activation of the effector cells to destroy tumor cells spread through the circulatory system after the surgical removal of the tumor.

**To stimulate the expression of tumor-specific surface antigens for the purpose of immunotherapy with the corresponding antibodies.** A number of data show that IFNγ increases the expression of tumor-specific antigens

that are not expressed on the surface of normal cells. This can be used for the purpose of diagnosis and for immunotherapy with specific antibodies, possibly coupled with toxins, and also for radioimmunotherapy. Examples of increased expression of tumor-specific antigens under the effect of IFNγ are given elsewhere (Steplewski et al. 1985; Borden 1988; Gross et al. 1989b; Marth et al. 1989a; O'Connell et al. 1989; Fuith et al. 1991; Fujisaki et al. 1993; Mobus et al. 1993; Shimada et al 1993; Sivinski et al. 1993, 1995; Clark et al. 1994a; Greiner et al. 1994, 1996; Imbert-Marcille et al. 1994; Hinoda et al. 1997; Verhaar et al. 1999).

**For adoptive immunotherapy by transfecting TIL with cytokine genes, including that of IFNγ.** TIL transfected with IFNγ are re-introduced into the organism, which leads to expression of the cytokine within the tumor invaded by the TIL cells. This method showed encouraging results for the treatment of melanoma (see Section 12.6).

**For transgenic immunotherapy through transfection of tumor cells with cytokine genes, including IFNγ** (for examples, see Sigal et al. 1990). The genetically modified tumor cells introduced into the organism cause an immune reaction against both the modified and the primary unmodified tumors. It is strange that an antitumor and antimetastatic effect was observed after transfection of tumor cells with IL-10 (Richter et al. 1993; Giovarelli et al. 1995; Kundu et al. 1996), a cytokine which belongs to the Th2 group and suppresses the Th1 cytokines. It is believed that this cytokine activates the NK cells and the cytotoxicity of the peripheral mononuclear cells which is not associated with MHC (Schwarz et al. 1994; Carson et al. 1995; Kundu et al. 1996), but the mechanism of this type of transgenic immunotherapy is not completely clear. The Th2 cytokines prevail in a number of malignant tumors. There also are data that TIL produce IL-10. All suppress the Th1 cytokines and favor tumor growth. The effect may be associated with suppression of the MHC class I antigens that helps NK cells recognize the tumor cells as foreign. It is also possible that the effect is associated with induction of an immune response against modified tumor cells.

**For enhancing the graft-vs.-host disease (GVHD) through the induction of MHC class II antigens by IFNγ.** The introduction of autologous bone marrow along with cyclosporine A leads to rejection of the graft by the host and the reaction is enhanced by IFNγ due to the induction of MHC class II antigens. This also causes an antitumor reaction and rejection of the tumor (Hess 1995).

**To increase apoptosis induced by ligands of the Fas/APO1 receptor** (e.g., antibodies against this receptor) by increasing its expression, as has been shown in malignant cells of the colon (Walther and Stein 1994; Stein et al. 1996).

**To overcome multidrug resistance (MDR) in some tumors through suppression of the mdr1 gene** (Reddy et al. 1991; Moulton et al. 1993; Efferth et al. 1996). Some multi–drug-resistant malignant lung cells appeared to be even more sensitive to IFNγ than the primary cell line (Jabbar and Twentyman 1990).

MDR is a resistance of cancer cells to a group of chemically and functionally unrelated antitumor chemotherapeutics. It arises following treatment of the tumor with any of these antitumor chemicals. It is due to amplification or overexpression of a gene responsible for the synthesis of a membrane glycoprotein (P-glycoprotein), which functions as an efflux energy-dependent pump that removes toxic compounds from the cell.

We should mention that instead of IFNγ, a different Th1 cytokine, IL-12, which induces secretion of IFNγ, also is used for treatment of malignant tumors. Actually, IFNγ plays a different role in different tumors. In the ovarian cancer cell line OV-HM IFNγ is necessary for T-cell migration into the tumor mass, whereas in CSA1 cell line it ensures the cytotoxic effect of TIL (Ogawa et al. 1998).

Examples of all of these possibilities are presented in the sections for the different tumor types.

It should be stressed that the tumor cells develop mechanisms counteracting these antitumor reactions. For example, melanoma cells treated with IFNγ or with tumor necrosis factor α (TNFα) increase their secretion of soluble ICAM-I (sICAM-I), which binds its ligand and hampers the contact between effector and target cells. Thus, the immunological control is blocked. The induction of sICAM-I as a result of IFNγ and TNFα may be one of the mechanisms leading to resistance directed against NK/LAK (see Becker et al. 1991; Dummer et al. 1994 and references therein). A study of sICAM-I secretion in different malignant cells showed that the peripheral mononuclear cell and B lymphoblastoid cell lines do not secrete the soluble molecule. In other malignant cells (melanoma M19, M26, Daudi, K562, SeAx, MyLa) such a secretion is observed with or without treatment with IFNγ or TNFα, but these cytokines increase the secretion except for the SeAx cell line in which IFNγ suppresses it (Dummer et al. 1994). This shows the high heterogeneity of the tumor cell lines with regard to their defense against the lytic activity of the effector cells and the differing effect of IFNγ.

Tumor cells also release other soluble receptors, such as those of TNF, which allows them to avoid its cytotoxic effect (Gatanaga et al. 1990), and of the p55 chain of IL-2R, which inhibits LAK proliferation (Dummer et al. 1990). The serum concentration of such soluble molecules correlates with an unfavorable prognosis (Nestle et al. 1990).

Another mechanism is the expression of the Fas ligand (FasL) on the surface of melanoma cells, which allows them to cause apoptosis of TIL, which express Fas on their surface (Hahne et al. 1996). The same has been found in cancer cells of the mammary gland. Unlike normal cells, they express FasL and lose expression of Fas (Muschen et al. 2000). Thus, the cancer cells avoid the immune response.

Another problem associated with using cytokines for stimulation of the cytotoxic activity of macrophages and other effector cells is the timing of cytokine application — as adjuvant therapy following the surgical removal of the tumor or as neoadjuvant therapy (preoperatively). The second approach theoretically should have an advantage, because it would

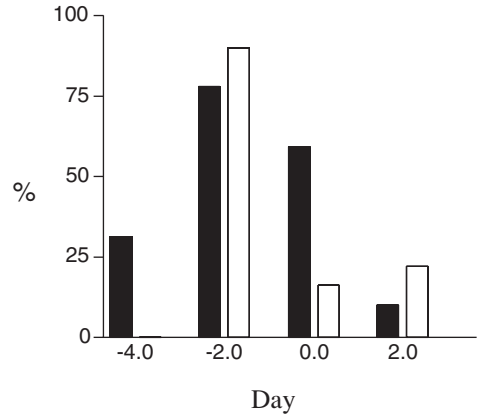


***Figure 12.3*** Percentage decrease (ordinates) of lung metastases after excision of the transplanted tumor (day 0.0) depending on the day of treatment (abscissas) with IFNγ (■) or with TNFα (□). (According to data by Schultz and Altom 1990.)

prepare the tumoricidal leukocytes to attack loose and dispersed cancer cells in the body, which can later develop into metastases. Experiments on mice that developed lung metastases after elimination of the primary tumor show the favorable effect of a pretreatment with IFNγ (Schultz and Altom 1990) (Figure 12.3).

## *12.2 Basocellular skin cancer (basalioma)*

The basocellular carcinoma (BCC) is a benign tumor that as a rule and very rarely shows invasive growth. It is surrounded by an infiltrate composed mainly of T lymphocytes which very rarely infiltrate the tumor. A large part of basalioma cells (93%) do not express ICAM-I and 73% do not express leukocyte function antigen-3 (LFA-3). Lymphocyte adhesion is important for the T-cell–dependent cellular immune reaction. This explains the lack of TIL and therefore the lack of a cellular immune response. Lymphocyte adhesion also depends on the binding of the antigens responsible for lymphocyte function (LFA-1 and -2) with the corresponding ligands ICAM-I and LFA-3, which are expressed on the surface of nonlymphoid cells. In basalioma cells the expression of the IFNγ receptor is also decreased. The expression of HLA class I and II also is low. Following incubation with IFNγ *in vitro* in 85% of the cases the BCC cells begin to express ICAM-I but to a lesser degree than normal keratinocytes. A secretion of soluble ICAM-I, which decreases the immune control, also is observed (Kooy et al. 1998). In some cases, induction of HLA class I and II was also found (Taylor et al. 1990; Kageshita et al. 1992).

This type of skin cancer is successfully treated by surgical removal, cryosurgery, electrosurgery, and chemosurgery. These methods lead to tissue damage. The aggressive forms penetrate deep into the skin and require surgical intervention that involves considerable tissue loss.

There are data of a healing effect after injection of 0.01 mg (20,000 IU) and 0.05 mg (100,000 IU) of recombinant IFNα-2b into the lesion (Bottomley and Keczkes 1991; Buechner 1991; Stenquist et al. 1992). There was 50% healing with the high dose and 7% with the low dose (Edwards et al. 1990). In a different study the low doses did not have an effect (Tank et al. 1989). We could not find data on the clinical application of IFNγ.

## *12.3 Cancer of the larynx*

The few data that we have found on this type of cancer with regard to IFNγ show that immunosuppression is observed even at its earliest stages, which can be counteracted with H2 histamine antagonists. In experiments with tonsil lymphocytes, histamine suppresses the production of IFNγ to 2% of control levels without affecting LAK activity (Richtsmeier et al. 1987). In later stages of laryngeal carcinoma IFNγ is not found in the blood (Beatrice et al. 1987).

These data justify the testing of the effect of IFNγ on this type of cancer.

## *12.4 Lung cancer*

The alveolar macrophages activated by IFNγ, produced by T helper cells after their interaction with the autologous cancer cells, are the immune effector cells that are responsible for the defense against lung cancer. We should also mention data showing that the normal lymphocytes of the lung exhibit a very low NK activity as compared to blood lymphocytes. This seems to be due to local inhibitory factors (Robinson et al. 1984).

In lung cancer patients the cytostatic function of the alveolar macrophages is also suppressed, which is an expression of a general decrease of the immune response due to a defect in IFNγ production by T lymphocytes. Under the effect of IFNγ, this function is considerably increased and reaches the same level as that in healthy people (McDonald and Atkins 1990). This is also confirmed by the fact that immunocompetent cells from patients with small-cell lung cancer or with non–small-cell metastatic carcinoma stimulated with mitogens secrete considerably less IFNγ, IL-2, and TNFα as compared to healthy individuals. The secretion of these cytokines is considerably improved after the tumor load is decreased following successful therapy (Fischer et al. 1995). There is also a report that the decreased tumoricidal function of the alveolar macrophages in patients with non–small-cell cancer remains unaffected by IFNγ (Siziopikou et al. 1991).

In small-cell lung cancer the cancer cells show a defect in antigen processing due to the low expression of HLA class I, which can be due in part to the decreased synthesis of TAP-1 and TAP-2 (Fisk et al. 1994). Following transfection of the cancer cells with IFNγ, the expression of HLA is increased (Traversari et al. 1997). However, IFNγ does not induce HLA class I in multi–drug-resistant cancer cell lines of this type (Colle et al. 1991).

Surface differentiation antigens are also induced under the effect of IFNγ (Ruff et al. 1986).

The mononuclear cells of patients with primary lung cancer show a lower cytotoxic activity than that in healthy people. This activity is normalized after treatment with IL-12, which induces IFNγ secretion by mononuclear cells (Haku et al. 1997). The same occurs following treatment of lymphocytes from lung cancer patients with IFNγ and IL-2 (Papamichail and Baxevanis 1992). The monocytes of such patients also produce less oxygen radicals as compared to the mononuclear cells of healthy individuals, which is also associated with their decreased cytocidal activity. The level of oxygen radicals produced by these cells and by alveolar macrophages reaches physiological levels under the effect of IFNγ, IL-2, and IFNα (Tohda et al. 1996). IFNγ also increases the expression of the carcinoembryonic antigen (CEA) on the cell surface, something which also occurs during chemotherapy, where both factors act synergistically (Takahashi et al. 1995).

Experiments on lung cancer cell lines have also shown the antitumor effect of IFNγ. This cytokine suppresses the growth of the small-cell lung metastastatic carcinoma human cell line H460 implanted in athymic mice and increases their survival rate (An et al. 1996). IFNγ also showed a local antitumor effect after it was administered in the pleural cavity during pleural efusion of lung cancer patients (Yanagawa et al. 1997).

Highly metastatic tumors in mice do not express the apoptosis mediator death-associated protein-kinase (DAP-kinase) (a $Ca^2$/calmodulin-dependent enzyme) as opposed to low metastatic tumors. IFNγ restores the DAP-kinase to its normal level in the highly metastatic Lewis carcinoma and suppresses the formation of metastases (Inbal et al. 1997). The angiostatic factor IP-10, which inhibits the growth and metastasis of the small-cell lung cancer, is also induced by IFNγ (Arenberg et al. 1996).

Small-cell lung cancer cells do not express ICAM-I, which is important for cancer cell binding to LAK cells. IFNγ induces ICAM-I expression in this carcinoma (Melis et al. 1996) and also in the alveolar macrophages (Fattal-German et al. 1996).

It has been shown in experiments with mice that the Lewis carcinoma induces bone marrow cells to exert a suppressor activity on T cells and macrophages. Low doses of IFNγ and TNFα eliminate this activity and decrease lung metastases (Young and Wright 1992). In the same type of cancer cell line implanted in mice the effect of IFNγ was increased in combination with LPS (Nagao et al. 1986). In the lung cancer cell line A549 experiments with IFNγ have shown that cancer cells die by apoptosis, showing the DNA fragmentation pattern typical of this type of death (Oh et al. 1996). Experiments with the mouse lung cancer cell line M109 showed a stronger growth-inhibiting effect of IFNγ as compared to TNFα (see Figure 12.2).

It has also been found that IFNγ activates NO production and the cytotoxicity of alveolar macrophages obtained by lavage of lung cancer patients. Heterogeneity with regard to the cytokine profile of the alveolar macrophages has been established in lung cancer patients. In some patients the

cytotoxicity of these macrophages was increased under the effect of IFNγ (IFNγ-dependent cytotoxicity). In other patients the macrophages normally exhibited cytotoxicity (IFNγ-independent cytotoxicity). In both cases the cytotoxicity was partially blocked by antibodies against TNFα and by inhibitors of NO production and was completely blocked by the combination of both factors (Yoshimatsu et al. 1996).

Incubation of the human non–small-cell cancer cell line with IFNγ led to squamous differentiation due to the induction of transglutaminase, a cross-linking enzyme. This also resulted in inhibition of cell proliferation (Kane et al. 1992). In the same type of cancer, suppression of proliferation was associated with induction of surface differentiation antigens (Ruff et al. 1986). It has also been shown that the angiogenic chemokines are not compensated for by the IFNγ-induced angiostatic chemokine MIG (membrane-bound immunoglobulin) (see p. 141). The overexpression of MIG by recombinant DNA approaches led to inhibition of cancer growth caused by the suppressed angiogenesis (Addison et al. 2000).

A synergistic effect of the combination of IFNγ with TNFβ (Shiiki et al. 1990b) or with TNFα (Shiiki et al. 1989) was found in the PC10 cell line.

Statistical analysis of the connection between laboratory data and favorable survival prognosis showed that an indicator of a favorable outcome is neopterin in the urine, which is produced by monocytes/macrophages under the effect of IFNγ (Kronberger et al. 1995).

These and other similar experiments supported the conduction of clinical trials involving the treatment of primary lung cancer with IFNγ (for lung metastases of other tumors, see the sections concerning the corresponding tumor types). Regretfully, even the earliest preliminary results did not meet the expectations. For small-cell lung cancer, the application of 1 mg/m$^2$ of recombinant IFNγ daily for 5 days followed by 0.5 mg/m$^2$ three times a week for 3 weeks was ineffective (Newman et al. 1987).

In clinical trials it was shown that IFNγ was not active in small-cell lung cancer even when the tumor load is decreased or eliminated by chemotherapy (Jett et al. 1994; Bitran et al. 1995; van Zandwijk et al. 1997). Daily treatment with $4 \times 10^6$ IU subcutanously for 5 months was unsuccessful. A combination of IFNγ, IFNα, and chemotherapy was not effective for treatment of non–small-cell lung cancer (Halme et al. 1994b), although there are some reports of some effect for small-cell lung cancer following chemotherapy (Bitran et al. 1990). Patients who respond well to chemotherapy subsequently die within 2 years as a result of the emergence of resistant tumors. Attempts to apply supporting IFNγ therapy in such cases (0.2 mg subcutaneously — approximately $4 \times 10^6$ units) resulted in a strong activation of the monocytes (increased expression of HLA-DR and of Fc receptors), but there was no activation of the T cells (Pujol et al. 1993).

The combination of IFNγ (2 mg intravenously) and IFNβ ($30 \times 10^6$ units) was also ineffective (Chachoua et al. 1990). IFNγ in combination with chemotherapy had some effect in small-cell lung carcinoma (Bitran et al. 1990; Zabel et al. 1990).

Combination therapy with IFNγ, cisplatin, and etoposide in advanced cases of non–small-cell lung cancer resulted in partial remission in 7%, a weak response in 28%, and disease progression in 62% of the cases. The average survival time was 7 months, and in 40% it was 1 year (Pirker et al. 1994).

IFNγ was applied in a 2-mg/m$^2$ dose three times a week for 12 weeks in combination with chemotherapy and radiotherapy for nonoperable non–small-cell lung carcinoma. Of ten patients, two recovered completely, five partially, and the state of three was stabilized. Of these three patients following chemotherapy, one had a partial response, one was stabilized, and the disease progressed in the last patient. The conclusion of the investigators is that high doses of IFNγ may be effective in non–small-cell lung cancer with a possibility for subsequent conventional therapy (Mattson et al. 1991). However, we have to pay attention to the fact that the combination of IFNγ and exposure to ionizing radiation is dangerous, because IFNγ increases the sensitivity to radiation not only of the tumor cells, but also of the normal lung cells. This combination led to the development of severe pneumonitis, sometimes with a fatal result, and therefore is not recommended (Shaw et al. 1995). In patients with small-cell lung cancer the application of IFNγ ($4 \times 10^6$ IU, approximately 0.2 mg, subcutaneously every other day) following chemotherapy and radiotherapy did not lead to increased survival (van Zandwijk et al. 1997).

What could be the reasons for the unsuccessful clinical trials given the proven antitumor activities of IFNγ? As the conducted clinical trials have shown, the tumor load, which is in itself an important factor, has been excluded in this case.

In the first place, we have to ask the question of whether IFNγ reaches the target malignant tissue. A number of experiments show that IFNγ cannot reach the lung alveoli from the blood stream (Halme et al. 1994a, 1995; Kawata et al. 1994; Kessler et al. 1994; Yano et al. 1994; Mizutani et al. 1996; 1997). It has been shown in humans that only IFNγ inhaled as an aerosol effectively reaches the alveoli (Martin et al. 1993b). Parenterally administered IFNγ does not reach the alveoli, and conversely IFNγ inhaled in the lung is not found in the blood (Jaffe et al. 1991; Halme et al. 1995), which shows the presence of a barrier for IFNγ between the lung and the blood stream. This was possibly the reason for using parenterally high doses. Following inhalation of 0.6 mg of IFNγ, it was found in the bronchoalveolar lavage after 3 hours (Halme et al. 1995).

This method of administration produced some healing effect for metastases of kidney carcinoma (Kawata et al. 1994). In mice with implanted Lewis carcinoma (3LL) IFNγ applied as an aerosol suppressed the formation of metastases in 50% of the animals and increased survival, although *in vitro* it did not exert a direct antitumor effect on the 3LL cells. The macrophages, however, exerted a strong antiproliferative effect on these cells (Kessler et al. 1994). In one case of bronchoalveolar carcinoma the inhalation of IFNγ led to stabilization of the disease for more than a year accompanied by high cytostatic activity of the macrophages (Yano et al. 1994). However, there are data that the result after aerosolic administration depends on the localization of the tumor and is not effective for tumors in the lung periphery.

Second, we have to mention that sometimes the malignancy is associated with mutations which disrupt the metabolic pathway of IFNγ to the nucleus. This can be due to inactivation of the interferon receptor or at downstream steps in the metabolic pathway. For example, cultured bronchial epithelium cells respond to the immunomodulatory, growth-inhibiting, and differentiation-inducing effects of IFNγ, as opposed to some lung carcinoma cell lines that are not sensitive (Saunders et al. 1994). Also, IFNγ induces NO production in normal lung epithelium cell lines but not in malignantly transformed cell lines (Thompson et al. 1998). Thus, the disrupted signaling pathways create tumors resistant to IFNγ. It has to be taken into consideration that the small-cell lung cancer, for example, is represented by heterogeneous cell lines with different sensitivities to IFNγ, IFNα, and TNFα (Suarez-Pestana et al. 1996), which are probably due to various disturbances of the metabolic pathways.

An anergic state of the organism could be another reason. For example, IFNγ increases the phagocytic and bacteriocidal activities of the alveolar macrophages of tuberculin-positive patients with lung cancer and tuberculosis. However, in anergic, tuberculin-negative patients, this cytokine did not have an effect (Kawatsu et al. 1991).

When cytokines are used for treatment of patients with lung cancer, it is important to know whether the cancer cells have the target molecules that are recognized and attacked by the cytotoxic T lymphocytes (CTL). Such molecules in lung cancer are p21 (ras), MAGE 1, MUC 1 (Yasumoto 1995).

The common opinion is that the parenteral administration of IFNγ for treatment of lung cancer produced unsatisfactory results, mainly because of the low permeability of the blood-lung barrier for IFNγ. From this point of view, the inhalation route of administration is more promising, but the denaturing effect of aerosolization should be considered (see Chapter 8).

## *12.5 Colorectal cancer*

Experimental studies on CD4+ T cells from patients with colorectal cancer led to the conclusion of a disturbed regulation between the Th1 and Th2 populations with an expansion of the Th2 and suppression of Th1 function, which is increased with tumor progression (Pellegrini et al. 1996). Disturbed cytokine production was found in blood cell cultures from patients with colorectal cancer as compared to healthy controls and patients with benign colorectal tumors (Elsasser-Beile et al. 1992).

It has been found that the serum of patients with colorectal cancer contains substances that suppress IFNγ synthesis by peripheral mononuclear cells stimulated with PHA (Muster-Bloy et al. 1996).

The endogenous IFNγ in the tissue of the colorectal carcinoma is elevated as compared to its levels in the surrounding normal tissues and decreases with tumor progression. The production of this cytokine by CD4+ T cells is a manifestation of the antitumor immune response (Numata 1992). It has also been shown that in colorectal tumors HLA-DR induction by IFNγ is

associated with less advanced stages, whereas in advanced cases this expression is lacking (Matsushita et al. 1996).

It appears that IFNγ does not exert a direct antiproliferative effect in this type of carcinoma. This could be the possible reason for the ineffectiveness of the TIL, which produce normal quantities of IFNγ in this type of cancer (Bateman et al. 1995). In *in vitro* experiments with eight different colorectal carcinoma cell lines it had an effect on only one of them, whereas IFNα did not have any effect, and IFNβ affected all of them (Wong et al. 1989). The reason why in some cases the direct antiproliferative effect of IFNγ is absent has not been elucidated. For example, rat intestinal epithelial cells immortalized by the E1A and T antigens are very sensitive to the direct antiproliferative effect of IFNγ but become insensitive after they are transformed with the human oncogene Ha-ras (Emami et al. 1990). Colon cancer cell lines appeared to be sensitive to the antiproliferative effect of IFNγ, since a direct effect was exhibited in five of seven cell lines and in three primary tumors (Pfuzenmaier et al. 1985).

In general, the colorectal tumors show high heterogeneity with regard to the effect of IFNγ and other cytokines, which is manifested both at the cellular level in the same tumor and in different tumors (Morikawa and Fidler 1989). It appears that if IFNγ has an effect in this type of cancer, this effect should be indirect, accomplished by the effector cells. Experiments with a mouse colon adenocarcinoma cell line revealed that the direct preliminary treatment of the cancer cells with IFNγ led to increased metastases (Hirano et al. 1996b).

The cytotoxic effect, specifically apoptosis, caused by monoclonal antibodies is also increased by IFNγ (Reali et al. 1994; Takamuku et al. 1996). In normal colon epithelium the apoptotic antigen Fas is constitutively expressed, but in colorectal carcinomas it is considerably decreased, such that anti-Fas antibodies do not induce apoptosis (experiments with the COLO201 cell line). Treatment with IFNγ (or with TNFα) increases Fas expression and the sensitivity to anti-Fas apoptosis (Koshiji et al. 1999). It was shown that IFNγ also induces ICAM-I expression in the DLD-1 cell line (Das et al. 1993).

The number of monocytes expressing the HLA-DR+ antigen is reduced in patients with this type of cancer. Treatment with IFNγ increases this antigen to the levels found in healthy individuals (Novellino et al. 2000).

Although all these data support the role of IFNγ in the immune response of the organism against colorectal carcinoma, clinical trials with this cytokine did not produce the expected results (O'Connell et al. 1989; Brown et al. 1991b). An intravenous infusion of IFNγ for 2 or 24 hours in such patients did not produce an antitumor effect, but this method of administration is not optimal.

The lack of results with the use of IFNγ alone led to experiments with different combinations.

Interesting results were obtained from experiments with IFNγ in combination with 5-fluorouracil (5-FU). This combination showed a synergistic

antitumor effect in a number of colorectal carcinoma cell lines (Maas et al. 1991). All three types of interferon increased the cytotoxicity of 5-FU (Wadler et al. 1990). It has been shown that this combination leads to apoptosis of the cancer cells. In the COLO201 colorectal cancer cell line the apoptosis caused by this combination is due to the suppressed expression of the anti-apoptotic gene Bcl-2 and the activation of the apoptotic gene Bax. Administration of 5-FU alone led to the induction of a protease which blocks apoptosis (Koshiji et al. 1997). Also, in the H630 cell line 5-FU increased severalfold the expression of thymidylate-synthetase, whereas in the presence of IFNγ this effect was suppressed (Chu et al. 1993b). These data support the clinical use of 5-FU in combination with IFNγ (Pavlidis et al. 1996; Grem et al. 1997). Of 34 patients with advanced metastatic colorectal carcinoma, 1 patient recovered completely, 3 partially, and 11 were stabilized (Pavlidis et al. 1996). Clinical trials were also conducted using a combination of IFNγ and TNF, but it was not particularly effective (Fiedler et al. 1991).

IFNγ and IL-1 in combination had an additive antitumor effect, as IL-1 increased the number of interferon receptors (Raitano and Kore 1993).

IFNγ in combination with TNFβ (Shiiki et al. 1990b) or with TNFα (Shiiki et al. 1989) had a synergistic antitumor effect on the colon cancer cell line RPMI4788. Synergism between IFNγ and TNF was also observed in other colon cancer cell lines (Schiller et al. 1990c). However, the clinical trials involving the combination of IFNγ and TNF were discontinued due to high toxicity before an effect was observed (Abbruzzese et al. 1990).

In three colorectal carcinoma cell lines the combination of IFNγ, TNF, and 5-FU had a strong antitumor effect *in vitro* (Schiller and Bitner 1990). A high percentage of remissions, 48%, was obtained in 30 patients treated with the complex combination of IFNγ, IFNβ, 5-FU, carboplatin, and mitomycin C (Klein et al. 1991a).

Monocytes/macrophages activated with IFNγ show increased antitumor activity in *in vitro* experiments. This supported the application of the so-called adoptive immunotherapy for patients affected by this type of cancer. Autologous macrophages activated *in vitro* with IFNγ and LPS were infused intravenously. Stabilization was observed in 1 of 9 patients (Hennemann et al. 1998). In another clinical trial with macrophages activated with IFNγ only, stabilization was observed in 3 of 14 patients (Eymard et al. 1996). We have to point out that, although activated, the macrophages probably could not eliminate the large tumor load in these cases.

There are data showing that IL-1a, IFNα, and IFNγ induce the expression of the thymidine phosphorylase in colorectal cancer cells, and this is an enzyme associated with angiogenesis (Iwagaki et al. 1995; Takebayashi et al. 1995, 1996), a factor that facilitates tumor growth (see p. 36).

*In vitro* IFNγ and dsRNA in combination had a synergistic effect (Chapekar and Glazer 1985, 1996).

It has been shown that IFNγ increased the expression of carcinoembryonic antigens in colorectal carcinoma (Toth and Thomas 1990; Dansky-Ullmann et al. 1995; Hinodo et al. 1997), which opens a possibility of its

being used in diagnostics and in therapy with monoclonal antibodies. IFNγ had the strongest effect on the expression of such antigens in moderately diferentiated colorectal tumors, whereas in highly or poorly differentiated tumors it was ineffective (Guadagni et al. 1990).

## 12.6 Malignant melanoma

The prognosis for malignant melanoma is very poor even with the modern therapy. In stage I of the disease (tumor depth < 1.5 mm) a wide excision leads to a 10-year cure rate in 85% of patients. When the depth is more than 4 mm, 50% of patients experience recurrence, those with metastases in the regional lymph nodes (stage III) 60 to 85%, and patients with metastases at distant sites 95%, which is associated with the worst prognosis (Barth and Morton 1995).

The cells of the malignant melanoma express many growth factors — bFGF, TGFα, PDGF, melanoma growth-stimulating activity (MGSA) — and also some of their receptors (for bFGF and MGSA), which leads to constant stimulation of cell proliferation (autocrine growth stimulation). Taking into consideration the effect of different cytokines, it can be seen that the regulation of cell growth in melanoma is a very complex network of growth factors and other cytokines that interact with each other (for review, see Krasagakis et al. 1993). A new gene was cloned recently, a member of the interferon-induced family of genes, which controls tumorigenesis in one model of melanoma (De Young et al. 1997).

During the last 15 years the effect of IFNγ and of its combination with other cytokines or with chemotherapy has been studied on melanoma cells *in vitro*, in mouse models of melanoma, and in patients in clinical trials.

A detailed study of melanoma patients showed that IFNγ increases the CD4+/CD8+ ratio, the HLA-DR, and HLA-DQ antigens, and the activity of the NK cells (Kirkwood et al. 1991, 1997). The supernatants of cultured melanoma tumors that react to therapy contain Th1-type cytokines (IFNγ, IL-2, IL-12) and those of progressing tumors — Th2-type cytokines (IL-10) (Enk et al. 1997; Okamoto et al. 1997; Vile et al. 1997). Also, increased Th1 cytokine mRNA was found in spontaneously regressing melanomas. Such tumors were infiltrated by a large number of CD4+ T cells. With regard to the Th2 cytokines, no difference was found between regressing and progressing melanomas. These data show that the CD4+ T cells lead to tumor regression caused by the secretion of Th1 cytokines (Lowes et al. 1997).

The early TIL play a defensive antitumor role, and according to some data are of critical importance for the generation of specific antitumor CD8+ cytotoxic lymphocytes (Kurosawa et al. 1995) and also for inducing NO production by IFNγ-activated macrophages (Abe et al. 1998).

IFNγ also increases the expression of ICAM-I — for the role of this molecule, see a review elsewhere (van de Stolpe and van der Saag 1996) — and activates the tumoricidal activity of the macrophages. ICAM-I expression is synergistically increased by a combination of IFNγ and TNFα (Jahnke

and Johnson 1995). Whereas in human melanoma cell lines IFNγ and TNFα lead to a clearly expressed increase in ICAM-I, in the mouse cell lines B16-F1 and F10 such an increase was not observed (Nakayama et al. 1997). IFNγ also increases the cytotoxic activity of peripheral monocytes and NK cells against freshly isolated melanoma cells (Itoh et al. 1985, 1987). In the human A375 melanoma it was shown that the interferons (γ and α) exerted their tumoricidal activity by a direct effect on the melanoma cells as well as by increasing the cytolytic properties of the monocytes (Gerrard et al. 1989; Webb and Gerrard 1990). The ability of LAK to lyse melanoma cells is increased by the synergistic action of IFNγ and IL-2 (Kaufmann et al. 1991). The significance of the successive or simultaneous treatment of LAK and B16 melanoma cells with IFNγ and IL-2 was also studied. With both procedures the cytotoxicity of the LAK and the sensitivity of the melanoma cells to lysis were increased, but simultaneous application produced better results (Lee et al. 1992).

Secretion of the growth-regulated oncogene (GROα) cytokine, a glycoprotein which is a chemoattractant for CD4+ and CD8+ T lymphocytes, is stimulated by IFNγ and TNFα and is suppressed by the Th2 cytokines IL-4, IL-10, and IL-13 (Jinquan et al. 1995).

Suppression of melanoma cell proliferation by IFNγ was observed a long time ago (Saito et al. 1986). IFNγ inhibits the expression of cellular oncogenes in melanoma cells. In the Cmel 453A melanoma cell line it inhibits c-myc, where this oncogene is overexpressed. Inhibition of c-myc precedes the blockade on cell proliferation (Osanto et al. 1992). In the melanoma cell line Colo38 it inhibits the Ha-ras1 oncogene, which also leads to suppression of proliferation (Giacomini et al. 1990). In some melanoma cell lines IFNγ suppressed the expression of the epidermal growth factor (EGF) receptors. This, however, did not affect cell proliferation (Worm et al. 1995).

A direct antiproliferative effect on melanomas has been shown for all interferons. Some investigators find that IFNγ is most active, whereas others find IFNβ to be more active. The effect is mostly cytostatic (for review, see Garbe and Krasagakis 1993). With regard to HLA class I antigen expression in the M14 melanoma cell line, the interferons are arranged as follows according to their activity: IFNγ>IFNβ>IFNα (Lanza et al. 1995). In the SK-MEL-118 melanoma cell line IFNγ and IFNβ showed a higher antitumor effect than IFNα, whereas IFNβ was more active than IFNγ (Horikoshi et al. 1995). In highly metastatic melanoma of the uvea (clones Mel 202 and 92.1) IFNγ inhibited the growth of clone 92.1 only, and IFNβ inhibited both clones (de Waard-Siebinga et al. 1995). IFNγ and IFNβ in combination exerted a synergistic antiproliferative effect on the melanoma cell line SK-MEL28 (Gomi et al. 1986).

These data show how different can be the *in vitro* effect of the individual interferons on different melanoma clones. The difference between the direct effect of IFNγ *in vitro* and its effect *in vivo* has to be taken into consideration. All three human melanoma cell lines, G-368, HT-144, and SK-MEL-3, were found to be sensitive to IFNγ *in vitro*, but only the SK-MEL-3 cell line reacted with growth inhibition *in vivo* (Trotta and Harrison 1987).

IFNγ also induces apoptosis in melanoma cells. In B16 melanoma it strongly increases the expression of the surface antigens Fas (CD95) and its ligand FasL (CD95L), which irreversibly programs the cells for apoptotic death, and at the same time it stimulates their lysis by cytotoxic T lymphocytes (Bohm et al. 1998). In the nonmetastatic melanoma cell line K 1735 the apoptosis induced by NO is associated with suppression of the antiapoptotic gene Bcl-2. In the metastatic cell line IFNγ does not induce NO, does not suppress this gene, and does not cause apoptosis (Xie et al. 1996, 1997).

In experiments *in vitro* IFNγ also increased the sensitivity of melanoma cells to ionizing radiation, but did not affect the normal skin fibroblasts. This can be explained by the delayed DNA repair caused by the combined effect of IFNγ and radiation (Kwok et al. 1991; Krajcik et al. 1993). The same effect was also observed *in vivo* upon increasing by 2°C the body temperature of mice with melanoma B16 (Anjum and Fleischmann 1992).

The combination of IFNγ and IFNβ suppressed *in vitro* the growth of the melanoma cell line HO-1, but this was not associated with increased differentiation (increased melanin synthesis and changes in antigen expression) (Graham et al. 1991).

Some investigators report that treatment of melanoma cells with IFNγ *in vitro* increases their invasive and metastatic capacity after their implantation in mice. In the first place, this appears to be dependent on the type of melanoma cell line, because the effect was observed in melanoma B16 but not in the B78H1 cell line, whose growth was suppressed. Apart from this, the effect on B16 decreases with increasing doses of interferon (Lollini et al. 1990). Transfection of melanoma cells with IFNγ led to suppression of tumor growth, but also to increased metastatic activity (Lollini et al. 1993). In a different highly mestastatic human cell line, C8161, IFNγ decreased its invasive properties and induced HLA-DR, which are not expressed in this line (Winters et al. 1990).

The metastasis-increasing effect of IFNγ in some melanoma cell lines at certain doses can be explained with increased expression of HLA class I antigens, which are responsible for NK cell reaction. The lack of these antigens on melanoma cells leads to their recognition as "foreign" and their attack by NK cells, whereas the recovery of antigen expression by the interferon makes them "self" and they avoid the elimination by these cells (Zoller et al. 1988; Garbe et al. 1990; Lollini et al. 1990; Garbe and Krasagakis 1993). (For other explanations, see Section 12.1.)

The increased invasive capacity of some melanoma strains (e.g., A2058) caused by IFNγ and IFNα could be due to the stimulation of collagenase IV (a metalloproteinase which degrades the basal membrane), and to stimulation of chemotaxis-migration of the melanoma cells toward laminin and their increased adhesive ability. However, all of these activities were suppressed upon a continuous (for 7 days) interferon application (Hujanen and Turpeenniemi 1991), which suppressed the invasiveness.

On the other hand, IFNγ increases the tumoricidal activity of the macrophages, increases ICAM-I expression, and increases the lytic activity of TIL,

activities which determine the final effect *in vivo*. For example, human IFNγ inhibits *in vivo* metastases of the human melanoma cell line Dx3-azak implanted in athymic mice and considerably prolongs their life (Ramani and Balkwill 1988). Also, in a different human melanoma cell line, SK-MEL-118, IFNγ had an inhibitory effect on growth accompanied by an anti-invasive effect in experiments with artificial membranes (Horikoshi et al. 1995).

In experiments on human xenografts in athymic mice, IFNγ and TNFα in combination suppressed the growth of the C5513 melanoma cell line (Gridley et al. 1989). In the mouse B16-BL6 melanoma the formation of metastases was similarly suppressed by IFNγ, and it had a synergistic effect with TNFα (Sylvester et al. 1990). In the same model the therapeutic effect correlated with the specific activity of the cytotoxic T lymphocytes and with the tumoricidal activity of the lung parenchymal mononuclear cells but not with that of the alveolar macrophages. (The latter could be explained with the poor penetration of parenterally administered IFNγ into the alveoli; see Chapter 8, Section 8.4.) A correlation with LAK activity was not found (Black et al. 1988a).

IFNγ and IL-2 in combination synergistically increase the antitumor activity of NK cells (Brunda and Davatelis 1985). In mice with subcutaneous or intraperitoneal B16 melanoma, IFNγ and IL-2 injected around the tumor in the first case and intraperitoneally in the second led to complete remission in 87 and 81% of animals, respectively. Separately, the two cytokines led to elimination of the tumors in 59% (IL-2) and 53% (IFNγ) of the mice with subcutaneous melanoma and in 17 and 29%, respectively, for tumors with intraperitoneal localization. It is interesting to note that the lower doses of IFNγ were more active. The local application was more effective than the systemic one (Silagi et al. 1988). The combination of IFNγ and IFNβ in mice with B16 melanoma exhibited a stronger antitumor effect without increasing the myelosuppression (Naldini and Fleischmann 1987).

Considering all of these data, what are the results of the clinical trials on the treatment of malignant melanoma with IFNγ? Such trials started as early as 1985, and different doses and routes of administration were used. In general, the clinical trials with IFNγ did not produce the expected results, as the effect was minimal (e.g., see Creagan et al. 1987; Landthaler and Braun 1987; Steiner et al. 1987; Meyskens et al. 1991). The result of one trial was a 23.5% response in 17 patients with metastatic melanoma (Haase et al. 1987). Patients with high-risk melanoma received IFNγ or levamisole and there was no difference in survival or recurrences during 1 year (Osoba et al. 1993).

The lack of a satisfactory result with the application of IFNγ alone led to trials with its combinations with other cytokines and chemostatics.

The use of IFNγ and IFNα in combination is justified considering that they have different receptors and different metabolic pathways for their cytostatic activity. There are data that in the B16 melanoma IFNγ exerts its effect by inducing p53, whereas IFNα acts by inducing the gene of a cyclin-dependent kinase inhibitor (Arany et al. 1997). However, the IFNγ/IFNα combination did not produce the expected effect (Osanto et al. 1989), and

the same applies to IFNγ in combination with IFNβ (Schiller et al. 1988b; Kowalzick et al. 1990) and with TNFα (Retsas et al. 1989; Selby 1990; Smith et al. 1991). Treatment with IFNγ and IL-2 in combination resulted in a partial remission of 4 months in one of seven patients (Taylor et al. 1990). In another clinical trial with 20 patients there was one partial remission for 7 months and one complete remission for 1 year (Kim et al. 1996). Also, in other clinical trials this combination had only a partial effect in a small number of melanoma patients (Margolin et al. 1992; Taylor et al. 1992).

A combination of IFNγ, TNFα, and M-CSF was proposed, because it showed an increased antitumor effect in mouse melanoma (Lasek et al. 1995).

Comparative experiments with IFNγ and IFNα have shown that in melanoma patients IFNα was more active (Steiner et al. 1987; Creagan et al. 1988, 1990). According to a review on the results of all clinical trials up to 1993, the most promising combination was that of cytokines (especially IFNα and IL-2) and chemotherapy (Parkinson 1993). For the clinical application of IFNα in malignant melanomas see elsewhere (Agarwala and Kirkwood 1996; Cole et al. 1996; Grob et al. 1996; Kirkwood et al. 1996; Punt et al. 1998). At present, the usefulness of IFNγ in melanoma is not completely clear and the advantage is given to IFNα and to IFNγ for locoregional therapy. According to some investigators, the optimal immunologically active dose of IFNγ is 0.1 mg/m$^2$ applied subcutaneously every day (Maluish et al. 1988).

For treatment of metastases with low tumor load (following chemotherapy and radiotherapy), intravenous administration of liposomal IFNγ was suggested for elimination by activated macropahges of disseminated cancer cells (Fidler 1990).

New studies on the metabolic pathways of the interferons show in a new light the possibilities for application of IFNγ and IFNα in combination. In order to stimulate certain genes (interferon-stimulated genes, ISG) the interferons induce the gene factor 3 (ISGF-3). The components of this factor (STAT-1, STAT-2, and p48-ISGFγ) are phosphorylated in order to form a complex, which is transported into the nucleus and activates the corresponding genes (see Chapter 4). The sensitivity of melanoma cells to type I interferons (α and β) correlates directly with the concentration of ISGF-3 and more specifically with STAT-1. The preliminary treatment of resistant melanoma cell lines with IFNγ (IFNγ priming) prior to their treatment with IFNα or IFNβ increases the level of ISGF-3 components and facilitates gene activation through their binding to the corresponding DNA control sequences. Thus, IFNγ increases the gene products induced by type I interferons, including, for example, the 2′-5′-oligoadenylate synthetase, the HLA class I antigens, the B7 molecules, and ICAM-I (Wong et al. 1998). In addition, it has been shown that IFNγ induces the A and B receptors of TNFα (Carrel et al. 1995).

All these data justify future trials with successive therapy for resistant melanomas with optimal priming doses of IFNγ and further treatment with IFNα or IFNβ and chemotherapy.

A systematic investigation of the antiproliferative effect of eight chemotherapeutics (bleomycin, dacarbazine [DTIC], doxorubicin, cisplatin, carboplatin, 5-FU, vindesine, and fotemustine) after preliminary treatment of four human melanoma cell lines with the three types of interferon has shown that the high resistance of melanoma cells to chemotherapeutics is not influenced by the interferons. However, there was an additive effect, since IFNβ and IFNγ in these experiments had a stronger effect than IFNα (Schadendorf et al. 1994). In the melanoma MmB16 subclone implanted in mice IFNγ and TNFα in combination showed an increased antitumor effect upon the addition of actinomycin D (Lasek et al. 1996).

Promising results were reported for inoperable melanomas of the limbs following isolated perfusion of the limb with a combination of IFNγ, TNFα, and melfalan, as the antitumor effect was increased by IFNγ (Noga and Hess 1991; Noga et al. 1992). There was a complete response in 80 to 90% even in close to 100% of patients, which prolonged the life of the limb. However, the remission was not long-lasting (Eggermont et al. 1992; Lienard et al. 1992a,b, 1994; Eggermont 1993; Vrouenraets et al. 1993; Lejeune et al. 1995; Thom et al. 1995; Fraker et al. 1996). Most investigators find this method to be safe, but a side effect similar to septic shock was reported in all patients subjected to this type of therapy (Vaglini et al. 1994a,b). Also according to other data, such a syndrome has been associated with this type of therapy, but it is transient and the recovery is rapid (Zwaveling et al. 1996). We should mention that when this method is used in combination with radiation therapy, there is a risk of tissue necrosis and disturbed wound healing (Vrouenraets et al. 1997).

This method is often combined with a moderate hyperthermia. It has been shown in mice that high body temperature increased the antitumor effect of IFNγ in B16 melanoma when the interferon was administered prior to 8 hours of hyperthermia. The investigators found that 2 hours of hyperthermia had an antagonistic effect, 5 hours an additive effect, and 8 hours a synergistic one (Fleischmann and Fleischmann 1994).

How could the unsatisfactory clinical results be explained, given the expressed antitumor activity of IFNγ in *in vitro* experiments and in mouse models?

In the first place, we have to point out that the primary melanoma tumor is composed of a very heterogeneous population of cells with various phenotypes and metabolism. Different sensitivity to the three interferons was found in five melanoma cell lines and it correlated with the induction of the 2′-5′ oligoadenylate synthetase. Resistance to the interferons is associated with a defect at an early step of the metabolic pathway, which in some cell lines leads to a decreased ability to induce tyrosine phosphorylation (Ralph et al. 1995). High heterogeneity in surface antigen and HLA expression was found in a study of 56 clones of the Me9229 and Me28 human metastatic melanomas. In the different cell lines IFNγ modulated to a different extent the expression of these antigens (Anichini et al. 1986). In two other melanoma

strains, Khm-1/4 and A101D, it also increased to a different extent the expression of HLA-DR and the melanoma-associated 97-kDa antigen (Kameyama et al. 1986). The lytic ability of the peripheral mononuclear cells toward the two melanoma strains — M19 and M26 — is stimulated by different combinations of IFNγ, IFNα, and IL-2 (Schultz et al. 1994).

Another well-studied factor is the influence of hypoxia. It has been found that melanoma cells are considerably less sensitive to IFNγ under hypoxic conditions, which are present in melanoma tumors (Bocci et al. 1991; Naldini et al. 1995).

Recently, attempts have started to treat melanomas by gene engineering approaches. In experiments with mice the IL-2 or IFNγ gene was introduced into B16 melanoma cells with a retroviral vector. They drastically reduced tumor formation after their subcutaneous administration in syngenic mice. Upon their intravenous introduction, most of the melanoma cells transduced with IFNγ were rejected, unlike those transduced with IL-2, which were tumorogenic and formed lung metastases (Abdel-Wahab et al. 1997a).

The first phase of clinical trials was conducted with specific immunization of melanoma patients with autologous melanoma cells transduced to express IFNγ and inactivated by radiation. Immunization with such cells was found to be harmless, and the results showed that this procedure was useful for treatment of patients with not very advanced stages of the disease (Abdel-Wahab et al. 1997b).

Successful experiments were also conducted with adoptive immunotherapy in mouse models using cytotoxic T lymphocytes genetically modified with cytokines (Abe et al. 1996).

## 12.7 Renal cell carcinoma (RCC)

There are data showing that renal cell carcinoma (RCC) in humans is due to a deletion in the short arm of chromosome 3 (Jacqmin 1994). It is believed that RCC is one of the few tumors which is immunogenic enough to be susceptible to immunotherapy (Graham 1994).

In kidney carcinoma IFNγ considerably increases ICAM-I expression at the level of transcription, which lasts for a long period, possibly due to a disturbed mechanism suppressing its expression (Hansen et al. 1993; Kawata et al. 1996; Tanabe et al. 1997). As it was already discussed (see Chapter 3, Section 3.4), high levels of this antigen favor tumor cell attack by tumoricidal lymphocytes. In most RCC lines the expression of the EGF receptor is also increased (Elsasser et al. 1999). Decreased MHC class I expression is found in some tumor lines (Kallfelz et al. 1999), which disturbs the function of the peptide-transporting system (TAP-1) and contributes to the emergence of a phenotype that can avoid the immune control. IFNγ increases the TAP-1 system of MHC class I associated with antigen presentation and also the LMP-2 proteosomal subunit, which facilitates RCC recognition by the immune system (Seliger et al. 1997).

In a number of clinical trials on kidney carcinoma it was found that IFNγ strongly activated the NK cells (Grups and Frohmuller 1990; Barna et al. 1991; Escudier et al. 1993; Kawata et al. 1993, 1995; Farace et al. 1994; Yasunaga et al. 1995; Yanagiyo 1996), activated the cytotoxic T cells, increased the T helper cells, and decreased the T suppressor lymphocytes, thus increasing the CD4+/CD8+ ratio, which is a manifestation of immunostimulation (Aulitzky et al. 1989a; Grups and Frohmuller 1990; Onishi et al. 1991; Ernstoff et al. 1992; Kawata et al. 1993; Yasunaga et al. 1995; Yanagiyo 1996).

The important role of IFNγ treatment for macrophage activation is revealed in experiments with the mouse kidney adenocarcinoma Renca (Hillman et al. 1997). In this tumor IFNγ also induced Fas overexpression, which leads to the effective killing of tumor cells through Fas ligand–induced apoptosis (Lee et al. 2000b). It was found that IFNγ also inhibits angiogenesis in patients with RCC (Yoshino et al. 2000), which leads to suppression of tumor growth.

An increase in CD8+ cells was observed in TIL (Kawata et al. 1993). In kidney carcinoma IFNγ also increases the expression of MHC classes I and II, which determine the immunogenicity of the tumor and thus play an important role in immunotherapy (Gastl et al. 1996). Different RCC lines, however, are heterogeneous with regard to growth inhibition and MHC antigen induction by IFNγ and IFNα (Angus et al. 1993).

The alveolar macrophages of patients with kidney cancer reacted to IFNγ with increased tumoricidal activity able of lysing neoblastic cells, but not normal cells (Thomassen et al. 1990; Barna et al. 1991). The high activity of prostaglandin E2 in such patients was decreased by IFNγ (Barna et al. 1991).

A metalloproteinase-2 (72-kDa type IV collagenase, gellatinase A) plays an important role in the invasiveness and metastatic potential of some tumors of the kidney. In experiments with the KG-2 cell line, bFGF and TGFβ stimulated the production of this enzyme, whereas IFNγ suppressed it. This decreased the invasive activity of the tumor (Gohji et al. 1998).

Studies on kidney cancer patients (Yoshino et al. 2000) and experiments with cell lines show that apart from the indirect effect exerted through the cellular immunity, IFNγ has also some direct antiproliferative activity. Thus, in the DNT-11 and HTB-44 cell lines IFNγ suppressed cell proliferation with 26 and 37%, respectively (Buszello 1995). In the TC-1 cell line the antiproliferative effect of IFNγ was associated with a suppressed expression of PCNA (a subunit of DNA polymerase δ) and of the cyclinB/cdc2 complex necessary for the G2/M transition (Hall et al. 1996). A direct inhibition of cell proliferation was also observed in the RAC cell line (Kuebler et al. 1987). Different RCC lines have different sensitivities to IFNγ. The latter suppresses the proliferaiton of three of four such cell lines (786-O, Cek-1, TK-10, TK-164) (Kavoussi et al. 1989).

The antiproliferative effect of IFNγ has also been shown in animal experiments. IFNγ suppressed the formation of lung metastases in an experimental model of kidney cancer in mice. It is interesting that the successive action

of IFNγ and IL-4 has a stronger suppressing effect on lung metastases than the action of IFNγ alone (Hillman et al. 1997). Still more effective was a combination of IFNγ and IL-2, as it had a synergistic effect and also suppressed the growth of subcutaneously implanted tumors (Masumori et al. 1995). IFNγ and TNFα in combination also suppressed tumor growth in rats with kidney carcinoma (Van Morselaar 1990).

With the human kidney cancer cell line KG-2 it was shown that IFNγ and IFNβ (but not IFNα) suppressed the transcription of the gelatinase gene independently of their antiproliferative activity. The lack of gelatinase activity considerably inhibited the invasive capacity of the tumor cells (Gohji et al. 1994).

The role of IFNγ as a protective cytokine has been shown by the fact that in blood cell cultures from patients with different stages of RCC the level of IFNγ is significantly lower than in healthy controls, and it decreases with increasing tumor burden. Also, IFNγ production was higher in stabilized patients than in patients with a progressing disease. These data led to the conclusion that low production of IFNγ was an indicator of tumor progression (Elsasser-Beile et al. 1989b).

The numerous clinical trials for treatment of kidney cancer with IFNγ gave very variable results, including remissions of 5 to 35% depending on the dose, the route, and the scheme of administration. Continuous as well intermittent administration has been used. Comparing the two procedures, it could be concluded that the second showed a stronger antitumor effect. Thus, whereas with continuous infusion there was no effect or the antitumor effect was minimal (6.0 to 8.6%), with intermittent application an antitumor effect was observed in 20.0 to 21.4% of the cases (Rinehart et al. 1986; Machida et al. 1987; Quesada et al. 1987; Takaku et al. 1987; Satake et al. 1993).

With regard to the dosage, different doses have been used — from 50 to 60 μg/m$^2$ to 1 to 3 mg/m$^2$. The administration of 1 mg/m$^2$ five times weekly (Mani and Poo 1996) and 0.15 to 0.2 mg/m$^2$ once a week (Scheibenbogen et al. 1993; Lummen et al. 1996) was ineffective. The very low dose of 50 to 60 μg/m$^2$ once a week also did not produce a satisfactory result (Hofmockel et al. 1993; Gleave et al. 1998; Small et al. 1998). The cyclic application of 0.25 mg a day for 8 days followed by an interval of 3 to 4 weeks produced some antitumor effect (Grups and Frohmuller 1989). The doses of about 100 μg/m$^2$ (approximately 3 to 5 × 10$^6$ IU) are believed to be optimal (Aulitzky et al. 1989a,b; Ellerhorst et al. 1992, 1994; Abratt et al. 1993). Unlike the preliminary data (Garnick et al. 1988), the high doses did not produce the expected result, which is not unusual considering that IFNγ is active at an optimal concentration (Kleinerman et al. 1986). The lower doses of IFNγ produced a smaller percentage of recurrences, and activated NK cells were found even 6 months after the interferon therapy was completed (Nakagawa et al. 1994).

It should be taken into consideration that the result of different clinical trials depended on the extent of disease progression. For example, in one clinical trial the low doses of IFNγ produced 23% response in patients with

unilateral tumors and affected regional lymph nodes only, whereas with widely spread metastases, the response was 5%. The investigators conclude that the low doses of IFNγ are effective and produce long-term remissions when the metastases are limited (Aulitzky et al. 1995) but not when the tumor load is large (Aulitzky et al. 1994).

When the activity of the effector cells was studied, it was found that the antitumor response resulted from stimulation of the NK cells (Kudo et al. 1990). Other investigators point out that the antitumor response is obtained when there is a decrease in CD8+ cells and an increase in CD4+ cells (Ernstoff et al. 1990, 1992). The increase in CD8+ cells after IFNγ therapy did not result in tumor regression (Aoyagi et al. 1992).

The increased activity of monocytes/macrophages under the effect of IFNγ was associated with elevated levels of neopterin. However, no correlation was found between this level and the course of the disease (Hobarth et al. 1994). The $\beta_2$-microglobulins are believed to be a more reliable marker for the antitumor response to IFNγ therapy. It correlates with an increase of the soluble HLA class I antigens induced by IFNγ (100 to 500 μg administered at intervals) (Aulitzky et al. 1991).

IFNγ was used as prophylactic postoperative adjuvant therapy ($3 \times 10^6$ IU weekly) after nephrectomy. The result was a significant stimulation of activated cytotoxic T lymphocytes (Miura et al. 1989).

Such an approach seems to be justified considering that a dominant Th2 cytokine profile is found in kidney cancer patients after nephrectomy (Onishi et al. 1999).

An important question is whether the objective remissions are associated with extended life span. According to some trials with 30% antitumor response, there was an increased survival (Otto et al. 1988). Analysis of 65 patients 3 to 4 years following interferon therapy (resulting in 26% remissions with IFNα and 36% with IFNγ) showed increased survival of patients who responded to therapy (Schneider et al. 1990). These data contradict the opinion that the remissions do not correlate with survival rate (Onishi et al. 1997).

Of interest are reports of single cases of RCC patients who responded to IFNγ with complete remission. Thus, a complete remission was observed in a patient with metastases in many organs after administration of low IFNγ doses (Otto et al. 1995) and also in another patient with lung metastases following inhalation and subcutaneous administration of IFNγ (Kawata et al. 1994). In one case a brain metastasis disappeared 2 weeks after IFNγ and radiotherapy (Kawakami et al. 1993). The last case is unusual, because in a different patient the metastases in other organs were affected by IFNγ, but the brain metastasis was not because of the hematoencephalic barrier (Otto et al. 1995). There is also a report on a liver metastasis that disappeared 6 months after IFNγ therapy (Ogi et al. 1992). Also, the intratumoral and transarterial administraiton of IFNγ eliminated metastases that appeared after operation of the affected kidney (Kitagawa et al. 1992). In a single case of kidney cancer metastasis a complete remission was observed 13 weeks

after therapy with IFNα-2b and IFNγ followed by IFNα-2b alone (Okaneya et al. 1999).

Clinical trials with IFNγ-1b for the treatment of metastatic kidney cancer showed minimal effectiveness (Gleave et al. 1998; Small et al. 1998). It is hard to say whether the reason for this result is the better (blind, placebo-controlled) trial, the inappropriate dose or scheme of application (60 μg subcutaneously once a week), or the modified IFNγ.

In a clinical trial with 22 patients different combinations of IFNγ and other cytokines also were tested. IFNγ in combination with TNFα resulted in 3 complete remissions, 1 partial remission, and 10 stabilized patients (Sohn et al. 1992).

The simultaneous administration of IFNγ and IFNα did not improve the results (de Mulder et al. 1991, 1995) regardless of the report that this combination strongly activated the NK cells and the cytotoxic T lymphocytes (Yanagiyo 1996). This combination was used for treatment of advanced kidney cancer (Naito et al. 1995). There are data that IFNγ applied before treatment with IFNα stimulates the antitumor activity of T cells (Ernstoff et al. 1992) and is important for the final result. Thus, injection of IFNγ 6 hours prior to IFNα in 30 patients resulted in 14 antitumor responses, which led to 2 complete remissions of 20 and 22 months (Ernstoff et al. 1990).

Already in the first trials it was found that the lower doses of IFNγ followed by high doses of IFNα produced more promising results. IFNγ and IFNα in combination produced a 20% response and stabilization longer than 12 months in more than 92.3% of the patients (Koga et al. 1999). In another clinical trial the successive administration of IFNα and IFNγ resulted in 25% complete plus partial responses in metastatic kidney cancer after nephrectomy (Fujii et al. 1999).

RCC metastases were suppressed after the simultaneous application of liposomes containing macrophage activators and subcutaneous injections of IFNγ (Dinney et al. 1992) and also after subcutaneous IFNγ in combination with liposomal muramyl peptides (Lautersztain et al. 1991), which act synergistically with IFNγ (Galligioni et al. 1994). In metastatic kidney carcinoma some successful trials have been conducted using the combination of IFNγ and 2-,2'-difluorodeoxycytidine (Gemcitabine) (Rohde et al. 1998).

Data showing that the monocytosis obtained upon combination of IFNγ with M-CSF is associated with thrombocytopaenia should be taken into consideration (Weiner et al. 1994).

The combination of IFNγ and IL-2 should be also considered. IL-2 is a powerful inducer of LAK activity against autologous and allogenic tumor cells. This activity is accomplished by the CD3-CD56+CD16+ lymphocytes. IL-2, however, is highly toxic. Treatment of kidney cancer patients with IFNγ (100 $\mu g/m^2$) 2 hours prior to IL-2 increased the LAK and NK activities more than did IL-2 alone (Weiner et al. 1991). The use of IFNγ in combination with IL-2 for treatment of metastatic kidney carcinoma increased the number of NK cells and resulted in 21% partial remissions, 39% stabilization, and only 13% progressive disease (Escudier et al. 1993). In another trial a 23% antitumor

response was observed (Hercend et al. 1991). Other investigators also obtained an effective result (33% response) (Tagliaferri et al. 1998). However, in a different trial the induction of IFNγ by IL-2 neither correlated with the antitumor response nor with the survival rate (Meffert et al. 1997).

An antitumor effect also was observed in a mouse model and in humans upon administration of IL-12, which induces IFNγ, as well as the angiostatic CXC chemokines IP-10 and MIG (Bukowski et al. 1999). Data from the clinical application of IL-12 in patients with kidney cancer and from experiments with mice show that the prolonged application of this cytokine leads to its decreased level in the serum together with that of IFNγ resulting in the loss of effect (Rakhit et al. 1999). We should mention some new data which show that IL-12 could not significantly stimulate the production of IFNγ by the peripheral T lymphocytes and by the kidney carcinoma–specific TIL (Ulchaker et al. 1999).

A new approach of intratracheal administration of liposomal IL-12 led to the induction of IL-12 and IFNγ in the bronchoalveolar lavage and to a strong suppression of lung metastases of mice kidney cancer (Blezinger et al. 1999).

The combination of IFNγ and vinblastine produced 20.6% complete plus partial responses (Bartoletti et al. 1990).

Finally, we shall mention the recent use of gene therapy for the treatment of kidney cancer. Experiments on mice have shown that radiation and multiple vaccination with cytokine-producing Renca cells (kidney cancer cells transformed to produce IFNγ, IL-2, or GM-CSF) considerably decrease the number of lung metastases (Nishisaka et al. 1999). However, we have to point out some studies which show that the effect of IFNγ on kidney cancer cells is the same as in cells modified to produce this cytokine — in both cases a 98% increase in HLA class I and ICAM-I and a 95% activation of HLA class II have been obtained (Nayak et al. 1999).

## *12.8 Breast cancer*

It has been found that the Th2 cytokines are dominant in primary cancer of the mammary gland and in established cell lines of this type of cancer. Thus, cancer cells avoid the cellular immune response (Karp and Chan 1994). A Th0-type cytokine, secreting large quantities of IFNγ and IL-4, has been found at the level of individual cells (Lorenzen et al. 1991). In six out of seven breast cancer cell lines studied (MCF-7, ZR-75.1, MB-231, MB-415, MB-468, SKBr3) and in five primary breast tumors there was no expression of Fas/Apo1 surface antigen, unlike the normal mammary gland epithelium where there is high expression of Fas leading to apoptotic death under the effect of anti-Fas antibodies (Keane et al. 1996). IFNγ increases Fas and ICAM-I (CD54) expression in MDA-MB453 and MCF-7 breast cancer cells but not in the estrogen receptor–negative cell line BT-474. The expression of ICAM-I positively correlates with the sensitivity of the breast cancer cells to lysis by NK/LAK cells (Salup et al. 1994). In the AU-485 cell line the increase in ICAM-I expression under the effect of IFNγ possibly occurs with the help of

protein kinase C (Bacus et al. 1993). A differentiation-inducing factor (a 44-kDa protein) has the same function and stimulates tyrosine phosphorylation of the c-erbB-2 (Her-2/neu) receptor. This also induces a phenotypic differentiation in some breast tumors, which stop proliferating and become milk-producing cells. Overexpression of c-erbB-2, found in 30% of breast cancer cases, has a poor prognosis (Salup et al. 1994). IFNγ suppresses this gene (Marth et al. 1990). The oncogenes c-myc, hst, and int-2 are also often expressed in breast cancer (Le Roy et al. 1991).

In general, the tumoricidal function of the effector cells (macrophages, NK, LAK, and others) is suppressed in mice carriers of the D1-DMBA-1 mammary gland adenocarcinoma (Sotomayor et al. 1993) and also in women with breast cancer (Marubayashi et al. 1991; Baxevanis et al. 1993). It has been shown that the production of LAK cells under the effect of IL-2 depends on the presence of IFNγ. The serum of patients with breast and ovarian cancer inhibits the production of IFNγ (Muster-Bloy et al. 1996). The addition of exogenous IFNγ recovers the suppressed LAK activity, whereas the latter is decreased by antibodies to IFNγ (Shiiba et al. 1986). It was reported that, unlike IFNγ, IFNα suppresses this activity (Kamamura et al. 1998). High doses of IFNγ and LPS recover the tumoricidal activity of these cells (Sotomayor et al. 1993). The cooperation between the T lymphocytes and the macrophages is dependent, at least in part, on IFNγ. In experiments on cancer of the mammary gland in mice it was found that IFNγ increased the transport into effector cells of L-arginine, which is necessary for production of NO (Cendan et al. 1996).

Some data indicate that the suppression of NK/LAK activity in breast cancer is due to the abnormally high production of PgE2, which suppresses IL-2 production. Indomethacin and IFNγ recover the NK/LAK activity to the levels in healthy organisms. The effect of PgE2 is due to the suppressed expression of IL-2 receptors on CD56+ cells (Baxevanis et al. 1993). IFNγ increases the cytotoxic activity of LAK, TIL, and the mononuclear cells in pleural exudates (Papamichail and Baxevanis 1992). In women with breast cancer the NK activity was normal in the peripheral blood mononuclear cells, but it was disturbed in the regional lymph nodes (Bonilla et al. 1988).

All these data justify the use of Th1-type cytokines to treat breast cancer.

This has been confirmed by a number of experiments with *in vitro* cell cultures. In several breast cancer cell lines the inhibition of cell proliferation correlated with HLA-DR expression (Gastl et al. 1985). Treatment of Fas-negative mammary gland cancer cells with IFNγ led to increased Fas expression and to recovered sensitivity to apoptosis (Keane et al. 1996). A number of breast cancer cell lines were sensitive *in vitro* to the antiproliferative activity of all three interferons. The strongest direct effect was obtained with IFNβ (Coradini et al. 1994). In the MCF-7 cancer cell line, when the LAK cells interacted with the tumor cells, IFNγ and TNFα were released (Chong et al. 1989).

The direct antiproliferative effect of IFNγ was increased syngergistically in combination with TNFα (Marth et al. 1987b; Shiiki et al. 1989), with TNFβ

(Shiiki et al. 1990b), and also with retinoic acid (Marth et al. 1986, 1987b, 1993; Windbichler et al. 1996). IFNγ in combination with IFNα also had a stronger antitumor effect (Gastl et al. 1985). However, the antiproliferative effect of IFNγ was absent in some cell lines, such as the T47 D cell line (Solary et al. 1991). It is interesting that the prolonged incubation (18 months) with IFNγ of the BT-20 cell line made it completely resistant to this cytokine, which was accompanied by a decreased number of IFNγ-binding sites (Marth et al. 1987a,b). This could be explained by the IFNγ-suppressed expression of the β-chain of its receptor (Bach et al. 1995, 1997).

The antiproliferative effect of IFNγ in breast cancer is also due to the induction of the cyclin-dependent kinase inhibitor p21(WAF1), which is synergistically activated by the tumor suppressor gene BRCA1, whereas the IRF-1 gene is not affected. It is important that the induction of p21 is disturbed in the presence of a mutant BRCA1 5382C allele (Ouchi et al. 2000).

An additive effect of all three types of interferon on the CG-5 estrogen-dependent cell line was obtained in combination with the antiestrogen tamoxifen (Iacobelli et al. 1986; Porzsolt et al. 1989). Other investigators find that the presence or absence of estrogen receptors does not influence the antiproliferative activity of IFNγ (Goldstein et al. 1989). TNFα exerts an antiproliferative effect on the hormone-dependent mammary gland cancer cells (during the early stage of their development), whereas the hormone-independent lines (late stage of breast cancer development) are not sensitive to this cytokine. However, in the presence of IFNγ, high concentrations of TNFα inhibit the growth of hormone-independent tumors (Mueller et al. 1994, 1996). In the MCF-7 cell line, which is relatively resistant to tamoxifen and to TNFα, the cytotoxicity is considerably increased when TNFα or tamoxifen is combined with IFNγ (Matsuo et al. 1992).

The synergism of IFNγ and retinoic acid in a number of cell lines (MDF-7, SKBr-3, BT-20) is due to the increased expression of retinoic acid gamma receptors (RAR-gamma) and also to the suppression of the activation of the cytoplasmic retinoic acid–binding protein (CRAB-I) by retinoic acid (Widschwendter et al. 1995a,b, 1996).

The effect of IFNγ on the MDA-468 cell line was due to increased secretion of TGFα, which suppresses the growth of this cell line (Hamburger and Gayatri 1993). The direct effect of IFNγ is also associated with the induction of HLA class II antigens in some breast cancer cell lines (CF-7, T47D, ZR-75.1, HSL-53, MDA-MB-231). In other cell lines, however, HLA-DQ is not induced. This could be important in connection with the activation of the immune response to different breast tumors (Jabrane-Ferrat et al. 1990b). In a number of cancer cell lines the induction of HLA-DR did not correlate with the antiproliferative activity (Gastl et al. 1985).

The suppressed proliferation of some cancer cell lines is due in part to the downregulation of the EGF receptors by IFNγ (Chakravarthy et al. 1991).

The antitumor activity of IFNγ in breast cancer has also been shown in experiments *in vivo*. Its intratumoral administration in combination with IFNα into xenotransplants of the human tumor cell lines MCF-7 and BF-20

led to complete regression of MCF-7 and partial regression of BF-20. Separately, each of the cytokines produced an incomplete antitumor reaction (Ozzello et al. 1988).

A synergistic antitumor effect was also observed with IFNγ in combination with IFNβ (Ozzello et al. 1990). In xenotransplants IFNγ combined with TNFα led to obstruction of tumor vascularization, apoptosis, and necrosis (De Kossado et al. 1995). Similar results were also obtained in tumors of the mammary gland induced with nitrosomethyl urea (Shah et al. 1989).

In contrast to these data, in the first clinical trials neither complete nor partial results were obtained. We should point out, however, that the doses administered were too high (2 mg/m$^2$, intravenously, 5 days every other week) (Muss et al. 1986), a regimen that appears to exceed the optimal dosage. In advanced stages of breast cancer a combination of IFNγ (or IFNβ) and hormonotherapy (Megace or Tamoxifen) was used. This combination, especially with tamoxifen, produced a favorable effect with a strong increase in the IFNγ level. The investigators believe that there is a favorable prognosis when the level of IFNγ increases and that of sIL-2R decreases, and these changes are the most sensitive parameters for making a prognosis (Barak et al. 1998).

The new approach of transgenic immunotherapy was also used. It involves cytokine (including IFNγ) gene transfer into tumor cells via retroviral vectors or vaccinia viruses (Teramura et al. 1993; Cornetta et al. 1994; Su et al. 1994; Matory et al. 1995; Peplinski et al. 1996; Nanni et al. 1998). Tumor cells transfected with IFNγ induce a strong immune response, which leads to the rejection of both the transfected and the primary unmodified tumors through the generation of active effector cells. IFNγ plays an important role in this process. This is demonstrated by the fact that antibodies to this cytokine block tumor rejection. In this reaction the cytotoxic CD8+ T cells (in myeloma) and the CD4+ helper cells (in breast cancer) play a role (Teramura et al. 1993; Su et al. 1994). Transfection with IFNγ of human breast tumors (cell lines MDA-MB-435 and -431) led to growth suppression, decreased invasiveness, IFNγ production, and increased synthesis of MHC antigens (Cornetta et al. 1994). The method was also tested in mouse mammary gland cancer (DA-3, EMT-6 and -410) (Matory et al. 1995). Transfection with GM-CSF alone or in combination with IFNγ was the most effective in the mouse cell lines (Peplinski et al. 1996).

Another approach for using IFNγ is the so-called graft-vs.-host disease (GVHD; see Section 12.1). The autologous GVHD is initiated by autotransplantation of bone marrow and treatment with cyclosporine A (CSA). It is enhanced by the application of IFNγ (Kennedy and Jones 1993; Kennedy et al. 1994; Hess 1995). A GVHD with allogenic bone marrow from a HLA identical donor has also been successfully used (Eibl et al. 1996).

The GVHD induces a powerful antitumor response in animal models. The effect is aimed at cells expressing MHC class II antigens, which is stimulated by IFNγ, which determines the strong antitumor activity (Kennedy et al. 1995, 1996). After treatment of metastatic breast cancer with

***Table 12.1*** Effect of GVHD,[a] HDC,[b] CSA,[c] and IFNγ combinations in breast cancer therapy

| Combination | Absence of progression (%) | Survival (%) |
|---|---|---|
| GVHD + HDC | 13 | 38 |
| GVHD + HDC +CSA | 24 | 44 |
| GVHD + HDC + CSA + IFNγ | 41 | 53 |

[a] Graft-vs.-host-disease.

[b] High doses chemotherapeutics

[c] Cyclosporine A

a combination of GVHD, high doses of chemotherapeutics, and IFNγ the following results were obtained for a period of 4 to 5 years (Table 12.1) (Kennedy et al. 1996). These results deserve further attention.

## *12.9 Cervical cancer*

Cervical cancer is caused by the human papillomaviruses 16 and 18. The mechanism is associated with inactivation of the p53 gene product.

This gene was discovered in 1979, and for the next 10 years it was believed to be an oncogene, because it had mutations in more than 50% of human tumors. Finally, it was found to be a tumor-suppressor gene, and it was named the "guardian" of the genome. It protects the genome from accumulation of damaged DNA during cell division. Normally, this gene is almost inactive, but it is activated whenever DNA is damaged — from UV or ionizing radiation, chemical agents, stress, heat shock, and others. The p53 protein encoded by this gene causes either apoptotic death of the cell with damaged DNA, or arrest of the cell cycle at the end of the G1 phase by inducing the kinase inhibitor p21/Waf1, which prevents the hyperphosphorylation of the retinoblastoma protein pRb. When the cell cycle is arrested, genes and mechanisms are activated leading to the repair of the damaged DNA, which makes possible its replication. The function of the p53 gene can also be impaired by the products of some viruses, which bind the p53 protein. This permits the replication of damaged DNA leading to malignant transformation. Such p53-binding proteins are the large T antigen of simian virus 40 (SV40), the 55-kDa protein of the adenoviral E1B gene, the IE84 protein of the cytomegalovirus, the mdm2 protein, the X protein of the hepatitis B virus, and the E6 protein of HPV16 and 18, which is the main cause of cervical cancer. The E6 proteins not only bind the p53 protein, but they also cause its degradation. An allelic variant which has an Arg at position 72 instead of a Pro is more easily degraded. In 77% of cervical cancer the patients had an Arg variant, whereas in the healthy controls it occurred in 37% (Storey et al. 1998).

It is important to point out that, in this case, the product of the p53 gene is only affected, but not the gene itself. This is why the use of ionizing

radiation and chemical cytostatics for cancer treatment could be a double-edged sword — they can cause mutations in this gene making the process irreversible and the cancer cells resistant to further therapy. This supports the rational use of antiviral therapy for virus-induced tumors.

Such an approach is also supported by a number of experimental data. In the first place, we have to mention that a significant shift from a Th1 to a Th2 cytokine profile has been observed in cervical intraepithelial neoplasia (Clerici et al. 1997). In human cervical epithelial cells immortalized by HPV16 and HPV18 IFNγ inhibited cell growth and reversibly decreased the E6/E7 viral RNA level. This effect of IFNγ was not due to affected RNA half-life but to selective suppression of the transcription of E6/E7 viral RNAs (Woodworth et al. 1990; Trizna et al. 1994). In other experiments the growth of human keratinocytes transformed with human papillomavirus (HPV) was strongly suppressed by IFNγ, and this effect was increased in combination with TNFα (Delvenne et al. 1995). In the SiHa cervical cancer cell line all three types of IFN selectively decreased the E6/E7 RNA of the HPV16 (De Marco and Marcante 1993). The peripheral blood mononuclear cells of cervical cancer patients had decreased production of IFNγ. It has been shown that this is due to an increase in the PgE2 level with disease progression and PgE2 suppression of IFNγ production (Mori et al. 1990). Decreased production of IFNγ was also found in the tissues of the intraepithelial and invasive cervical carcinoma (Pao et al. 1995). Peritoneal macrophages of patients with cervical and ovarian cancer treated with IFNγ increase their secretion of TNFα, IL-1, and IFNγ but not of PgE2. A strong correlation also has been observed between the secretion of IFNγ and TNFα (Chen et al. 1990).

An example of growth suppression of the human cervical cancer cell line SKGIIIb is shown in Figure 12.4 (Iwasaka et al. 1987).

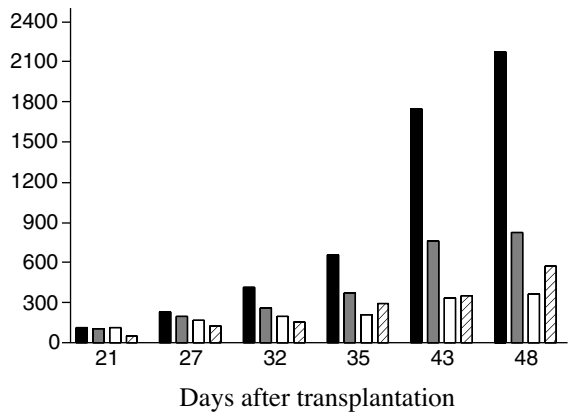


***Figure 12.4*** Effect of IFNγ ($10^6$ IU intraperitoneally every day) on the growth of human cervical tumor SKGIIIb. Ordinates — size of the tumors in $mm^3$. Controls (■); IFNγ injected from the day of transplantation (▨); IFNγ started 3 weeks after transplantation (■); IFNγ injected into the tumor (□). (According to data by Iwasaka et al. 1987.)

IFNγ showed an antiproliferative effect in the human cervical cancer cell lines ME-180, SiHa, HT-3, and MS751, whereas TNFα did not have an effect, but it synergistically increased the effect of IFNγ (Mutch et al. 1990). In another experiment the lysing ability of IFNγ on the same cell lines plus the C-33A cell line was tested. None of them was susceptible to lysis. However, if protein synthesis was inhibited after the preliminary treatment with IFNγ, the ME-180 and MS751 cell lines underwent considerable lysis (Massad et al. 1990).

As in the other types of cancer, here IFNγ also increases the expression of HLA antigens (Okamura et al. 1986). Using neutralizing antibodies, it was shown that the antitumor activity of the lymph node cells in cervical cancer was due to the secretion of IFNγ (Okadome et al. 1991). In the cervical cancer cell lines HeLa, CAC-1, and TMCC it was found that the antiproliferative activity of the peripheral blood and lymph node lymphocytes was due to IFNγ production (Saito et al. 1994). Treatment with IFNγ and TNFα increases ICAM-I expression in cervical cancer too (Okamoto et al. 1993; Cristoforoni et al. 1994; Evans and Baker 1996; Bornstein et al. 1997). IFNγ induces the membrane expression of ICAM-I, whereas IFNβ suppresses it (Bornstein et al. 1997). In this type of cancer the combination of IFNγ and retinoids also synergistically increases the antitumor effect (Bollag 1994; Widschwendter et al. 1994). The cytotoxic effect of low doses of cisplatin is increased by IFNγ, IFNα, and TNFα (Verma et al. 1996).

In patients with cervical cancer the long-term cultivation (28 days) of peripheral blood leukocytes with IL-2 or a mixture of IL-2, IFNγ, and TNFα in the presence of cancer cells generates cytotoxic lymphocytes with high lytic activity toward autologous cancer cells, poor activity toward the heterologous ones, and a lack of cytotoxicity directed against other cancer cells and normal cervical cells (Rangel et al. 1995).

The direct antitumor effect of IFNγ and its synergism with IFNα highly vary depending on the cancer cell line. The optimal scheme for a direct antitumor effect is a long-term treatment with interferons (Higashihara et al. 1988). An indirect anti-tumor effect of IFNγ was observed in human cervical tumors transplanted in nude mice (Iwasaka et al. 1987).

Unlike all of these data, the results of the clinical trials are difficult to interpret. Injection of IFNγ ($2 \times 10^6$ IU/day) around the cervical tumor lesion for 3 days prior to radical hysterectomy neither showed immune cell infiltration nor a decrease in tumor size (Honma et al. 1991). Due to the short duration of treatment, these data do not allow conclusions regarding the effect of IFNγ on the tumor. A review of the literature on the clinical application of interferons and retinoids in patients with cervical cancer did not reach a firm conclusion about the effectiveness of this treatment. For intraepithelial cervical cancer there are data showing 39.7% remissions with IFNα, 64.3% with IFNβ, and 51.8% with IFNγ. However, it has been noted that in double-blind placebo-controlled trials the effect of the interferons and retinoids has not been confirmed, and the results for invasive cervical cancer are inconclusive (Gunter et al. 1995). The combination of interferons with ionizing

radiation is recommended as useful (Angioli et al. 1992), but the ability of IFNγ to increase the radiosensitivity of normal tissues should be considered (see p. 126).

As with the other malignant tumors, the contradiction between the theoretical and experimental data on the one hand and the clinical results on the other also shows that the optimal scheme (e.g., dosage, route of administration) for treatment with IFNγ of viral tumors has not yet been established.

## 12.10 Gastric cancer

The activity of NK cells in advanced (stage IV) gastric cancer is considerably decreased as compared to stages I to III and to healthy controls. IFNγ increases the activity of these cells without increasing their number (Kuwahara et al. 1987). The LAK activity of the lymphocytes in the regional lymph nodes is also decreased (Karimine et al. 1994), although according to another study (Kusugami et al. 1990), the regional lymph nodes of patients with gastric cancer produce high levels of IL-2 and IFNγ. In the peripheral blood lymphocytes, however, the activity of IL-2 and IFNγ is decreased, especially in the advanced stages of stomach cancer (Kusugami et al. 1990; Wen et al. 1994; Zhu et al. 1995).

IFNγ increases the expression of HLA-DR in gastric cancer (Shibata et al. 1992) and also of ICAM-I (Ishii et al. 1994; Ishigami et al. 1996). IFNγ together with TNFα stimulates the expression of IL-8, which is a chemotactic agent for neutrophils, basophils, and T lymphocytes (Yasumoto et al. 1992).

The expression of the apoptotic antigen Fas highly varies in different stomach cancer cell lines. Relatively high expression was found in the MKN-74 and MKN-75 cell lines (both with normal p53), and decreased expression was observed in the cell lines with deleted p53 (KATO-III cell line) or with mutated p53 (MKN-1, MKN-7, and MKN-28 cell lines). Fas expression was increased by IFNγ except in the cell lines with mutated p53 (Hayashi et al. 1997).

In *in vitro* experiments on gastric cancer cell lines the interferons exhibited an antiproliferative activity in the following order; IFNγ>IFNβ>IFNα regardless of whether they were natural or recombinant products (Kimoto et al. 1986).

In the MKN-1 and MKN-28 cell lines a synergistic effect was observed upon the application of IFNγ in combination with TNFβ (Shiiki et al. 1990b) or with TNFα (Shiiki et al. 1989). It has been shown that IFNγ does not change the number nor the affinity constant of the TNFα-binding sites. This indicates that the synergism is due to postreceptor mechanisms (Yasumoto et al. 1992).

The effect of IFNγ in combination with chemotherapeutics was increased by low doses of 5-FU or methotrexate if the latter was administered prior to the interferon therapy (Kimoto et al. 1986). In the SNU-1, SNU-5, SNU-16, and NCI-N87 cell lines the combination of IFNγ with 5-FU showed an additive, but not a synergistic, effect (Park et al. 1991). There is a report on a case

of stomach cancinoma with multiple metastases in the liver that reacted to the combination of IFNγ and 5-FU with a considerable decrease in the number and size of the metastases and in the size of the primary tumor, which permitted a partial resection of the stomach and the liver (Katano et al. 1987).

Unexpected was the report that IL-4 (a Th2 cytokine) inhibited the growth of stomach cancer cell lines, 5 to 85% of which expressed the receptor for this cytokine (IL-4R). The effect was increased synergistically after combining IL-4 with IFNγ and TNFα (Morisaki et al. 1992).

## *12.11 Prostate cancer*

The data in the literature are exclusively experimental, with indications for a possible favorable effect of the application of IFNγ and other Th1 cytokines for treatment of prostate cancer.

The MHC class I antigens are strongly expressed in the cells of the benign prostatic hyperplasia, whereas in malignant transformation different levels of expression are found: from complete lack of expression in the LNCaP cell line to normal expression in the PC-3 and DU-145 cell lines. MHC class II antigens are neither expressed in normal nor cancer cells of the prostate. They were slightly induced by IFNγ (Bander et al. 1997). Increased expression of MHC class II by IFNγ was also found in other studies (Blumenfeld et al. 1993; Sokoloff et al. 1996).

The direct antiproliferative activity of IFNγ and TNFα on prostate cancer cells has been shown in *in vitro* experiments. TNFα inhibited the androgen-dependent cell line LNCaP, whereas it had no effect or a very poor effect on the androgen-independent cell lines PC-3 and JCA-1. IFNγ had a different effect: it inhibited the PC-3 and JCA-1 cell lines and had no effect on the LNCaP cell line. These data show the advantage of using the two cytokines in combination for treatment of some types of gastric cancer and of IFNγ alone for others (Nakajima et al. 1995). A synergistic effect of IFNγ in combination with TNFα was also observed in a rat model with androgen-dependent and androgen-independent cell lines (van Moorselaar et al. 1991a). In *in vivo* experiments with the human cell lines PC-3 and DU-145 transplanted in athymic mice, the combination of IFNγ and IFNα had a considerable antitumor effect on PC-3 cell line but not on DU-145 cell line (both cell lines are hormone independent) (van Moorselaar et al. 1991b). In connection with these cell lines, we shall point out that in the DU-145 and PC-3 cell lines, which are sensitive to the antiproliferative activity of IFNγ, this cytokine induced the expression of p21 but not in the resistant cell line LNCaP. Also, in the sensitive cell lines IFNγ suppressed the proto-oncogene neu (HER-2) and induced phosphorylation of STAT-1 (Kominsky et al. 2000).

In some types of prostate cancer IFNγ also induces ICAM-I (Sokoloff et al. 1996). Interesting data were obtained in the DU-145 cancer cell line, which has mutations in the genes encoding p53 and pRb. It was found that IFNγ and IFNα induce the expression of the kinase inhibitor p21/WAF1, which blocks the cell cycle and is normally induced by p53 if its gene is not

inactivated (see Section 12.9). Thus, both interferons inhibit cell proliferation, but only IFNγ leads to phenotypic changes and at the same time causes considerable reduction in the number of EGF receptors and increases the expression of ICAM-I. All of these changes point to the appearance of a nontumorigenic state associated with decreased invasive capacity regardless of the state of p53 and pRb. In other words, IFNγ bypasses the metabolic pathway of the mutated tumor-suppressor genes (Hobeika et al. 1998). A synergistic cytostatic activity against hormone-insensitive prostatic cancer cells was observed with the combination of Fenretinide (a less toxic derivative of retinoic acid) and nontoxic doses of IFNγ (Fine et al. 1994). Increased expression of IFNγ has been observed in cell lines with mutated p53, which can be considered as an attempt, although insufficient, to inhibit the uncontrolled cell proliferation (Royuela et al. 2000).

In prostatic cancer patients it was observed that the ability to produce interferons (IFNγ and IFNα) decreases with disease progression. Thus, the IFN level may have a prognostic value (Kita et al. 1990).

Recently, clinical trials have been started to study the immunotherapy of prostatic cancer with the combination of natural cytokines (IL-1, IL-2, GM-CSF, IFNγ, TNFα) (Harris et al. 1999). It is interesting to note that in a mouse model of prostatic cancer the metastatic cells were resistant to CTL, unlike the primary cancer cells. This was not due to the defective production of molecules associated with antigen presentation (Lee et al. 2000a).

## 12.12 Ovarian cancer

In ovarian cancer IFNγ suppresses the c-erbB-2 oncogene (Her2/neu), whose overexperssion is associated with a poor prognosis, as also is the case of breast cancer (Marth et al. 1990, 1992; Nehme et al. 1995). At the same time, IFNγ decreases the constitutive phosphorylation of the tyrosine on c-erbB-2 and inhibits its kinase activity (Mishra and Hamburger 1994). On the other hand, IFNγ increases the expression of MHC class I and induces the expression of MHC class II antigens (Boyer et al. 1989; Marth et al. 1989b, 1996; Allavena et al. 1990; Fuith et al. 1991; Mobus et al. 1993; Santin et al. 1996) and of ICAM-I (Santin et al. 1996). IFNγ also increases or induces the expression of specific tumor-associated antigens of ovarian cancer such as CA-125, HMFG, CEA, MUC-1, O3 (Marth et al. 1989a; Fuith et al. 1991; Mobus et al. 1993; Clark et al. 1994a; Imbert-Marcille et al. 1994). This helps the specific discrimination between malignant and benign cells in radioimmunoscintigraphy.

Studies of the direct antiproliferative activity of IFNγ on ovarian cancer showed that the various cell lines had different sensitivity (Marth et al. 1989a,b; Massad et al. 1990; Mobus et al. 1993; Klouche et al. 1995). In some cell lines IFNγ increases the cytotoxicity of cisplatin (Clark et al. 1994b; Nehme et al. 1995) both *in vitro* and *in vivo* experiments in mice with xenotransplants (Dumont et al. 1995). We should mention that although the treatment with platinum compounds is very effective in the beginning, some

tumors develop multidrug resistance (MDR; see p. 120) after long treatment. In such tumors IFNγ also increases the expression of MHC class I and class II, as well as the expression of tumor-specific antigens, which enhances their immunogenicity and therefore the therapeutic effect (Mobus et al. 1993).

In *in vivo* experiments on athymic mice with intraperitoneal xenotransplants of human ovarian carcinoma, the combination of IFNγ and TNFα exhibited a strong antitumor effect — only 1 of 20 mice had an intraperitoneal tumor, whereas all of the controls expressed intra-abdominal canceromatosis (Manetta et al. 1989). The indirect effect of IFNγ in ovarian cancer shown in *in vivo* experiments is also associated with stimulation of the tumoricidal activity of the peritoneal mesothelial cells (Michelini-Norris et al. 1996).

The resistance of some ovarian cancer cell lines to lysis by IFNγ appears to be dependent on a mechanism connected to protein synthesis. Treatment with IFNγ followed by inhibitors of protein synthesis leads to considerable lysis in some resistant cell lines (Massad et al. 1990). Cell lines resistant to IFNγ and TNF react with suppressed growth when the two cytokines are combined (Li et al. 1994), and also when they are combined with cytostatics (Li et al. 1995).

The direct antitumor effect of IFNγ on some ovarian cancer cell lines is also associated with the induction of IDO (see Section 3.2) leading to tryptophan starvation. At the same time, however, the tryphophanyl-tRNA synthetase is also induced (Reano et al. 1993). This appears as a compensatory mechanism that functions to continue protein synthesis when the concentration of free tryptophan is decreased. The lack of a direct connection between the tryptophan decrease and the antitumor activity shows that other mechanisms also are involved (Burke et al. 1995).

Also, there are data that patients with ovarian cancer show a higher level of sICAM-I, which is increased by IFNγ (Giavazzi et al. 1994).

The antitumor activity of IFNγ on ovarian cancer initiated early clinical trials with its intraperitoneal administration for treatment of resistant tumors and residual tumors after previous treatment. (For the early clinical trials, see review by Welander 1988.) In one of the first trials no objective response was observed in 27 resistant tumors (D'Acquisto et al. 1988). In another clinical trial there was a complete response in 29% of the cases of residual ovarian cancer following a primary therapy, and a correlation was found between the size of the tumor and the response to IFNγ (for sizes less than 5 mm there was a 37.5% response; for sizes between 5 and 20 mm there was a 21% response; and there was a 9% response for larger tumors) (Pujade-Loraine et al. 1991). In another clinical trial an effect of IFNγ also was observed when the tumor load was small (Colombo et al. 1992).

For the prognosis of an IFNγ response, apart from the size of the tumor, the age of the patients also is of importance. When $20 \times 10^6$ IU/m$^2$ of IFNγ was administered intraperitoneally twice a week for 3 to 4 months, 31 of 98 patients responded positively, including 23% with a complete response. Forty-one percent of patients younger than 60 years with tumors smaller than 2 cm had a complete response. A 3-year follow-up study showed that

62% of patients with a complete response had survived (Pujade-Loraine et al. 1996).

## 12.13 Cancer of the bladder

In *in vitro* experiments IFNγ showed a direct antiproliferative effect on different uroendothelial cancer cell lines (Jackson et al. 1994). Sensitive cell lines included BT1, RT4, EJ, 468P, 253J, SD, TCCSUP, and SW1738, relatively less sensitive were T24, 647V, VMCUB2, and J82, and the cell lines VM-CUB1, 639V and SW1710 appeared to be resistant. In some cell lines (RT4 and RT112) IFNγ was cytostatic and cytotoxic, whereas in others (MGH-U1) it was only cytostatic. The combination of the three interferons (α, β, and γ) appeared to be more effective (Grups and Frohmuller 1988). In an experiment with the T24 cell line the cytotoxicity of the interferons was arranged as follows: IFNβ>IFNα>IFNγ (Hara 1989). IFNγ, as well as TNFα, exerted a powerful antiproliferative effect on cancer cells derived from bladder tumors at the G1 and G2 stages. Cells from advanced G3-stage tumors were the least sensitive (Hawkyard et al. 1992b, 1993). There also are data that IFNα had a direct antiproliferative effect on 5 of 10 human bladder cancer cell lines, whereas IFNγ was effective only in 1 (Niell et al. 1994). IFNγ had a stronger antiproliferative effect on the malignant cell lines HCV29-T112Cl, Hu1703He, and T24 than on the premalignant HEV29 and Hu609 cell lines (Ottesen et al. 1990). In general, IFNγ is an inhibitor of proliferation in the earlier stages of bladder cancer (Hawkyard et al. 1993). A 2-hour incubation of MBT-2 cells with IFNγ increased their sensitivity to TNF and actinomycin D (Bahnson and Ratliff 1990).

In bladder cancer IFNγ induced rapidly the expression of sICAM-I (in less than 4 hours) even after being applied for a short period (less than 10 seconds) (Jackson et al. 1992a,b; Campbell et al. 1994). It is important to point out that this increase in sICAM-I occurs at the expense of the membrane antigen molecule, which decreases, and this blocks the effect of the cytokine. Treatment with cycloheximide after IFNγ increases the level of membrane ICAM-I (Jackson et al. 1993). The excretion of sICAM-I in the urine of bladder cancer patients may be used as an indication of successful immunotherapy (Jackson et al. 1993).

All of these data show the high diversity in the sensitivity of different uroendothelial cancer cell lines to the different interferons and their combinations.

A study of the IFNγ receptors in the RT4, RT112, and MGH-U1 cell lines showed that the antiproliferative effect was not directly connected to the absolute number of its receptors, nor with their affinity constant (Jakse et al. 1988; Hawkyard et al. 1992a).

The indirect effect of IFNγ in bladder cancer is associated with increased MHC class I expression and with the induction of the MHC class II antigens necessary for the cytotoxic effector cells to exert their effect (Oliver et al. 1989; Ottesen et al. 1990; Hawkyard et al. 1991, 1994). However, IFNγ did

not induce MHC class II antigens in some bladder cancer cells (Furukawa et al. 1996). In addition, IFNγ suppressed the secretion of the human chorionic gonadotropin β, which in some tumors of the bladder is associated with increased metastatic activity and resistance to radiotherapy and chemotherapy (Oliver et al. 1989).

In experiments with the human cancer cell line UM-UC-9 it was found that IFNγ increased the expression of some integrins, but the treated cells still lose their ability to adhere to laminin. This shows that IFNγ leads to dissociation between the expression and function of some laminins (Liebert et al. 1991).

An important step in the process of tumor invasion and metastasis is the binding of tumor cells to the extracellular matrix of the basal membrane. The integrins are a family of transmembrane proteins that form heterodimers, some of which serve as receptors in the extracellular matrix.

Several reports show that the successful therapy of bladder cancer with BCG (bacille Calmette-Guérin) is due to the secretion of cytokines, including IFNγ (Sargent and Williams 1992; Bohle et al. 1994; Kurisu et al. 1994), but there also are other data that the effect of BCG exceeds the effect of the cytokines IFNα, IFNγ, and IL-2 applied separately (Pryor et al. 1995). A stronger effect had the combination of IFNγ and IL-2 or of IFNγ and BCG (Riggs et al. 1992). The same holds true for recombinant BCG secreting the cytokines IL-2, IFNγ, or GM-CSF (Murray et al. 1996b).

The growth of bladder tumors is associated with secretion of IL-4. Treatment with BCG leads to a decrease in IL-4 and to increased expression of IFNγ mRNA; that is, to a favorable shift from Th2- to Th1-type cytokines (McAveney et al. 1994).

No correlation was found between the level of IFNγ in patients with invasive or noninvasive bladder cancer, but the level of IFNγ decreased with progression of the cancer stage (Shapiro et al. 1994).

In surface cancer of the bladder the intravesicular instillation of IFNγ during 4 weeks led to a decrease of proliferating cell nuclear antigen (PCNA) and of the proliferating cell fraction (Stavropoulos et al. 1999).

Gene immunotherapy with tumor cells transfected with IFNγ and IL-2 via a retrovector was also used for treatment of bladder cancer. In mice the growth of such a tumor is strongly suppressed and the animals also become resistant to the primary unmodified tumor. The obtained antitumor immunity is specific for the tumor type used (Hashimura et al. 1993; Connor et al. 1993). Gene immunotherapy had the strongest effect in the MBT-2 tumor cell line after transfection of the tumor with IL-2 and GM-CSF as compared to transfection with IL-1α, IL-1β, or IFNγ. The induction of the cytotoxic T lymphocytes did not correlate with the antitumor effect (Saito et al. 1994).

## *12.14 Hepatocellular carcinoma*

In this section we shall only discuss the data on IFNγ for hepatocellular cancer (HCC), but not that for liver metastases of other types of cancer.

It has been found that the spontaneous NK activity of HCC patients is decreased. IFNγ considerably increases the NK and LAK activity of the patients, but without reaching the level that is seen in healthy controls (Hirofuji et al. 1987). Defective LAK activity has been observed more than 6 months before the development of HCC (Saibara et al. 1993a). Some data show that the primary cause for the defective NK and LAK activity is not the decreased production of IFNγ. Whereas 64.6% of the patients had defective LAK activity, only 2.1% had a decreased production of IFNγ (Saibara et al. 1993a). However, the role of IFNγ may be seen from the difference between two groups of patients with regard to LAK activity — those with high activity and those with low activity. Patients with low activity had a defect in IFNγ production in contrast to those with high activity. On the other hand, anti-IFNγ antibodies suppressed this activity, whereas IFNγ induced it (Saibara et al. 1990).

The direct effect of IFNγ in *in vitro* experiments was manifested differently in the various HCC cell lines concerning antigen expression and its antiproliferative activity. In the PLC/PRF/5, HepG2, Hep3B, and SK-Hep1 cell lines IFNγ induced high levels of MHC class I but not MHC class II (Fukusato et al. 1986; Ren et al. 1988). Both MHC class I and II were stimulated and induced by IFNγ in the HA22T/VGH and TONG PHC cell lines, and none of these molecules was expressed in the HA597/VGH cell line (Wadee et al. 1994). In experiments with the Huh6 cell line it was found that PKC participated in the induction of MHC class I by IFNγ (Towata et al. 1991).

Unlike the *in vitro* data cited above, no induction of MHC class I was found after local injection of IFNγ 7 days before operation in HCC patients, but there was induction of ICAM-I (Shibata et al. 1992). IFNγ also induced ICAM-I in the hepatoma cell line HepG2 (Volpes et al. 1992). In different cancer cell lines IFNγ increased to a different extent the membrane and the free soluble form of ICAM-I, which suppressed the MHC-independent cytotoxicity of NK and LAK cells (Shimizu et al. 1994; Momosaki et al. 1995). In normal hepatocytes IFNγ did not have this effect (Saito et al. 1996). It is believed that the secretion of the free soluble form of ICAM-I could be one of the mechanisms through which the hepatic cancer cells escaped the local immune control (Shimizu et al. 1994). The expression of ICAM-I and LFA-3 was increased after local injection of IFNγ, which was also associated with increased tumor infiltration by TIL (Shibata et al. 1995).

The induction of MHC and ICAM-I molecules is associated with activation signals for CD4+ helper cells and CD8+ cytotoxic T lymphocytes. Thus, the HA22T/VGH cell line which constitutively expresses MHC class II antigens stimulates the CD4+ T lymphocytes, whereas the class II–negative Li7A cell line stimulates these cells only after treatment with IFNγ. Both cell lines stimulate purified CD8+ T cells. The HepG2 cell line, which does not express MHC class II neither constitutively nor after treatment with IFNγ, is unable to activate a proliferative response of the CD4+ and CD8+ T cells (Paroli et al. 1994). In the HLF cell line TNF suppressed the expression of the ornithin

decarboxylase, of histone H2b, and of the oncogenes c-myc and c-Ha-ras (Takeda et al. 1991).

Considering the Fas-induced apoptosis and the ability of IFNγ to induce this antigen, Fas expression was studied in six hepatoma cell lines. Only two of these cell lines showed high constitutive expression of Fas both in the cytoplasm and on the cell surface, whereas the other cell lines expressed mostly cytoplasmic Fas (Yano et al. 1996).

The antiproliferative activity of the interferons was different in the various hepatoma cell lines. In the HLF cell line IFNα and IFNβ had a stronger effect than IFNγ. The inhibition was manifested as a blockade of the S/G2 transition, resulting in the accumulation of cells in S phase (Takeda et al. 1989, 1991). In the H7 cell line IFNγ in combination with TNFβ (Shiiki et al. 1990b) or with TNFα (Shiiki et al. 1989) had a synergistic antitumor effect. In the PLC/PRF/5 cell line TNFα exhibited a significant antitumor effect only in combination with IFNγ (Takayama et al. 1990). *In vivo* experiments were conducted on rats with Morris 3924A hepatoma implanted in the liver. IFNγ was administered by intra-arterial injection after ligation of the hepatic artery. The application of IFNγ and TNFα produced a better result, as they suppressed tumor growth and eliminated the tumors in 60% of the animals (Yang et al. 1995). However, in the human HCC cell line HepG2 IFNγ decreased the number of TNF receptors in a process mediated by PKC (Aggarwal and Pardita 1994). In another experiment with a rat model of hepatic carcinoma, it was also shown that the regional intra-arterial administration of IFNγ in the liver had a greater therapeutic effect than its intravenous application (Codde et al. 1990).

In a clinical trial with seven patients the intravenous administration of IFNγ (8 to 24 $\times$ $10^6$ IU/day for 5 days every other week) produced only a partial response (Kanda et al. 1988). Here the route of administration and the dosage do not seem to be optimal. Also, in another early clinical trial, which was discontinued because of unacceptable toxicity, IFNγ did not produce a result (Forbes et al. 1985).

We have to point out that there are several single cases of HCC remarkably affected by IFNγ (Narita et al. 1986; Urabe et al. 1989).

Adoptive immune therapy with LAK in combination with IFNγ also was applied. A decrease in the level of α-fetoprotein (AFP) was observed, which, however, increased again after four courses of treatment (Shibata et al. 1993).

Taking into consideration the experiments on animals (Yang et al. 1995), the locoregional therapy appears to be more promising. In humans transarterial locoregional chemotherapy and immunotherapy with IFNγ and IL-2 (emulsified in a mixture of Lipiodol-Urografin) has been applied for treatment of inoperable HCC. A decrease in tumor size was observed in 14 of 20 patients, as well as a decrease of AFP to normal levels in 12 patients and an average survival rate of 18 months (4 to 22 months). The investigators believe that this approach deserves further evaluation (Lygidakis et al. 1995).

## 12.15 Sarcomas

The role of the cytokines in the emergence and progression of tumors has also been shown in sarcomas. Th2-type cytokines (IL-10) predominate in the sarcoma bed of soft tissues (Spellman et al. 1996). The immunomodulator AS101 (trichloro[dioxyethylene-O,O′]telurate) induces a change in the cytokine profile in mice and in humans — a strong induction of the Th1 profile (IL-2, IFNγ, IL-12) and a significant suppression of the Th2-type cytokines (IL-4, IL-10). This is accompanied by activation of the cytotoxic NK and LAK cells and an antitumor effect (Sredni et al. 1996). In mice the synthesis of IFNγ by spleen cells is suppressed during tumor progression, and IL-12 restores this synthesis (Zou et al. 1995).

In the mouse methylcholantrene sarcoma (MethA) the rejection of the tumor is associated with expression of Th1 cytokines — IL-12 and an IFNγ-inducing factor, synthesis of NO, and tumor cell death by apoptosis. In this case, mice with an inactivated IFNγ gene are unable to lyse the tumor cells (Sanchez-Bueno et al. 1996). Macrophages stimulated with IFNγ also kill MethA cells through apoptosis initiated by NO (Sveinbjornsson et al. 1996).

Blocking IFNγ with antibodies accelerated the growth of MCA105 sarcoma in mice (Doherty et al. 1996). The effective elimination of lung metastases of this sarcoma through adoptive immunotherapy with TIL depends on the ability of the TIL to produce IFNγ and GM-CSF (Nagoshi et al. 1998).

IFNγ recovers the apoptotic ability of osteosarcomas with a defective Fas/CD95 receptor (Fellenberg et al. 1997). IFNγ showed a direct antitumor activity *in vitro*, as well as an indirect one *in vivo* in a number of sarcoma cell lines (MG-63, SAOS-2, TE-85, G-292, CE-2, MCB-8, MSO-76) implanted in athymic mice (Giovarelli et al. 1986; Gomi et al. 1986; Dubinett et al. 1989; Jia and Kleinerman 1991). The combination of IFNγ and IL-2 showed a significant antitumor effect on the highly aggressive mouse sarcoma MCA105 (Mao et al. 1995).

IFNγ induces the transcription factor IRF-1 (interferon-regulatory factor 1), which functions as a tumor suppressor in experiments with mice implanted with the highly aggressive sarcoma MCA105 transformed with IRF-1 cDNA. This transformation leads to the reversion of the malignant phenotype and to increased immune recognition (Yim et al. 1997). In addition, this factor (recently named IL-18) also increases the NK activity of human and mouse peripheral mononuclear cells cultured *in vitro*. IL-18 has a significant antitumor activity on MethA implanted in BALB/c mice, which is not observed *in vitro*. It also induces the immune memory by generating cytotoxic CD4+ cells (Micalef et al. 1997). There are data showing that IL-18 exerts an IFNγ-independent antitumor effect, since the latter is exhibited in mice with an inactivated IFNγ gene (Osaki et al. 1998).

The role of IFNγ also is supported by the fact that tumor regression of the mouse fibrosarcoma due to a massive lymphocyte infiltration of the tumor is completely blocked by anti-IFNγ antibodies. This is accompanied by an inhibited synthesis of NO and of the IP-10 gene product, which is

induced by IFNγ (Yu et al. 1996a, 1997; see also Fujiwara and Hamaoka 1996) and has an antitumor effect *in vivo* (Luster and Leder 1993).

The mouse fibrosarcoma FS29 is killed more effectively by the effector lymphocytes when transformed to synthesize IFNγ or IL-2. The supernatants of the IFNγ-synthesizing cells increased the sensitivity of the unmodified tumor cells, which was associated with increased expression of MHC in both types of tumor cells (Flemming et al. 1997). IFNγ suppressed the invasiveness of the HT-180 fibrosarcoma through model membranes, unlike TNFα which increased it (Schirren et al. 1992). Again in the mouse fibrosarcoma, the local expression of IL-18 had a powerful antitumor effect (Osaki et al. 1999).

It appears that the effect of the interferons on sarcomas is also influenced by their localization. In experiments with the mouse M5076 sarcoma both types of interferon (γ and α) prevented the formation of liver metastases. IFNγ was more active in suppressing the subcutaneous tumor growth, whereas IFNα was more active in suppressing the metastases (Brunda et al. 1987).

IFNγ was found to have a practical application in the isolated limb perfusion, as it did in the treatment of melanomas (see Section 12.6). The combination of IFNγ, TNFα, and melphalan was successfully used for treatment of inresectable soft tissue sarcomas. The limbs were saved in 84% of cases followed up for 20 and 50 months (Eggermont et al. 1996). The 4-year experience of Swiss investigators also showed a 87.7% complete remission in soft tissue sarcomas and a 90% remission in melanomas (Lejeune et al. 1995). There also are results from a multicenter study with this method used for the treatment of inresectable soft tissue sarcomas (Brodowicz et al. 1997).

### *12.15.1 Kaposi's sarcoma*

Kaposi's sarcoma, which often occurs in AIDS patients, presents a special case. It is characterized by the proliferation of spindle-shaped cells, increased angiogenesis, infiltration of the lesions by leukocytes, and edema. These processes are initiated by the secretion of bFGF and EGF, which is stimulated by TNF, IL-1, and IFNγ (Sirianni et al. 1998). Some investigators even believe that Kaposi's sarcoma is in fact a malignant angioproliferative disease associated with HHV8. The infiltrating cells (CD8+ T lymphocytes and CD14+/CD68+ monocytes/macrophages) produce large quantities of IFNγ, which acts synergistically with the *tat* protein of HIV-1 to stimulate proliferation of the endothelial cells (Fiorelli et al. 1998) and to induce them to acquire the spindle-shaped Kaposi's form with angiogenic properties. Moreover, that the inflammatory cytokines induce vascular endotheoluim growth factor (VEGF) (Samaniego et al. 1998). Thus, some investigators believe that IFNγ plays a key role in the development of this sarcoma (Fiorelli et al. 1998), although this contradicts the data that IFNγ induces IP-10, which has angiostatic properties (Angiolillo et al. 1996; Arenberg et al. 1996).

HHV8 is believed to participate in the pathogenesis of Kaposi's sarcoma (Sirianni et al. 1998; Zimiring et al. 1998). A G-protein–connected receptor (GPCR), encoded by the genome of this virus, leads to oncogenic transformation.

The IFNγ-induced IP-10 chemokine specifically inhibits the signaling pathway of the viral GPCR (Geras-Raaka et al. 1998), and Kaposi's sarcoma patients express a phenotype dominated by IFNγ (Sirianni et al. 1997).

The conclusion that can be drawn from these data is that IFNγ should not be used for treatment of Kaposi's sarcoma.

## 12.16 Neuroblastoma

The neuroblastoma is the most common extracranial solid tumor that occurs in childhood (Cornaglia-Ferraris et al. 1993). This tumor has a neuroectodermal origin and contains neurons, melanocytes, and Schwann cell precursors (Ridge et al. 1996). The neuroblastoma is a fatal disease resisting surgical intervention or chemotherapy. Neuroblastomas express a wide spectrum of tumor-associated antigens but are devoid of the MHC molecules necessary for their presentation to the cellular immune system (Pistoia et al. 1994).

The N-myc oncogene plays a key role in the biology of the neuroblastoma. Most neuroblastoma cell lines have amplification or overexpression of this oncogene. Its overexpression leads to lost differentiation of normal neuroblasts, which migrate from the neural tube during embryogenesis. The overexpression of N-myc also is associated with the development of metastases. IFNγ alone (Imanishi et al. 1987; Watanabe et al. 1989a) or in combination with retinoic acid suppresses the expression of N-myc and induces the differentiation of the neuroblastoma cells by acting at the level of transcription and by decreasing the half-life of its mRNA (Wada et al. 1997). The decreased expression of N-myc is inversely correlated with the expression of the *trk* proto-oncogene and with growth suppression (Shikata et al. 1994). In the human neuroblastoma cell lines GOTO and KP-N-RT (which overexpress N-myc), IFNγ suppresses the expression of the N-myc gene and induces morphological differentiation (Watanabe et al. 1989a).

The opinion exists that there is a negative correlation between MHC class I expression and the overexpression of N-myc. However, some data show that the expression of these two molecules is regulated by IFNγ independently of one another (Gross et al. 1990). The neuroblastoma cells are sensitive to lysis by NK cells, but patients with Kaposi's sarcoma have a very low or lack of NK activity (Reynolds et al 1989). Low doses of IFNγ render the neuroblastoma cells more sensitive to the cytotoxic action of the LAK cells, i.e., they become more sensitive to treatment with IL-2 (Sigal et al. 1991).

Unlike IFNα and IFNβ, many studies show that only IFNγ has a powerful differentiating effect on the neuroblastoma cells and inhibits their growth (Imanishi et al. 1987; Parodi et al. 1989; Bruchelt et al. 1990; Higuchi et al. 1990; Lanciotti et al. 1990, 1992; Martin et al. 1993a; Ponzoni et al. 1993b, 1994). However, there are cell lines resistant to IFNγ (Higuchi et al. 1989; Ponzoni et al. 1992b). It appears that the induction of the 2′-5′-oligoadenylate-synthetase is not sufficient to induce differentiation (Corrias et al. 1995).

The combination of IFNγ and retinoic acid has a synergistic effect on the induction of differentiation and on the proliferation inhibition of the neuroblastoma cells (Ponzoni et al. 1990, 1991a,b; Wuarin et al. 1991; Cornaglia-Ferraris et al. 1992; Lanciotti et al. 1992). The combination of IFNγ and TNFα also has the same synergistic effect (Ponzoni et al. 1992a; Munoz-Fernandez et al. 1994; Ridge et al. 1996), which is due in part to the induction of TNF receptors by IFNγ. This makes it necessary that the treatment should begin with IFNγ (Montaldo et al. 1994). It has to be taken into consideration that TNF stimulates the proliferation of some neuroblastoma cell lines (SKNFI and SKNBE), an effect which is suppressed by IFNγ. There are speculations that this is due to the induction of IFNγ receptors by TNF (Favrot et al. 1991). It appears that IFNγ and TNF reciprocally increase the expression of their receptors (Ponzoni et al. 1992c). However, it was found that IFNγ decreases the specific binding of TNF to mouse macrophages (Drapier and Wietzerbin 1991), which raises the question of the importance of the cell type for these interactions.

It seems that the use of IFNγ and IFNα for the treatment of neuroblastoma is not advisable, considering that in some cell lines (T98G) IFNγ suppresses the expression of IFNα receptors (Hannigan et al. 1984).

The effect of IFNγ also is due to the induction of NO (Munoz-Fernandez et al. 1994), which is inhibited by dexamethasone (Ogura and Esumi 1996). The IFNγ-induced differentiation is associated with suppressed expression of the antiapoptotic gene Bcl-2 (Kim et al. 1995). In some cell lines IFNγ induces apoptosis before the beginning of differentiation (Montaldo et al. 1997).

In these tumor cells IFNγ also induces the expression of ICAM-I (Naganuma et al. 1991). There are data that PKC (Ponzoni et al. 1993c; Bouillon and Audette 1994) and the $Ca^{2+}$/calmodulin metabolic pathway (Bouillon et al. 1992) participate in the induction of this molecule and in differentiation. In different neuroblastoma cell lines it was found that the induction of differentiation is associated with the induction by IFNγ of laminin and fibronectin.

IFNγ increases the expression of MHC class I, whereas MHC class II is rarely induced in some neuroblastoma cell lines (Lampson and Fisher 1984; Lampson and George 1986; Gross 1987; Main et al. 1988; Sugimoto et al. 1989; Naganuma et al. 1991; Sigal et al. 1991; Ponzoni et al. 1992b). As in other tumors, PgE2 suppresses the expression of MHC class II induced by IFNγ (Hong et al. 1991).

The indirect effect of IFNγ is associated with an increase in the cytotoxicity of NK and LAK cells against the neurblastoma. Unlike some other tumors (see Section 12.1), the incubation of neuroblastoma cells with IFNγ or IL-2 increases their sensitivity to lysis, and also to the antibody-dependent cellular cytotoxicity (Handretinger et al. 1989, 1990a,b; Naganuma et al. 1991). These data show that the increased MHC class I expression is not always associated with decreased sensitivity of the tumor cells to lysis by NK, but that it can also lead to increased sensitivity (Handretinger et al. 1989). IFNγ also increases the cytotoxic activity of the monocytes against the neuroblastoma cells (Shimizu and Fujimoto 1990a,b). In very young children,

however, the mechanism of NK cell activation is not completely developed, unlike the LAK activity (Pierson et al. 1990).

The role of IFNγ may be also seen from gene engineering experiments. The subcutaneous introduction of the neuroblastoma cell line C1300, which produces large quantities of IFNγ, into A/J mice strongly suppresses the growth of the tumor, and this effect is suppressed by a monoclonal antibody to IFNγ. At the same time, the mice also become resistant to the original unmodified tumor cell line (Watanabe et al. 1989b). Continuous cytokine production and phenotypic changes were also observed in other experiments with human neuroblastoma cells transduced with IFNγ (Ucar et al. 1995). Also, retroviral constructs of N-2a neuroblastoma cells expressing IL-18 inoculated into mice become nontumorigenic and rapidly elicit immunity against the parent cell line. Tumors developed only in mice lacking CD4+ and CD8+ T cells, which shows the role of the T lymphocytes in tumor elimination. The immune response to IL-18 was characterized by a dominating Th1 component (IFNγ and GM-CSF) (Heuer et al. 1999). Double-transduced tumor cells expressing IFNγ and GM-CSF were used for effective immunization against neuroblastoma (Bausero et al. 1996).

In spite of all the experimental data promising a therapeutic effect of IFNγ and its combinations with TNF, IL-2, retinoic acid, and others (Cornaglia-Ferraris et al. 1993), there are still no detailed clinical trials for its application. A clinical trial (phase I) showed that IFNγ activates the cytotoxicity of the peripheral blood monocytes of neuroblastoma patients (Shimizu and Fujimoto 1990b). An increase in NK activity and induction of MHC class I was found in another clinical study without, however, a significant clinical response (Evans et al. 1989). Adoptive immunotherapy with TIL, IFNγ, and IL-2 was used for the treatment of refractory neuroblastoma in children, but the results were not encouraging (Wexler et al. 1992).

## *12.17 Gliomas/glyoblastomas*

In several studies it was found that the peripheral monocytes and T lymphocytes of glioma patients show defective production of IFNγ upon stimulation. This is considered to be the main immune defect in these tumors (Bogdahn et al. 1986; Shimizu et al. 1986; Maleci et al. 1988; Urbani et al. 1995). The production of IFNγ induced by IL-2 is already defective in the early stages of the disease.

Various cell lines and glioma clones show high heterogeneity with regard to the induction of HLA-DR by IFNγ, which is not due to differences in its receptors (Piguet et al. 1986a,b; Hong et al. 1991). On the other hand, however, it was shown that the induction depended on the dose, since it was absent with the low doses (Takiguchi et al. 1985). Here, as with the other cell types, IFNγ also increases the expression of ICAM-I (Kuppner et al. 1990).

In a number of glioma cell lines IFNγ did not show an antiproliferative effect, but a considerable effect was obtained by its combination with dsRNA, IFNα, or IFNβ (Dick and Hubbell 1987). The combination of IFNγ with IFNβ,

TNFα, or MDP exerts a strong lytic effect on glioblastoma cell lines *in vitro* (Vita et al. 1988; Kirsch et al. 1994). IFNγ combined with IFNβ was found to be effective in multi–drug-resistant glioblastoma cell lines (Reddy et al. 1991; Moulton et al. 1992). Glioma cell lysis by cytotoxic lymphocytes depends on IFNγ (Dhib-Jalbut et al. 1990).

In experiments with mouse gliomas the cytotoxicity of T lymphocytes transfected with the IFNγ gene was dependent on the secretion of this cytokine (Nishihara et al. 1988).

As with other tumors, the sensitivity of the glioma to lysis by LAK decreases under the effect of IFNγ due to the increased expression of MHC class I on tumor cells (Yin et al. 1994) (see Section 12.1).

In spite of these data supporting the protective role of IFNγ in this tumor type, the clinical trials did not produce the expected results. In relatively small recurrent tumors IFNγ (2 mg/m$^2$) administered intravenously two times a week for 8 weeks produced a response in one of four patients. Twelve- to 86-week stabilization was observed in the other patients (Mahaley et al. 1988). The result was evaluated as being disappointing, although in our opinion the dose and the scheme of administration were not the most suitable ones.

Gene engineering approaches also are under development. The human IFNγ gene cloned in an eukaryotic vector and included in positively charged liposomes has been used for transfection of glioma cell lines (SK-MG-1, U-251-MG). The introduction of these cells led to a considerably stronger inhibition of cell growth than the administration of exogenous IFNγ. The mechanism appears to be associated with the expression of ICAM-I on the glioma cells. The combination of this approach with LAK cells is considered to be promising (Mizuno et al. 1992, 1994).

## *12.18 Hematological malignancies*

Although IFNα has a much larger application in hematological malignancies, we shall discuss some facts that define the role of IFNγ. First, we have to point out that the receptors of IFNγ are expressed in the cells of almost all malignant hematological diseases. They were found in chronic lymphatic leukemia, acute lymphoblast leukemia, hairy cell leukemia, and promyelocytic leukemia. A higher expression was observed in myeloproliferative, than in lymphoproliferative, disorders (Watson et al. 1990). Resistance to interferons occurs often in leukemia, especially in the acute forms (Delforge et al. 1990).

### *12.18.1 Chronic myelogenous leukemia*

Chronic myelogenous leukemia (CML) affects the pluripotent hematopoietic stem cells common for the myeloid, monocytic, megakaryocytic, and erythrocytic clones. It is characterized by the presence of the Philadelphia chromosome, a result of a reciprocal translocation between the 9th and the 22nd chromosomes (t:(9:22) (q34;q11)), which transfers the cellular oncogene

*abl* from chromosome 9 to the region of breakpoint *bcr* in chromosome 22. This leads to the expression of an abnormally large hybrid mRNA (8.5 kb). Its presence detected by polymerase chain reaction (PCR) is an indication for a minimal residual disease after treatment due to the Philadelphia chromosome (Opalka et al. 1991; Dhingra et al. 1992). The 210-kDa protein translated by this mRNA has an elevated tyrosine kinase activity (see references in the review by Ozer 1988).

This chronic period lasts for 3 to 5 years and in many cases is followed by a blast transformation, which cannot be differentiated from acute leukemia and has a rapid fatal result. According to some new data, the blast transformation does not occur at the level of the stem cells but somewhere along the pathway to their maturation into different clones. The defect is not in an increased proliferation of the precursor cells but in their prolonged life span and a higher number of additional cell divisions (see references in the review by Clarkson and Strife 1993).

An immune deficiency was found in CML — defective NK cytotoxicity, defective adhesion of the NK cells to the target cells, and an IFNγ deficit (Chang et al. 1991). It is important to point out that the precursor myeloid cells have a defect in adhesion, which is probably the reason why they appear outside the bone marrow.

A family of transcription factors (ICSBP — a member of the IRF) which bind to consensus sequences in genes that are induced by IFNγ are important for the regulation of these genes. Mice with defective ICSBP show hematological changes similar to those in human CML (Giese et al. 1997). According to some new data, such changes in ICSBP can play an important role in the genesis of the human myeloid leukemia. Thus, no expression of these proteins was found in 79% of CML cases and in 66% of acute myelogenous leukemia (AML) cases, whereas in healthy individuals this occurred in 6% only. Treatment of leukemia cells from CML patients with IFNγ led to the induction of ICSBP (Schmidt et al. 1998). It was found that another transcription factor, Spi-1/Pu-1 (from the Ets family), is necessary for normal hemopoiesis. Treatment of the K562 cell line (isolated from a CML patient in blast crisis) with IFNγ, IFNα, or IFNβ leads to a four- to eightfold increase in Spi expression and to inhibition of cell proliferation (Gutierrez et al. 1997).

IFNγ (but not IFNα) strongly stimulates the expression of ICAM-I and LFA-1 that could contribute to the therapy (Grander et al. 1994). It was also reported that IFNγ (but not IFNα) suppressed the expression of endogenous G-CSF, which stimulates the proliferation of the precursor cells in CML (Riedel et al. 1990).

In CML both interferons α and γ show a direct antiproliferative effect *in vitro*, and both act through different mechanisms, which is a prerequisite for a synergistic effect (Rubin and Gupta 1980; Toretsky et al. 1986; McGlave et al. 1987). In chronic granulocytic leukemia IFNγ showed a synergistic and subadditive effect in combination with 5-FU and cytosine arabinoside C (Ara-C), respectively (Kafka et al. 1989).

TNFα exerted a stronger antiproliferative effect than IFNγ, but the latter synergistically increased the effect of TNFα (Herrman et al. 1988).

The combination of IFNγ and IFNα in *in vitro* experiments showed a stronger synergistic effect — small doses of IFNγ enhanced the effect of increasing doses of IFNα (Carlo-Stella et al. 1988).

Treatment with interferons should start before the blast transformation occurs, at which time they become ineffective. In the numerous clinical trials with CML patients both IFNγ and IFNα showed an antileukemic activity, but the effect of IFNγ was much weaker. In some clinical trials with IFNγ there was a complete hematological remission in 23% of the cases and partial remission in 15% (Cortes et al. 1996; Freund et al. 1996; Kantarjian 1987a,b; Kurzrock et al. 1987; Ozer et al. 1991; Bolinger and Taeubel 1992), whereas IFNα produced 55 to 91% complete hematological remissions (Thaler 1993).

Some investigators find that the combination of IFNγ and IFNα produces a stronger cytostatic effect than either applied separately (see references in Visani et al. 1988). However, a number of trials with this combination did not produce better results than trials with IFNα alone (Herrman et al. 1990; Schiffer 1991; Talpaz et al. 1991; Wandl et al. 1992b; Niederle et al. 1993). This combination led to hematological remission in 51% of the patients, to partial remission in 24%, and there was no response in 21%. According to some data, treatment with IFNγ prior to IFNα treatment improved the result. For example, IFNγ had a minimal effect in accelerated blast crisis, but IFNγ followed by IFNα showed an improved result. Other data also show that patients who did not respond to one of the interferons responded to the other (for review, see Ozer 1988). In one case, a complete hematological remission resulted from treatment with IFNα and IFNγ in much lower doses than those used when the interferons were applied separately (Kloke et al. 1990).

An autologous transplantation with bone marrow treated *ex vivo* with IFNγ was used successfully, but it was often followed by a leukemia recurrence (McGlave et al. 1990, 1993).

Regardless of the very promising results with IFNα, it seems reasonable to believe that the finding of a suitable scheme for its combination with IFNγ would improve the results. The high heterogeneity of the leukemic cells creates a serious problem. This is illustrated by the behavior of different leukemic cell lines, as shown by the following examples of two such cell lines isolated from two CML patients in blast crisis. The proliferation of one cell line — IMS-BC2 — was inhibited by IFNγ and its cells died by apoptosis, whereas the other cell line — IMS-BC1 — was resistant to this cytokine (Nagamura et al. 1998). The proliferation of another leukemia cell line KT-1 (again isolated from a CML patient in blast crisis) was suppressed by both IFNγ and IFNα. The combination of the two cytokines had a synergistic action (Yanagisawa et al. 1998). These examples show how different the effect of IFNγ can be in different individuals with the same diagnosis.

### 12.18.2 B and T chronic lymphatic leukemias

The B and T chronic lymphatic leukemia (B-CLL and T-CLL, respectively) are characterized by the presence of a small cellular fraction with low proliferative activity and an increasing fraction of long-lived lymphocytes. It is assumed that cells resistant to apoptosis arise with the progression of the disease (Robertson et al. 1994).

Experiments with CLL cells cultured *in vitro* show that IFNγ has an inducing effect on differentiation and proliferation (Ostlund et al. 1986; Tremisi et al. 1991; Alfinito et al. 1994) except in the absence of serum when only differentiation is stimulated (Totterman et al. 1987). IFNγ inhibits the IL-4 receptors on human lymphocytes (Byron et al. 1991).

On the other hand, it has been shown that IFNγ inhibits the apoptosis of B-CLL cells, which prolongs their life. This is probably due to its antagonistic effect on IL-10, which induces apoptosis specifically in the B-CLL cells (Fluckiger et al. 1994). An increased serum level of IFNγ was found in most such patients, since the leukemia cells also secrete this cytokine (Buschle et al. 1993). The extent of apoptosis inhibition is greatest in the patients' group of highest risk (Rojas et al. 1996). The stimulation of proliferation and inhibition of apoptosis are probably factors which lead to an increase in the number of leukemia cells in B-CLL.

IFNγ induces the expression of the apoptotic factor Fas (see Section 3.3). However, the CLL cells remain resistant to apoptosis even when Fas binds a ligand, such as the anti-Fas antibody. This is possibly due to the increased activity of the Bcl-2 gene (Panayiotidis et al. 1995).

These data do not support the application of IFNγ in CLL. Clinical trials with intravenous infusion of IFNγ in CLL patients did not produce a beneficial effect (Vadhan-Raj et al. 1986a,b), although the scheme and the high dose of $20 \times 10^6$ units applied were not probably the most suitable. In the light of these data, the results of clinical trials in Japan in which a 40% response rate to IFNγ was reported were unexpected (Kobayashi and Urabe 1988).

The antibody-dependent lytic activity deserves some consideration. The combination of IFN with a monoclonal antibody against the Lym-1 surface antigen led to 80% lysis of the target cells isolated from CLL patients. The effector cells were found to be neutrophils (Cemerlic et al. 1991).

### 12.18.3 Acute leukemias

Two groups of facts support the role of IFNγ in acute leukemia — the changes in the production of this cytokine and its effect on this type of malignant cells. In addition, disruptions of the metabolic pathway of IFNγ, such as the constitutive expression of STAT (see Chapter 4), could be a factor in leukemia genesis. A transcription factor — LIL-STAT — was found to be constitutively active in undifferentitated leukemia hematopoietic cells but not in mature cells (Tuyt et al. 1998).

The production of IFNγ and IL-2 by peripheral mononuclear cells from patients with acute leukemia in complete remission is higher than in those with early recurrences (Tajima et al. 1996). The activity of NK cells and the production of IFNγ in the supernatants of cultures from lymphocytes isolated from children with acute lymphoblast leukemia (ALL) are considerably lower than those in healthy children (Wakiguchi et al. 1994). The PMA-induced IFNγ secretion by peripheral blood monocytes also is strongly decreased (Nash et al. 1993).

When the GVHD (see p. 144) was used as immunotherapy for the treatment of AML (Klingemann 1996), it was noted that the serum level of IFNγ and neopterin were increased, which is dependent on the presence of T lymphocytes in the transplant (Ferm et al. 1996).

A number of experiments with different forms of acute leukemia — stable cell lines (HL-60, U-937, RC-2A, ML-1) as well as primary cultures of blast cells from AML — have shown that treatment with IFNγ induces cellular differentiation and inhibits their growth (Ball et al. 1984; Koeffler et al. 1984; Takei et al. 1984; Harris et al. 1985; Matsui et al. 1985; Rigby et al. 1985; Lyons and Ashman 1987; Dubreuil et al. 1988; McCachren et al. 1988; Stella et al. 1988; Testa et al. 1988; Craig and Buchan 1989; Howell et al. 1990; Iwanami et al. 1990; Nakamaki et al. 1990; Hassan et al. 1995) In an experiment with ALL IFNγ also caused apoptosis (Grawunder et al. 1993). There are data showing that in blast cells from AML IFNγ can exert a growth-stimulating effect in the presence of hematopoietic growth factors (IL-3) (Murohashi and Hoang 1991). In the HL-60 and U-937 cell lines the activity of INFγ was also increased when it was combined with antineoplastic agents such as 5-FU, Ara-C (Kafka et al. 1989, 1990), methotrexate (Sur et al. 1991), and other cAMP-inducing agents (Iwanami et al. 1990).

There are data showing that differentiation of these cell lines is accompanied by reorganization of the microtubular network (Treon et al. 1991).

In the same cell line, HL-60, IFNγ suppressed the cellular oncogene c-myc and also induced cellular differentiation to mature macrophages (Matsui et al. 1985). In the same time it increased the expression of HLA-DR (Sariban et al. 1987).

The HL-60 cell line expresses CSF-1 (colony-stimulating factor-1) under the effect of IFNγ, and thus the combination of the two cytokines induces terminal cell differentiation (Vassiliadis and Guilbert 1991).

The combination of IFNγ and TNFα exerts a synergistic effect on cell differentiation and growth inhibition in acute leukemia cells (Broxmeyer et al. 1986; Price et al. 1987; Takuma et al. 1987; Weinberg and Larric 1987; Herrman et al. 1988; Craig and Buchan 1989; Geissler et al. 1989; Ruth et al. 1989; Nara 1991). A synergistic effect also has combinations of IFNγ and retinoic acid (Gullberg et al. 1985; Hemmi and Breitman 1987; Trinchieri et al. 1987; Ruth et al. 1989; Peck and Bollag 1991; Ferrero et al. 1992; Kumar and Korutla 1995; Geary and Ashman 1996; Gianni et al. 1996), IFNγ and 1,25 — vitamin $D_3$ (Gullberg et al. 1985; Matsui et al. 1985; Murao et al. 1985; Toshimitsu et al. 1985; Ball et al. 1986; Weinberg et al. 1986; Kelsey et al.

1994). It was shown in experiments with the U-937 cell line that the differentiation under the effect of IFNγ, TNFα, 1,25-vitamin $D_3$, and retinoic acid is accompanied by the expression of the cytochrome b-558 subunit (Kikuchi et al. 1994).

It has been reported that IFNγ enhances the lytic activity of adenosine triphosphate (ATP) on AML blast cells. The investigators conclude that the combination of ATP and IFNγ could be a potential therapeutic regimen (Blanchard et al. 1993; Spranzi et al. 1993).

The various cytokines may lead the process of differentiation into different directions. Thus, in primary cultures of AML cells IFNγ in the presence of retinoic acid leads to monocytic differentiation, whereas IFNα induces granulocytic differentiation. In acute megakaryocytic leukemia IFNγ stimulates monocytic differentiation of the blast cells, whereas IFNα and IFNβ direct the differentiation toward megakaryocytes (Hassan et al. 1995). This pluripotentiality of the leukemia cells shows that, at least in some cases, the malignization occurred at the level of stem cells. In the acute promyelocytic leukemia NB-4 cell line the combination of interferons and retinoic acid inhibited the growth synergistically by inducing the retinoic acid α receptor, which was not induced by each agent applied separately (Kumar and Korutla 1995). There is an interesting observation that AML patients often are infected with post-transfusional hepatitis, which prolongs their life. Data show that this is due to the increased release of LPS and increased secretion of IFNγ and TNFα (Treon and Broitman 1992).

The expression of MHC antigens, which are necessary for specific signaling, and also of the nonspecific costimulatory molecules B7-1, B7-2, and ICAM-I, showed high variability in different cases. Examination of this expression in 94 leukemic samples showed expression of B7-1, B7-2, ICAM-I, and MHC class I in 5, 22, 16, and 100%, respectively. In AML B7-1 was expressed in only one case (Hirano et al. 1996a). Increased expression of MHC class I was observed after treatment of ALL cells with IFNγ (Guha et al. 1993). In ATL (acute T-lymphoblastic leukemia) IFNγ induces resistance of the malignant cells to LAK lysis (Cesano et al. 1993) (see Section 12.1).

As in solid tumors, here also IFNγ induces the expression of MHC class II antigens (mostly HLA-DR) (Koeffler et al. 1984; Weinberg and Larric 1987; Lecchi et al. 1989; Willheim et al. 1995; Hirano et al. 1996a). In the promonocytic cell line THP-1 this induction occurs with the participation of PKC (Gumina et al. 1991), and in the HL-60 cell line through the $Ca^{2+}$/calmodulin pathway without the participation of PKC (Koide et al. 1988). The emergence of the cellular subline U-937 (designated as C119/9) shows how different can be the changes associated with malignant transformation. Unlike the parental cell line, in this new subline IFNγ suppresses the induction of these antigens when induced by TNFα (Willheim et al. 1995).

It deserves mentioning that in AML patients the CD4+ T cells that express HLA-DR under the effect of IFNγ are capable of suppressing erythropoiesis (Hansz et al. 1992).

In leukemia cells IFNγ induces the apoptotic surface antigen Fas and ICAM-I (Munker and Adreeff 1996), but this expression is heterogeneous concerning both constitutive and IFNγ-induced expression (Heil et al. 1994). IFNγ and M-CSF in combination increase the expression of ICAM-I and TNFα and also the cytotoxicity of the monocytes toward target cells (Kimball et al. 1995). An increase in LFA-1 and ICAM-I expression caused by IFNγ was also found in the esinophilic leukemic cell line Eol-3 and in U-937 but without the participation of PKC and cAMP (Nambu et al. 1992). In the leukemic cell line U-937, IFNγ and M-CSF synergistically with 1,25-vitamin $D_3$ increase the secretion of neopterin by the monocytes — a sign of their immunostimulation (Kelsey et al. 1994).

The few experimental data with the HL-60 cell line on the effect of IFNγ on c-myc expression are contradictory. A lack of effect (Dubreuil et al. 1988; McCachren et al. 1988) as well as suppressed expression of this oncogene (Yuan and Shen 1994) have been reported.

The sensitivity of the leukemic cells to the monocytic cytotoxicity varies during the different phases of the cell cycle. In the HL-60, U-937, and THP-1 cell lines the cells were most sensitive in G1 and least or not at all sensitive in the S, G2, and M phases. This is associated with the expression of $\beta_2$-integrins on the surface of the cell, which is the highest during the G1 phase (Van de Loosdrecht et al. 1993b).

The tumorogenicity of AML cells transfected with the IFNγ gene in mice is strongly decreased and the life expectancy of the mice is considerably increased (Morecki et al. 1998).

We found few data on the clinical application of IFNγ in acute leukemia. In AML and MDS patients the continuous intravenous infusion of IFNγ in doses of $2 \times 10^6$ to $20 \times 10^6$ IU/day for 14 days has led to severe toxic side effects and a lack of hematological remission (Stone et al. 1993).

Children in remission from acute leukemia were treated subcutaneously with doses from $1 \times 10^6$ to $7.5 \times 10^6$ IU/m$^2$ for 14 days, followed by application three times a week. In one child this resulted in a hematological response, and two children were stabilized for 138 and 148 days. Some side effects were reported — one reversible kidney insufficiency and one death from gastrointestinal hemorrhage due to thrombocytopenia (Mahmoud et al. 1992).

An attempt was made in children with acute leukemia to control with immunotherapy (IL-2 and IFNγ in low doses) the residual minimal disease after chemotherapy or autologous bone marrow transplantation. An increase in the average remission period was found (Baumgarten et al. 1994; Kolecki et al. 1994). Phase I/II clinical trials were also conducted in children with acute leukemia in order to control and extend the remission following autologous bone marrow transplantation. The conclusion was that this is a promising therapeutic approach (Baumgarten et al. 1991). Of four patients with AML, in one patient the intravenous administration of IFNγ has led to a shift of the leukocyte profile from immature blast cells to maturing myeloid cells, and in another patient there was a complete hematological

remission. These data show the potential possibilities for treatment of some patients with IFNγ (Beran et al. 1987). IFNγ strongly stimulated the NK activity in immunocompromised ALL patients (McKolanis et al. 1991).

With regard to the combination of IFNγ and IFNα, there is an opinion that it is more toxic and less effective than IFNα administered alone (Ozer 1991). The combination of IFNα and low doses of INFγ used in myeloproliferative diseases led to an increased titer of antinuclear antibodies in 72% of the patients and symptoms of systemic lupus erythematosis (SLE) appeared in 3 out of 25 patients (Wandl et al. 1992a).

### *12.18.4 Lymphomas*

Experimental data demonstrate the role of IFNγ in protecting the organism against the emergence of lymphomas. In experiments with C57BL/Ka mice the total exposure to ionizing radiation led to the appearance of T-cell lymphomas in 90% of the animals. The injection of IFNγ after exposure to irradiation inhibited the appearance of lymphomas (Boniver et al. 1989). The expression of the human HTLV, the virus which causes T-cell lymphoma/leukemia, has been found to be inversely proportional to the constitutive production of IFNγ (Moore et al. 1985).

The data on the skin form of the T-cell lymphoma are interesting. It represents an invasive growth of CD4+ T helper lymphocytes expressing a Th2 cytokine profile (IL-4 and IL-5) and a defective Th1 profile (IL-2 and IFNγ). This also applies to its leukemic form (Cezari's syndrome) (Vowels et al. 1994; Yagi et al. 1996; Rook et al. 1997) and to another skin form, mycosis fungoides (Hansen 1996), in which the progression is associated with decreased expression of IFNγ and increased expression of IL-10 (Asadullah et al. 1996).

These data support the expectation that IFNγ could change the cytokine profile and may lead to a beneficial effect. Such an effect has been shown in clinical trials. A group of Japanese investigators reported a curative response in 67% of mycosis fungoides patients treated with IFNγ (Kobayashi and Urabe 1988). Others also found such a treatment for the dermal form of T-cell lymphoma to be effective (Kaplan et al. 1990). The combination of IFNγ and local hyperthermia has also been applied successfully (Nakayama et al. 1989). The intratumoral administration of IFNγ also produced a healing effect in mycosis fungoides (Yamamoto et al. 1995). We should mention the unusual fact that in the EL-4 T-cell lymphoma, which expresses a high level of IFNγ receptors, IFNγ increases cell proliferation (Fassio et al. 1988).

It also is of interest that IFNγ has a different effect in the different stages of malignant B-cell proliferation. Thus, in the RAMOS-1 Burkitt's lymphoma cell line IFNγ suppressed proliferation and induced apoptotic death, whereas in the KM-3 B cell progenitor cell line it did not have such an effect (Trubiani et al. 1994). Contrary to these data, in another experiment it was reported that IFNγ inhibited the p53-induced apoptosis in Burkitt's lymphoma (Sangfelt et al. 1996).

In clinical trials conducted in Japan treatment of non-Hodgkin's lymphoma and Burkitt's lymphoma with IFNγ resulted in a clinical response in 16.7 and 11.8% of the cases, respectively (Kobayashi and Urabe 1988). Partial response was observed after treatment with IFNγ and IL-2 in combination (Redman et al. 1990). In children this combination in low doses was applied to maintain the remission following autologous bone marrow transplantation (Kolecki et al. 1994).

### *12.18.5 Myelodysplastic syndrome (MDS)*

The myelodysplastic syndrome (MDS) is a disorder with various manifestations resulting from disturbed clonal hematopoiesis of the hematopoietic stem cells. It is characterized by pancytopenia and progression, which can widely vary from a relatively benign, slowly progressing disease to a fast-progressing, life-threatening cytopenia or AML (Greenberg 1991; Heyman 1991; Kizaki and Koeffler 1992).

Because the maturation of the stem cells is disturbed, it is logical to include in the treatment of this disease factors stimulating cell differentiation, such as IFNγ, IFNα, retinoic acid, and vitamin $D_3$ (Greenberg 1991; Yoshida et al. 1991).

It was found in *in vitro* experiments that IFNγ stimulated the differentiation of blast cells from patients with acute nonlymphatic leukemia and MDS (Stella et al. 1988). The effect was increased additively in combination with vitamin $D_3$ (Tohyama et al. 1989). Other experiments show that IFNγ cannot recover the defective NK function of MDS patients, but it can stimulate the suppressed cytotoxicity of the polymorphonuclear leukocytes (De Santis et al. 1992).

In most MDS patients IL-12 and IL-2 in combination increased the production of IFNγ and TNFα by the free mononuclear cells (Ogata et al. 1995).

The effect of IFNγ in clinical trials appeared to be generally weak (Greenberg 1991). After treatment with low doses (0.01 mg/m$^2$ subcutaneously every other day for 1 month) a slight improvement in the growth of the precursor cells was observed *in vitro* (Rosti et al. 1989). In another clinical trial with higher doses (0.1 mg/m$^2$ subcutaneously every day) there was neither a complete nor a partial response (Schwarzinger et al. 1990). In a trial with 30 patients treated with high doses of 0.1 mg/m$^2$ and with low doses of 0.01 mg/m$^2$ three times a week encouraging results were obtained. This justified the belief that this type of therapy for MDS was promising (Maiolo et al. 1990).

In spite of the scarce data, it appears that the effect of IFNγ in MDS is very poor unless it is assumed that here, as in the other malignant diseases, the optimal dosage, combination, and regimen of administration have not yet been found.

### *12.18.6 Multiple myeloma (MM)*

Multiple myeloma (MM) is a fatal disease for which there is no successful chemotherapy. It makes up to 10 to 15% of the hematological neoplasias in the United States (Niesvizky et al. 1993).

Initially, MM was believed to be a neoplasia of differentiated plasma cells, but in fact it turned out to be a heterogeneous population of cells originating from much earlier stages of B-cell development even before their determination and maybe at the level of stem cells. Thus, MM represents a mixture of cells arrested at different stages of B-cell differentiation. Overexpression of the antiapoptotic gene Bcl-2 is found in almost all myeloma cell lines as well as in clinical samples (Niesvizky et al. 1993). In addition, IL-6 plays a central role as a growth factor and the production of this cytokine is increased in MM patients (King and Nelson 1989; Gottlieb et al. 1990; Bataille and Klein 1993; Niesvizky et al. 1993; Portier et al. 1993; Huang and Vitetta 1995; Palumbo et al. 1995a; Shima et al. 1995). Cell lines independent of IL-6 have also been obtained (Jernberg-Wiklund et al. 1991).

IFNγ as a Th1 cytokine suppresses the expression of IL-6. According to some studies, this is probably due to the fact that the different transcription factors activated by these two cytokines bind to common DNA sequences (Yuan et al. 1994). Due to this competition, it is logical to expect a beneficial effect of IFNγ. A number of experimental data show that IFNγ as well as antibodies against IL-6 suppress the proliferation of MM cells and decrease the number of viable cells (Einhorn et al. 1988; Jernberg-Wiklund et al. 1991; Bataille and Klein 1993; Portier et al. 1993; Ogata et al. 1994; Palumbo et al. 1994). They also suppress the synthesis of IgE (Matsumoto et al. 1991). That IFNγ exerts its inhibitory effect by suppressing IL-6 can be seen from the fact that MM cell lines independent of IL-6 are resistant to IFNγ (Jernberg-Wiklund et al. 1991; Palumbo et al. 1995a) (see Chapter 3, Figure 3.3). The effect of IFNγ is probably due also to the suppression of IL-6 receptor and to a blockade of its metabolic signaling pathway (Palumbo et al. 1995a). Some data have to be considered that IFNγ increases IL-6 production by monocytes if the cells are previously treated with LPS (Biondillo et al. 1994).

Regardless of the logical expectations resulting from these experimental data, the few clinical trials did not produce the expected result. The first clinical data from the application of interferons for treatment of MM are summarized elsewhere (Ohno et al. 1987), with the conclusion that these cytokines, especially IFN, have a certain effect. In 15 MM patients IFNγ was administered in daily doses of 0.125 to 0.5 mg/m$^2$ without a response — only one patient's disease was stabilized for more than 16 months (Quesada et al. 1988). In another clinical trial the combination of IL-2 and IFNγ did not cause tumor regression in MM patients resistant to chemotherapy, although it led to an increase and activation of NK cells (Pecherstofer et al. 1994). However, IFNγ increased the antitumor effect of the autoimmune GVHD (see Section 12.1) induced by cyclosporine A (Noga et al. 1992). The combination of IFNγ with retinoic acid seems justified, since it also suppresses the growth of MM cell *in vitro* (Palumbo et al. 1995b). In Japan clinical trials for treatment of MM with IFNγ resulted in a 11.8% response (Kobayashi and Urabe 1988).

Due to the limited clinical experience and the lack of an optimal scheme, dosage, and combination it would be still early to conclude (Schwarzinger et al. 1988) that IFNγ is not effective in MM.

## *12.19 Conclusions on the use of IFNγ in malignant diseases*

We may draw the following general conclusions without respect to all other types of neoplasia, where the results are more or less similar:

1. Theoretical considerations and all experimental data on cancer cell lines *in vitro* and on animal models *in vivo* show that IFNγ as well as the whole group of Th1 cytokines have an antitumor activity which is manifested both directly and indirectly through the activation of the cellular immunity and the suppression of angiogenesis.
2. IFNγ has an optimal dosage of activity and schemes of application that have not yet been well determined for different malignancies. It appears to be more effective when intermittently administered (instead of long-term continuous infusion), or when it is applied locally and in combination with other cytokines and cytostatics, as well as upon neoadjuvant (preoperative) administration.
3. Several single cases of a unusually effective antitumor activity of systemically administered IFNγ justify its clinical use, considering the fatal issue of most of these diseases.
4. Different reasons may be discussed for the lack of a final antitumor result in the clinical trials:
   - The heterogeneous composition of the tumors comprising both sensitive cells and cells resistant to IFNγ due to different mutations and different gene variants (for review, see Kwiatkowski 2000). The absence of a direct effect in a number of cases is due to the development of many different cell lines in the process of carcinogenesis and during tumor progression. They contain various mutations affecting the signaling metabolic pathway of IFNγ, beginning with its receptor. A high quantitative heterogeneity is found in the expression of IFNγ receptors in malignant hematological diseases (Watson et al. 1990). Of 77 different malignant cells studied, 6 leukemia cells of lymphoid origin did not express the IFNγ receptor (Ucer et al. 1986). Also, in other studies it was shown that malignant cells lacking IFNγ receptors were not sensitive to IFNγ (Fassio et al. 1988). Disruptions in the IFNγ metabolic pathway also were found. For example, the lack of MHC class I stimulation by IFNγ in some tumors, such as the methylcholantrene sarcoma in mice, is due to disruptions in the Jak/Stat signaling pathway (see Chapter 4) (Svane et al. 1997). Mutants with a genetic defect in Jak-1 do not respond to any of the three interferons (Loh et al. 1994). The lack of antiretroviral effect of IFNγ is also due to a defect in the induction of the JAK/STAT metabolic pathway (Bovolenta et al. 1999). The mutations that lead to IFNγ resistance in breast cancer disturb the relationship between cdk2 kinase, cycline E, and the level of p27, so that IFNγ cannot inhibit the hyperphosphorylation of pRb and block the cell cycle (Harvat et al. 1997). Mutants with

a defect in the metabolic signaling pathway leading to IDO synthesis were also resistant to IFNγ (Feng et al. 1991).
- Disturbances in the IL-2 metabolic pathway preventing IFNγ to induce LAK activity (Shiiba et al. 1986).
- Disturbance in the molecular mechanisms leading to apoptosis of the malignant cells.
- Poor vascularization, especially in tumors of large size, which prevents the access of the cytokine to all tumor cells. The large tumor mass can also play a negative role creating hypoxia, especially in necrotic regions. Hypoxia makes the cancer cells more resistant to the antiproliferative activity of the interferons (Naldini et al. 1995).
- Suppression by tumor cells of TIL cell locomotion and their penetration through the cellular matrix (Applegate et al. 1990).
- Tumor localization in organs where the access of IFNγ after its parenteral administration is limited, such as the brain and the alveoli of the lung (see Chapter 8).
- Resistance to IFNγ arising after long contact of the cells with this cytokine (and also with IFNα), which has been observed in cell cultures (see Einhorn and Grander 1996). This could explain clinical trials where the initial antitumor effect was followed by a resumed tumor progression. There are two possibilities that could explain this result — the selection of resistant cells in the heterogeneous cell population and/or IFNγ suppressing the expression of its β-receptor chain (Bach et al. 1997).
- Shedding of soluble receptors. For example, sIFNγR or sICAM-I, which bind the corresponding ligands in solution and prevent the contact between target and effector cells, thus blocking the immune reaction (Dummer et al. 1994; Kooy et al. 1998).
- Shedding of soluble Fas, which blocks the apoptosis induced by anti-Fas ligands. For example, a primary nonmetastatic melanoma cell line, WM793, and its highly metastatic variants, P1N1 and P2N1, did not differ in their surface Fas expression nor in their Bcl-2 expression, but the metastatic cell lines secreted a tenfold higher level of soluble Fas (Angelo et al. 1994).
- Decreased anti-Fas sensitivity was observed with tumor progression of melanoma (Meterissian et al. 1994).
- The expression of Fas ligands (FasL) on the surface of the tumor cells (melanoma) leading to the apoptotic death of TIL which express Fas. Thus, an immune privilege of the tumor arises (Hahne et al. 1996; Naujokat et al. 1999).
- Intracellular superoxide anions in the tumor cells can prevent the Fas-induced apoptosis in cells that are constitutively sensitive to such a death. Thus, oxidative stress can increase the resistance of the tumor cells to apoptosis (Clement and Stamenkovic 1996).
- Because the identification of the tumor cells occurs through antigen recognition mediated by the MHC molecules, the inability of

tumors to initiate an immune response may be due to the lack of costimulatory signals to the T lymphocytes. The absence of such signals prevents T-cell activation and induces a state of immune tolerance of the tumor (Guckel et al. 1996).

- The synthesis of proteins that block the effect of IFNγ. In some cancer cell lines a factor (YY1) was discovered that binds to the IFNγ promoter and blocks IFNγ expression (Ye et al. 1996). Other malignant cells (of the colon, breast, and lung) secrete a factor that prevents their lysis by NK cells and is not connected with the level of IFNγ (Marubayashi et al. 1991). Sone malignant melanomas and colorectal cancer cell lines (G361, colo320, HT-29) also suppress LAK activity by producing factors inhibiting their generation (Guillou et al. 1989). Cancer cell lines may also express a negative regulator of transcription which suppresses DNA-binding factors that regulate the synthesis of IFNγ (Petricoin et al. 1994). In some ovarian and cervical tumors it was found that protein synthesis makes them resistant to IFNγ. Inhibition of protein synthesis recovers their sensitivity to this cytokine (Massad et al. 1990).
- The tendency to apply maximum tolerated dose (MTD) of IFNγ in clinical trials leads to the opposite effect, considering that this cytokine has an optimal concentration of activity.
- An anergic state of the organism. For example, IFNγ increases the phagocytic and bacteriocidal activities of the alveolar macrophages in tuberculin-positive patients with lung cancer and tuberculosis. However, in tuberculin-negative (anergic) patients this cytokine was without effect (Kawatsu et al. 1991).

Some of these negative factors could be eliminated, others could not, but the possibility, how small it may be, of obtaining a beneficial effect justifies the use of IFNγ in malignant diseases, provided that it is not contraindicated.

With the exception of some cases of active tuberculosis (see Section 10.2.1.1.1), the induction of IFNγ-neutralizing antibodies in the organism has not been observed, as is the case of IFNα (Einhorn and Grander 1996), and so this factor does not play a role. The high toxic doses necessary to reach an effect with IFNα (Einhorn and Grander 1996) probably do not have the same importance for IFNγ, considering the need of an optimal concentration that is lower that MTD.

5. It is justified and necessary to continue the scientific research on the molecular mechanisms of IFNγ activities, of the mechanisms of resistance, and of the optimal regimens for its administration in malignant tumors.
6. The possibility of immunoprophylaxis of cancer with interferons deserves special attention, as it has been pointed out by some investigators, who discuss the problem of the preventive use of interferons (Einhorn and Grander 1996; Tannenberger and Hrelia 1996). IFNγ would have an advantage in this approach due to the possibility of

using lower doses and the data from animal experiments showing the prevention of carcinogenesis by this cytokine (see p. 115).

7. Of special interest for the prophylaxis of metastases under low tumor load is the use of liposomal IFNγ with muramyl peptides for systemic activation of the macrophages (Fidler et al. 1989; Philips 1989; Fidler 1990).

# *chapter thirteen*

# *General conclusions*

Immune interferon (INFγ) is one of the key cytokines, because it has several important functions in the defense of the organism against foreign pathogens. This defense is effected by stimulating cellular immunity (mainly the macrophages) and by maintaining the domination of T helper 1 (Th1) function over that of the Th2 lymphocytes. In addition, this cytokine modulates gene activity by activating the expression of some genes and suppressing the function of others.

These properties of IFNγ determine its various activities — antiviral, antibacterial, antiproliferative, antitumor, and antiallergic. An important peculiarity is that these activities are manifested at optimal concentrations of IFNγ, and the maximum tolerance dose (MTD) is not a suitable dose.

Due to its various activities, IFNγ can be used to treat different diseases: viral, bacterial, malignant, and metabolic disorders. However, this does not mean that it is a universal curative drug. Its application should be in accordance with the cytokine profile of the disease and the known experimental and clinical results. Based on the available data, four groups of diseases can be differentiated from the point of view of using this cytokine and in general the Th1 cytokines for stimulating the body's cellular immunity:

**Group A.** Diseases in which IFNγ has a proven curative effect. Examples of such diseases are:

A number of *viral infections* (e.g., those caused by herpes simplex 1 and 2, herpes zoster, human papillomaviruses (condylomata acuminata), adenoviruses, respiratory viruses, including influenza and others.

*Bacterial infections* such as, for example, those caused by mycobacteria. It is believed that IFNγ is the only cytokine that can successfully fight tuberculosis mycobacteria.

*Protozoal infections* such as leishmaniasis.

*Fibroproliferative diseases* such as systemic sclerosis, scleroderma, idiopathic pulmonary fibrosis, and others.

*Metabolic disorders* such as chronic granulomatous disease, allergic states, osteopetrosis, and others.

**Group B.** Diseases where IFNγ has a certain, although variable effect, and does not lead to a final recovery with the present methods of administration or the clinical data are contradictory. Examples of such diseases are:

Some *malignant diseases* such as renal cell carcinoma, melanoma, sarcoma, (applied in isolated limbs), and probably in breast cancer to enhance graft-vs.-host disease.

Some *viral infections* such as that caused by the human immunodeficiency virus, in which it has no curative effect but can prolong the asymptomatic latent period.

*Metabolic disorders* such as *rheumatoid arthritis* in which the data are controversial and others.

**Group C.** Diseases in which there are experimental and theoretical reasons to expect an antitumor, antiviral, and antibacterial effect, but there are not enough clinical data and the optimal dosage, combinations, and regimens of administration have not been established. Examples of such diseases are:

*Malignant tumors* (tumors with viral etiology such as cervical cancer, laryngeal cancer), neuroblastoma, interleukin IL-6–dependent multiple myeloma, and others.

*Viral papillomas* such as laryngeal papillomas and others caused by human papillomaviruses.

*Bacterial infections* such as those caused by chlamydia.

**Group D.** Diseases in which IFNγ is contraindicated. Presently such diseases are multiple sclerosis, Kaposi's sarcoma, and possibly psoriasis and systemic lupus erythematosus.

It is evident that more experimental data and clinical trials are needed to complete the list of diseases in the different groups. More experimental studies also are needed to increase the stability of the pharmaceutical INFγ preparations and for obtaining suitable liposomal, inhalation, transdermal, and other pharmaceutical formulations.

# *References*

## A


**Aarvak T**, Chabaud M, Thoen J et al (2000) Changes in the Th1 or Th2 cytokine dominance in the synovium of rheumatoid arthritis (RA): a kinetic study of the Th subsets in one unusual RA patient. Rheumatology (Oxford) **39**, 513.

**Abbruzzese JL**, Levin B, Ajani JA et al (1990) A phase II trial of recombinant human interferon-gamma and recombinant tumor necrosis factor in patients with advance gastrointestinal malignancies: results of a trial terminated by excessive toxicity. J Biol Response Modif **9**, 522.

**Abdel-Wahab Z**, Dar M, Osanto S et al (1997a) Eradication of melanoma pulmonary metastases by immunotherapy with tumor cells engineered to secrete interleukin-2 or gamma interferon. Cancer Gene Ther **4**, 33.

**Abdel-Wahab Z**, Weltz C, Hester D et al (1997b) A Phase I clinical trial of immunotherapy with interferon-gamma gene-modified autologous melanoma cells: monitoring the humoral immune response. Cancer **80**, 401.

**Abe J**, Wakimoto H, Tsunoda R et al (1996) In vivo antitumor effect of cytotoxic T lymphocytes engineered to produce interferon-gamma by adenovirus-mediated genetic transduction. Biochem Biophys Res Commun **218**, 164.

**Abe K**, Harada M, Tamada K et al (1998) Early-appearing tumor-infiltrating natural killer cells play an important role in the nitric oxide production of tumor-associated macrophages through their interferon production. Cancer Immunol Immunother **45**, 225.

**Aboulafia D**, Mitsuyasu RT (1992) Potential role of Interferon-gamma in human immunodeficiency virus–associated disease. In: Antiinfective application of interferon gamma (Jaffe HS, Bucalo LR, Sherwin SA, eds). Marcel Dekker, New-York, p 37.

**Abratt RP**, Pontin AR, Ball HS (1993) Activity of a short course of interferon-gamma for metastatic renal cell carcinoma. A phase2 study. Cancer Immunol Immunother **37**, 140.

**Ackermann L**, Harvima IT, Pelkonen J et al (1999) Mast cells in psoriatic skin are strongly positive for interferon-gamma. Br J Dermatol **140**, 624.

**Adams LB**, Dinauer MC, Morgenstern DE, Krahenbuhl JL (1997) Comparison of the roles of reactive oxygen and nitrogen intermediates in the host response to Mycobacterium tuberculosis using transgenic mice. Tuber Lung Dis **78**, 237.

**Addison CL**, Arenberg DA, Morris SB et al (2000) The CXC chemokine, monokine induced by interferon-gamma, inhibits non–small cell lung carcinoma tumor growth and metastasis. Hum Gene Ther **11**, 247.

**Adolf GR** (1985) Structure and effects of interferon-gamma. Oncology **42**, 33.

**Agarwal SK**, Marshall GD Jr (1998) In vivo alteration in type-1 and type-2 cytokine balance: a possible mechanism for elevated total IgE in HIV-infected patients. Hum Immunol **59**, 99.

**Agarwala SS**, Kirkwood JM (1996) Interferons in melanoma. Curr Opin Oncol 8, 167.

**Aggarwal BB**, Pandita R (1994) Both type I and type II interferons down-regulate human tumor necrosis factor receptors in human hepatocellular carcinoma cell line Hep G2: Role of protein kinase C. FEBS Lett **337**, 99.

**Agrewala JN**, Mishra GC (1995) A 38kDa antigen of Mycobacterium tuberculosis predominantly induces the secretion of interleukin-2, interferon-gamma and IG2a antibodies. Microbiol Immunol **39**, 801**.**

**Aguet MZ**, Dembic G, Merlin (1988). Molecular cloning and expression of human interferon-γ receptor. Cell **55**, 273.

**Aguet MZ** (1990) The interferon-gamma receptor: A comparison with other cytokine receptors. J Interferon Res **10**, 551.

**Ahn MC** (1995) Regulatory role of cytokines in the development of tumoricidal function in mononuclear phagocytes from different anatomical sites of cancer patients. Diss Abstr Int B **56**, 2546.

**Aihara Y**, Mori M, Katakura S, Yokota S (1998) Recombinant IFN-gamma treatment of a patient with hyperimmunoglobulin E syndrome riggert autoimmune thrombocytopenia. J Interferon Cytokine Res **18**, 561.

**Akahoshi M**, Nakashima H, Tanaka Y et al (1999) Th1/Th2 balance of peripheral T helper cells in systemic lupus erythematosus. Arthritis Rheum **42**, 1644**.**

**Akaike T**, Noguchi Y, Ijiri S et al (1996) Pathogenesis of influenza virus-induced pneumonia: involvement of both nitric oxide and oxygen radicals. Proc Natl Acad Sci USA **93**, 2448.

**Akinaga S**, Gomi K, Oka T, Morimoto M (1987) Characterization of receptors for human recombinant interferon-γ in human cells. J Pharmacobiol Dyn **10**, 272.

**Ala-aho R**, Johanson N, Grenman R et al (2000) Inhibition of collagenase-3 (MMP-13) expression in transformed human keratinocytes by interferon-gamma is associated with activation of extracellular signal-regulated kinase-1, 2 and STAT1. Oncogene **19**, 248.

**Albert F**, Hus C, Truneh A et al (1985) Distinction between antigen receptor and IL-2 receptor triggering events in the activation of alloreactive T cell clones with calcium ionophore and phorbol ester. J Immunol **134**, 3649.

**Alcami A**, Smith GL (1995) Vaccinia, cowpox, and camelpox viruses encode soluble gamma interferon receptors with novel broad species specificity. J Virol **69**, 4633.

**Alfinito F**, Lamberti A, Capobianchi MR et al (1994) T cell growth-promoting activity of interferon-gamma. Mitogenic effect of the recombinant cytokine on cells from a human T-chronic lymphocytic leukemia. Leukemia **8**, 1294.

**al-Janadi M**, al-Balla S, al-Dalaan A, Raziuddin S (1993) Cytokine profile in systemic lupus erythematosus, rheumatoid arthritis, and other rheumatic diseases. J Clin Immunol **13**, 58.

**Allavena P**, Peccatori F, Maggioni D et al (1990) Intraperitoneal recombinant gamma-interferon in patients with recurrent ascitic ovarian carcinoma: Modulation of cytoxicity and cytokine production in tumor-associated effectors and of major histocompatibility antigen expression on tumor cells. Cancer Res **50**, 7318.

**Altare F**, Durandy A, Lammas D et al (1998) Impairment of mocobacterial immunity in human interleukin-12 receptor deficiency. Science **280**, 1432.

**Altfeld M**, Addo MM, Kreuzer KA et al (2000) T(H) 1 to T(H) 2 shift of cytokines in peripheral blood of HIV-infected patients is detectable by reverse transcriptase polymerase chain reaction but not by enzyme-linked immunosorbent assay under nonstimulated conditions. Acquir Immune Defic Syndr **23**, 287.

**Altobelli G**, Nacheva G, Todorova K et al (2001) Role of the C-terminal chain in human interferon-γ stability: An electrostatic study. Proteins Struct Funct Genet **43**, 125.

**An Z**, Wang X, Astoul P et al (1996) Interferon gamma is highly effective against orthotopically-implanted human pleural adenocarcinoma in nude mice. Anticancer Res **16**, 2545.

**Anderson DJ**, Berkowitz RS (1985) γ-Interferon enhances expression of class I MHC antigens in the weakly HLA human choriocarcinoma cell line BeWo, but does not induce MHC expression in the HLA choriocarcinoma cell line Jar. J Immunol **135**, 2498.

**Anderson PM**, Hanson DC, Hasz DE et al (1994) Cytokines in liposomes: preliminary studies with IL-1, IL-2, IL-6, GM-CSF and interferon-gamma. Cytokine **6**, 92.

**Angelo LS**, Meterissian SH, Owen-Schaub LB (1994) Soluble Fas/APO-1 antigen may provide partial protection from anti-Fas mediated apoptosis in human metastatic melanoma (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **35**, A41.

**Angioli R**, Sevin BU, Perras JP et al (1992) Rationale for combining radiation and interferon for the treatment of cervical cancer. Oncology **49**, 445.

**Angiolillo AL**, Sgadari C, Tosato G (1996) A role for the interferon-inducible protein 10 in inhibition of angiogenesis by interleukin 12. Ann New York Acad Sci **795**, 158.

**Angus R**, Collins CMP, Symes MO (1993) The effect of alpha and gamma interferon on cell growth and histocompatibility antigen expression by human renal carcinoma cells in vitro. Eur J Cancer A Gen Top **29**, 1879.

**Anichini A**, Mortarini R, Fossati G, Parmiani G (1986) Phenotypic profile of clones from early cultures of human metastatic melanomas and its modulation by recombinant interferon γ. Int J Cancer **38**, 505.

**Anjum A**, Fleischmann WR Jr (1992) Effect of hyperthermia on the antitumor actions of interferons. J Biol Regul Homeostatic Agents **6**, 75.

**Anthony LSD**, Chatterjee D, Brennan PJ, Nano FE (1994) Lipoarabinomannan from mycobacterium tuberculosis modulates the generation of reactive nitrogen intermediates by gamma interferon-activated macrophages. FEMS Immunol Med Microbiol **8**, 299.

**Aoyagi T**, Murai M, Kimura F et al (1992). Immunological study of interferon-gamma therapy for advanced renal cell carcinoma. Acta Urol Jpn **38**, 1357.

**Appleberg R** (1994) Protective role of interferon-gamma, tumor necrosis factor alpha and interleukin-6 in Mycobacterium tuberculosis and M. avium infections. Immunobiology **191**, 520.

**Applegate KG**, Balch CM, Pellis NR (1990) In vitro migration of lymphocytes through collagen matrix: Arrested locomotion in tumor-filtrating lymphocytes. Cancer Res **50**, 7153.

**Arakawa T**, Hsu YR, Chang D et al (1986a) Structure and activity of glycosylated human interferon γ. J Interferon Res **6**, 687.

**Arakawa T**, Hsu YR, Parker CG, Lai PH (1986b) Role of polycationic C-terminal portion in the structure and activity of recombinant human interferon-gamma. J Biol Chem **261**, 8534.

**Arakawa T**, Parker CG, Lai PH (1986c) Sites of phosphorylation in recombinant human interferon-gamma. Biochem Biophys Res Commun **136**, 679.

**Arakawa T**, Hsu YR, Yphantis DA (1987) Acid unfolding and self-association of recombinant *Escherichia coli* derived human interferon γ. Biochemistry **26**, 5428.

**Arakawa T**, Narachi MA, Hsu YR et al (1989) The effect of C-terminal processing on the activity of human interferon-gamma. Drug Des Deliv **4**, 217.

**Arakawa T**, Hsu YR, Narachi MA et al (1990) Reversibility of acid denaturation of recombinant interferon-gamma. Biopolymers **29**, 1065**.**

**Aramaki Y**, Suda H, Tsuchiya S (1995) Interferon-gamma inductive effect of liposomes as an immunoadjuvant. Vaccine **13**, 1809.

**Aramaki Y**, Nitta F, Matsuno R, Morimura Y, Tsuchiya S (1996) Inhibitory effects of negatively charged liposomes on nitric oxide production from macrophages stimulated by LPS. Biochem Biophys Res Commun **220**, 1.

**Arany I**, Tyring SK (1996) Status of local cellular immunity in interferon-responsive and non responsive human papillomavirus-associated lesions. Sex Transm Dis **23**, 475.

**Arany I**, Goel A, Tyring SK (1995a) Interferon response depends on viral transcription in human papillomavirus-containing lesions. Anticancer Res **15** B, 2865.

**Arany I**, Nagamani K, Tyring SK (1995b) Interferon resistance is dependent from copy numbers in benign HPV-induced lesions. Anticancer Res **15**, 1003.

**Arany I**, Fleischmann CM, Tyring SK, Fleischmann WR (1997) Interferon regulates expression of mda-6/WAF1/CIP1 and cyclin-dependent kinases independently from p53 in B16 murine melanoma cells. Biochem Biophys Res Commun **233**, 678.

**Arao Y**, Ando Y, Narita M, Kurata T (1997) Unexpected correlation in the sensitivity of 19 herpes simplex virus strains to types I and II interferons. J Interferon Cytokine Res **17**, 537.

**Arenberg DA**, Kunkel SL, Polverini PJ et al (1996) Interferon-gamma–inducible protein 10 (IP-10) is an angiostatic factor that inhibits human non–small cell lung cancer (NSCLC) tumorigenesis and spontaneous metastases. J Exp Med **184**, 981.

**Armstrong JA** (1981) Cytopathic effect inhibition assay for interferon: Microculture plate assay. Methods Enzymol **78** A, 381.

**Arnaudova P**, Rashkov R, Tsanev R (1993) Application of interferon-γ–containing gel for local treatment of skin ulcers in autoimmune vasculitis and skin changes in progressive systemic sclerosis. J Rheumatol **20**, 445.

**Arvin AM** (1992) Herpes simplex as a target for anti-infective application of interferon-gamma: preclinical in vitro and in vivo observations. In: Antiinfective application of Interferon gamma (Jaffe HS, Bucalo LR, Sherwin SA, eds). Marcel Dekker, New York, p 189.

**Asadullah K**, Docke WD, Haeussler A et al (1996) Progression of mycosis fungoides is associated with increasing cutaneous expression of interleukin-10 m RNA. J Invest Dermatol **107**, 833.

**Ashkar S**, Weber GF, Panoutsakopoulou V et al (2000) Eta-1 (osteopontin): an early component of type-1 (cell-mediated) immunity. Science **287**, 860.

**Asselin S**, Conjeaud H, Fradelizi D, Breban M (1998) In vitro differentiation of peripheral blood T cells towards a type 2 phenotype is impaired in rheumatoid arthritis (RA). Clin Exp Immunol **114**, 284.

**Aston C**, Rom WN, Talbot AT, Reibman J (1998) Early inhibition of mycobacterial growth by human alveolar macrophages is not due to nitric oxide. Am J Respir Crit Care Med **157**, 1943.

**Atamas SP**, Yurovsky VV, Wise R et al (1999) Production of type 2 cytokines by CD8+ lung cells is associated with greater decline in pulmonary function in patients with systemic sclerosis. Arthritis Rheum **42**, 1168.

**Aubock J**, Niederwieser D, Romani N et al (1985) Human interferon-γ induces expression of HLA-DR on keratinocytes and melanocytes. Arch Dermatol Res **277**, 270.

**Aulitzky WE**, Aulitzky W, Ellerhorst J et al (1995) Intermittent low-dose IFN gamma treatment for metastatic renal cell carcinoma: Analysis of factors predicting clinical response and long-term survival. Oncologie **18**, 339.

**Aulitzky W**, Gastl G, Aulitzky WE et al (1989b) Successful treatment of metastatic renal cell carcinoma with a biologically active dose of recombinant interferon-gamma. J Clin Oncol **7**, 1875.

**Aulitzky WE**, Aulitzky W, Gastl G et al (1989a) Acute effects of single doses of recombinant interferon-gamma on blood cells counts and lymphocyte subsets in patients with advanced renal cell cancer. J Interferon Res **9**, 425.

**Aulitzky WE**, Grosse Wilde H, Westhoff U et al (1991) Enhanced serum levels of soluble HLA class 1 molecules are induced by treatment with recombinant interferon gamma (IFN gamma). Clin Exp Immunol **86**, 236.

**Aulitzky WE**, Huber C, Peschel C (1993) Cytokine therapy of neoplastic and inflammatory disease. Int Arch Allergy Immunol **101**, 221.

**Aulitzky WE**, Lerche J, Thews A et al (1994) Low-dose gamma-interferon therapy is ineffective in renal cell carcinoma patients with large tumour burden. Eur J Cancer Part A Gen Top **30**, 940.

**Ault BH**, Stapleton FB, Gaber L et al (1988) Acute renal failure during therapy with recombinant human gamma-interferon. N Engl J Med **319**, 1397.

**Austin LM**, Ozawa M, Kikuchi T et al (1999) The majority of epidermal T cells in Psoriasis vulgaris lesions can produce type 1 cytokines, interferon-gamma, interleukin-2, and tumor necrosis factor-alpha, defining TC1 (cytotoxic T lymphocyte) and TH1 effector populations: a type 1 differentiation bias is also measured in circulating blood T cells in psoriatic patients. J Invest Dermatol **113**, 752.

**Autenrieth IB**, Beer M, Bohn E et al (1994) Immune responses to Yersinia enterocolitica in susceptible BALB/c and resistant C57BL/6 mice: an essential role for gamma interferon. Infect Immun **62**, 2590.

**Ayala A**, Wang P, Chaudry IH (1993) Insights into the mechanism by which interferon-γ improves macrophage function following hemorrhage and resuscitation. J Surg Res **54**, 322.

**Aziz N**, Nishanian P, Mitsuyasu R et al (1999) Variables that affect assays for plasma cytokines and soluble activation markers. Clin Diagn Lab Immunol **6**, 89.

## B

**Baba M**, Ito M, Shigeta S, De Clercq E (1984) Synergistic antiviral effects of antiherpes compounds and human leukocyte interferon on varicella-zoster virus in vitro. Antimicrob Agents Chemother **25**, 515.

**Bach EA**, Szabo SJ, Dighe AS et al (1995) Ligand-induced autoregulation of IFN-gamma receptor beta chain expression in T helper cell subsets. Science **270**, 1215.

**Bach EA**, Aguet M, Schreiber RD (1997) The IFNγ receptor: a paradigm for cytokine receptor signaling. Annu Rev Immunol **15**, 563.

**Bach EA**, Tanner JW, Marsters S et al (1996) Ligand-induced assembly and activation of the gamma interferon receptor in intact cells. Mol Cell Biol. **16**, 3214.

**Bacus SS**, Gudkov AV, Zelnik CR et al (1993) New differentiation factor (heregulin) induces expression of intercellular adhesion molecule I: Implications for mammary tumors. Cancer Res **53**, 5251.

**Badaro R**, Johnson WD, Jr (1993) The role of interferon-γ in the treatment of visceral and diffuse cutaneous leishmaniasis. J Infect Dis **167**(Suppl. 1), S13.

**Badaro R**, Falcoff E, Badaro FS et al (1990) Treatment of visceral leishmaniasis with pentavalent antimony and interferon gamma. N Engl J Med **322**, 16.

**Bahnson A**, Ratliff (1990) In vitro and in vivo anti-tumor activity of recombinant mouse tumor necrosis factor (TNF) in a mouse bladder tumor (MBT-2). J Urol **144**, 172.

**Bailer RT**, Holloway A, Sun J et al (1999) IL-13 and IFN-gamma secretion by activated T cells in HIV-1 infection associated with viral suppression and a lack of disease progression. J Immunol **162**, 7534.

**Baker BS**, Powles AV, Valdimarsson H, Fry L (1988) An altered response by psorriatic keratinocytes to gamma interferon. Scand J Immunol **28**, 735.

**Baker JN**, Karabin GD, Stoof TJ et al **(**1991**)** Detection of interferon gamma mRNA in psoriatic epidermis by chain polymerase reaction. J Dermatol Sci **2**, 106.

**Baker JN**, Goodlad JR, Ross L et al (1993) Increased epidermal cell proliferation in normal human skin in vivo following local administration of interferon-gamma. Am J Pathol **142**, 1091.

**Balachandra K**, Thawaranantha D, Ayuthaya PI et al (1994) Effects of human alpha, beta and gamma interferons on varicella zoster virus in vitro. Southeast Asian J Trop Med Public Health **25**, 252.

**Balish MJ**, Abrams ME, Pumfery AM, Brandt CR (1992) Enhanced inhibition of herpes simplex virus type 1 growth in human corneal fibroblasts by combinations of interferon-alpha and -gamma. J Infect Dis **166**,1401.

**Ball ED**, Guyre PM, Shen L et al (1984) Gamma interferon induces monocytoid differentiation in the HL-60 cell line. J Clin Invest **73**, 1072.

**Ball ED**, Howell AL, Shen L (1986) Gamma interferon and 1,25 dihydroxyvitamin D-3 cooperate in the induction of monocytoid differentiation but not in the functional activation of the HL-60 promyelocytic leukemia cell line. Exp Hematol **14**, 998.

**Bander NH**, Yao D, Liu H et al (1997) MHC class I and II expression in prostate carcinoma and modulation by interferon-alpha and gamma. Prostate **33**, 233.

**Barak V**, Kalickman I, Nisman B et al (1998) Changes in cytokine production of breast cancer patients treated with interferons. Cytokine **10**, 977.

**Barcova M**, Kacani L, Speth C et al (1998) Gp 41 envelope protein of human immunodeficiency virus induces interleukin (IL)-10 in monocytes but not in B, T, or NK cells, leading to reduced IL-2 and interferon-gamma production**.** J Infect Dis **177**, 905.

**Barker JN**, Karabin GD, Stoof TJ et al (1991) Detection of interferon-gamma MRNA in psoriatic epidermis by polymerase chain reaction. J Dermatol Sci **2**, 106.

**Barna BP**, Thomassen MJ, Sergi J et al (1991) Immunologic changes in renal cell carcinoma patients receiving gamma interferon. Oncology **48**, 464.

**Barnes PF**, Lu S, Abrams JS et al (1993) Cytokine production at the site of disease in human tuberculosis. Infect Immun **61**, 3482.

**Barnes PJ** (1996) Immunomodulation as asthma therapy: where do we stand? Eur Respir J Suppl **22**, 154.

**Baron S**, Tyring S, Klimpel G et al (1985) Marked cytolysis of human tumor cells by interferon gamma and leukocytes. In: Mediators in cell growth and differentiation (Ford RJ, Maizel, AL eds). Raven Press, New York, p 257.

**Baron S**, Tyring SK, Fleischmann RW et al (1991) The interferons. Mechanisms of action and clinical applications. JAMA **266**, 1375.

**Baroni GS**, D'Ambrosio L, Curto P et al (1996) Interferon gamma decreases hepatic stellate cell activation and extracellular matrix deposition in rat liver fibrosis. Hepatology **23**, 1189.

**Barr CL**, Saunders GF (1991) Interferon-γ inducible regulation of the human invariant chain gene. J Biol Chem **266**, 3475.

**Barth A**, Morton DL (1995) The role of adjuvant therapy in melanoma management. Cancer **75**, 726.

**Bartoletti R**, Dami A, Tosto A, Rizzo M (1990) The role of interferons in the treatment of urinary tract cancer. Acta Urol Ital **4**, 43.

**Basham TY**, Merigan TC (1983) Recombinant interferon-γ increases HLA-DR synthesis and expression. J Immunol **130**, 1492.

**Baskin H**, Ellermann-Eriksen S, Lovmand J, Mogensen SC (1997) Herpes simplex virus type 2 synergizes with interferon-gamma in the induction of nitric oxide production in mouse macrophages through autocrine secretion of tumour necrosis factor-alpha. J Gen Virol **78**, 195.

**Bataille R**, Klein B (1993) Cytokines and lymphoplasmocytic proliferations: Major role of interleukin 6 (IL-6). Rev Prat **43**, 275.

**Batcheler LM**, Bonham DG, Collins R, Liddell HS (1986) Topical interferon cream for the treatment of herpes genitalis: a double-blind controlled trial. NZ J Obstet Gynecol **26**, 239.

**Bateman WJ**, Donnellan I, Fraser IA et al (1995) Lymphocytes infiltrating colorectal cancer have low proliferative capacity but can secrete normal levels of interferon gamma. Cancer Immunol Immunother **41**, 61.

**Baumgarten E**, Schmid H, Baurmann H et al (1991) Phase I/II trial with low-dose interferon-gamma and interleukin-2 in children with high-risk malignancies after autologous bone marrow transplantation (meeting abstract). Cytokines in Hemopoiesis, Oncology and AIDS, June 19–22.

**Baumgarten E**, Schmid H, Pohl U et al (1994) Low-dose natural interleukin-2 and recombinant interferon-gamma following autologous bone marrow grafts in pediatric patients with high risk acute leukemia. Leukemia **8**, 850.

**Baumgarth N**, Kelso A (1996) In vivo blockade of gamma interferon affects the influenza virus-induced humoral and the local cellular immune response in lung tissue. J Virol **70**, 4411.

**Bausero MA**, Panoskaltsis-Mortari A, Blazar BR, Katsanis E (1996) Effective immunization against neuroblastoma using double-transduced tumor cells secreting GM-CSF and interferon-gamma. J Immunother **19**, 113.

**Baxevanis CN**, Reclos GJ, Gritzapis AD et al (1993) Elevated prostaglandin E2 production by monocytes is responsible for the depressed levels of natural killer and lymphokine-activated killer cell function in patients with breast cancer. Cancer **72**, 491.

**Bayad J**, Galteau MM, Siest G (1993) Viral theory of atherosclerosis. Role of cytomegalovirus. Ann Biol Clin Paris. **51**, 101.

**Beaman L** (1987) Fungicidal activation of murine macrophages by recombinant gamma interferon. Infect Immun **55**, 2951.

**Beatrice F**, Giordano C, Succo G et al (1987) Basal serum IFN (gamma) production, assessed by monoclonal antibody, in hayfever patients, patients with laryngeal carcinoma and normal controls. Med Sci Res **15**, 393.

**Beatty WL**, Byrne GI, Morrison RP (1993) Morphologic and antigenic characterization of interferon gamma-mediated persistent Chlamydia trachomatis infection in vitro. Proc Natl Acad Sci USA **90**, 3998.

**Beck J**, Rondot P, Catinot L et al (1988) Increased production of interferon gamma and tumor necrosis factor precedes clinical manifestation in multiple sclerosis: Do cytokines trigger off exacerbations? Acta Neurol Scand **78**, 318.

**Becker JC**, Dummer R, Hartmann AA et al (1991) Shedding of ICAM-1 from human melanoma cell lines induced by IFNγ and tumor necrosis factor α. Functional consequences on cell-mediated cytotoxicity. J Immunol **147**, 4398.

**Becker S** (1985a) Interferon-γ accelerates immune proliferation via its effect on monocyte HLA-DR expression. Cell Immunol **91**, 301.

**Becker S** (1985b) Effect of interferon-gamma on class-II antigen expression and accessory cell function. Surv Immunol Res **4**, 135.

**Beilke MA** (1989) Vascular endothelium in immunology and infectious disease. Rev Infect Dis **11**, 273.

**Beldarrain A**, Lopez-Lacomba JL, Furrazola G et al (1999) Thermal denaturation of human gamma-interferon. A calorimetric and spectroscopic study. Biochemistry **38**, 7865.

**Bello I**, Perez A, Torres AM et al (1998) High levels of soluble IFN gamma receptor alpha chain in the plasma of rheumatoid arthritis patients. Biotherapy **11**, 53.

**Bennett MR**, Littlewood TD, Hancock DC et al (1994a) Down-regulation of the c-myc proto-oncogene in inhibition of vascular smooth-muscle cell proliferation: A signal for growth arrest? Biochem J **302**, 701

**Bennett MR**, Evan GI, Newby AC (1994b) Deregulated expression of the c-myc oncogene abolishes inhibition of proliferation of rat vascular smooth muscle cells by serum reduction, interferon-gamma, heparin, and cyclic nucleotide analogues and induces apoptosis. Circ Res **74**, 525.

**Benson M**, Strannegard IL, Wennergren G, Strannegard O (1997) Cytokines in nasal fluids from school children with seasonal allergic rhinitis. Pediatr Allergy Immunol **8**, 143.

**Benson M**, Strannegard IL, Wennergren G, Strannegard O (2000) Low levels of interferon-gamma in nasal fluid accompany raised levels of T-helper 2 cytokines in children with ongoing allergic rhinitis. Pediatr Allergy Immunol **11**, 20.

**Benyoucef S**, Lion G, Gerard Y et al (1998) Imbalance in cytokine production by whole blood related to presence of cytopathogenic HIV-1 strains in HIV-1–infected patients. Infection **26**, 109.

**Beran M**, Andersson B, Kantarijan H et al (1987) Hematologic response of four patients with smoldering acute myelogenous leukemia to partially pure gamma interferon. Leukemia **1**, 52.

**Beresford JN**, Taylor GT, Triffit JT (1990) Interferons and bone. A comparison of the effects of interferon-α and interferon-γ in cultures of human bone-derived cells and an osteosarcoma cell line. Eur J Biochem **193**, 589.

**Berger DP**, Homann D, Oldstone MB (2000) Defining parameters for successful immunocytotherapy of persistent viral infection. Virology **266**, 257.

**Berger S**, Ballo H, Stutte HJ (1996) Distinct antigen-induced cytokine pattern upon stimulation with antibody-complexed antigen consistent with a Th1→Th2-shift. Res Virol **147**, 103.

**Berman B**, Flores F (1998) The treatment of hypertrophic scars and keloids. Eur J Dermatol **8**, 591.

**Berman B**, Duncan MR (1989) Short-term keloid treatment in vivo with human interferon alfa-2B results in a selective and persistent normalization of keloidal fibroblast collagen, glycosaminoglycan and collagenase production in vitro. J Am Acad Dermatol **21**, 694.

**Bermudez LE**, Kaplan G (1995) Recombinant cytokines for controlling mycobacterial infections. Trends Microbiol **3**, 22.

**Bernassola F**, Scheuerpflug C, Herr I et al (1999) Induction of apoptosis by IFNgamma in human neuroblastoma cell lines through the CD95/CD95L autocrine circuit. Cell Death Differ **6**, 652.

**Berner B**, Akca D, Jung T et al (2000) Analysis of Th1 and Th2 cytokines expressing CD4+ and CD8+ T cells in rheumatoid arthritis by flow cytometry. J Rheumatol **27**, 1128.

**Berrih S**, Arenzana-Seisdedos F, Cohen S et al (1985) Interferon-γ modulates HLA class II antigen expression on cultured human thymic epithelial cells. J Immunol **135**, 1165.

**Berthet B**, Di Costanzo J, Arnaud C et al (1994a) Treatment of caustic burns of the esophagus with interferon-gamma. Comparison with epidermal growth factor. Experimental study in rats. Gastroenterol Clin Biol **18**, 680.

**Berthet B**, Di Costanzo J, Arnaud C et al (1994b) Influence of epidermal growth factor and interferon gamma on healing of oesophageal corrosive burns in the rat. Br J Surg **81**, 395.

**Berthold J**, Schopf E, Volkers W, Brzoska J (1989) Topical application of interferon β as an adjuvant in the treatment of recurrent genital warts: results of a placebo-controlled double-blind study. J Invest Dermatol **93**, 541.

**Berthou C**, Bourge JF, Zhang Y et al (2000) Interferon-gamma-induced membrane PAF-receptor expression confers tumor cell susceptibility to NK perforin-dependent lysis. Blood **95**, 2329.

**Beutnar KR** (1989) Human papilloma viruses infection. J Am Acad Dermatol **20**, 114.

**Beutner KR**, Becker TM, Stone KM (1991) Epidemiology of human papilloma viruses. Dermatol Clin **9**, 211.

**Bhatnagar PK**, Chattopadhya D, Sharma GL et al (1996) Aspergillus fumigatus specific antibodies in multitransfused children with human immunodeficiency virus (HIV) infection in relation to serum levels of interleukin-2, gamma interferon and tumor necrosis factor. J Trop Pediatr **42**, 85.

**Bienkowski RS**, Gotkin MG (1995) Control of collagen deposition in mammalian lung. Proc Soc Exp Biol Med **209**, 118.

**Billiau A** (1981) Interferon therapy: Pharmacokinetic and pharmacological aspects. Arch Virol **67**, 121.

**Billiau A** (1987) Interferons and inflammation. J Interferon Res **7**, 559.

**Biondillo DE**, Konicek SA, Iwamoto GK (1994) Interferon-gamma regulation of interleukin 6 in monocytic cells. Am J Physiol **267**, 564.

**Biotechnology Report** in UBS Warburg. Global Life Sciences Conference Issue 2000. <www.twst.com/info/info182.htm>

**Bird JL**, Tyler JA, (1995) Tumor necrosis factor alpha, interfero gamma and dexamethasone regulate IGF-I–maintained collagen production in cultured human fibroblasts. J Endocrinol **147**, 167.

**Bitran J**, Goutsou M, Clamon J et al (1990) A test of gamma interferon (G-INF) after initial chemotherapy for previously untreated extensive disease (PUED), small cell lung cancer (SCLC) (meeting abstract). Proc Annu Meet Am Soc Clin Oncol **9**, A893.

**Bitran JD**, Green M, Perry M et al (1995) A phase II study of recombinant interferon-gamma following combination chemotherapy for patients with extensive small cell lung cancer. CALGB. Am J Clin Oncol **18**, 67.

**Bjerke JR**, Livden JK, Serge M et al (1983) Interferon in suction blister fluid from psoriatic lesions. Br J Dermatol **108**, 295.

**Black CA** (1999) Delayed type hypersensitivity: current theories with an historic perspective. Dermatol Online J **5**, 7**.**

**Black PL**, Phillips H, Tribble HR et al (1988a) Correlation of immunomodulatory and therapeutic activities of interferon and interferon inducers in metastatic disease. J Cell Biochem **36**, 377.

**Black RJ**, Yu ZP, Brown D, Chang E (1988b) Modulation of oncogene expression by epidermal growth factor and γ-interferon in A431 squamous carcinoma cells. J Biol Regul Homeost Agents **2**, 35.

**Blanchard DK**, Djeu JY, Klein TW et al (1986) Interferon-gamma induction by lipopolysaccharide: Dependence on interleukin 2 and macrophages. J Immunol **136**, 963.

**Blanchard DK**, Djen JY, Klein TW et al (1988) Protective effects of tumor necrosis factor in experimental *Legionella pnumophila* infections of mice via activation of PMN function. J Leuk Biol **43**, 429.

**Blanchard DK**, Spranzi E, Djeu JY (1993) Lysis of human monocytic leukemia cells by extracellular ATP: Mechanism and characterization of the ATP receptor (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **34**, 1739.

**Bletry O**, Laraki R, Cabane J et al (1993) Long-term results of the treatment of diffuse systemic scleroderma with interferon-gamma. Rev Med Interne **14**, 978

**Blezinger P**, Freimark BD, Mutar M et al (1999) Intratracheal administration of interleukin 12 plasmid-cationic lipid complexes inhibits murine lung metastases. Hum Gene Ther **10**, 723.

**Blumenfeld W**, Ye JQ, Dahiya R et al (1993) HLA expression by benign and malignant prostatic epithelium: augmentation by interferon gamma. J Urol **150**, 1289.

**Bocci V** (1984) Evaluation of roots of administration of interferon in cancer: A review and a proposal. Cancer Drug Deliv **1**, 337.

**Bocci V**, Naldini A, Carraro F (1991) Hypoxia modifies the antiproliferative activity of interferon on a human melanoma cell line (meeting abstract). FASEB J **5**, A858.

**Bogdahn U**, Fleischer B, Rupniak HTR, Ali-Osman F (1986) Cell mediated cytotoxicity in human glioma. In: Biology of Brain Tumour (Walker MD, Thomas DGT, eds). Boston, Martinus Nijhoff, p 501.

**Boguniewicz M**, Leung D (1992) Atopic Dermatitis. In: Antiinfective application of interferon gamma (Jaffe HS, Bucalo LR, Sherwin SA, eds). Marcel Dekker, New York, p 67.

**Boguniewicz M**, Schneider LC, Milgrom H et al. (1993) Treatment of steroid-dependent asthma with recombinant interferon-gamma. Clin Exp Allergy **23**, 785.

**Bohle A**, Thanhauser A, Ulmer AJ et al (1994) On the mode of action of intravesical bacillus Calmette-Guerin: In vitro characterization of BCG-activated killer cells. Urol Res **22**, 185.

**Bohm I**, Bauer R (1997) Th1 cells, Th2 cells and atopic dermatitis. Hautarzt **48**, 223.

**Bohm W**, Thoma S, Leithauser F et al (1998) T cell-mediated, IFN-gamma–facilitated rejection of murine B16 melanomas. J Immunol **161**, 897.

**Bohn E**, Heesemann J, Ehlers S, Autenrieth IB (1994) Early gamma interferon mRNA expression is associated with resistance of mice against Yersinia enterocolitica. Infect Immun **6**, 3027.

**Bolinger AM**, Taeubel MA (1992) Recombinant interferon gamma for treatment of chronic granulomatous disease and other disorders. Clin Pharm **11**, 834.

**Bollag W** (1994) Experimental basis of cancer combination chemotherapy with retinoids, cytokines, 1,25-dihydroxyvitamin D-3, and analogs. J Cell Biochem **56**, 427.

**Bonecchi R**, Bianchi G, Bordignon PP et al (1998) Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. J Exp Med **187**, 129.

**Bonecini-Almeida MG**, Chitale S, Boutsikakis I et al (1998) Induction in vitro of human macrophage anti–Mycobacterium tuberculosis activity: requirement for IFN-gamma and primed lymphocytes. J Immunol **160**, 4490.

**Bonfichi R**, Sottani C, Colombo L et al (1995) Preliminary investigation of glycosylated proteins by capillary electrophoresis and capillary electrophoresis/mass spectrometry using electrospray ionization and by matrix-assisted laser desorption ionization/time-to-flight mass spectrometry. Rapid Commun Mass Spectrom Spec No: S95.

**Bonilla F**, Alvarez-Mon M, Merino F et al (1988) Interleukin-2 induces cytotoxic activity in lymphocytes from regional axillary nodes of breast cancer patients. Cancer **61**, 629.

**Boniver J**, Humblet C, Defresne MP (1989) Tumor necrosis factor and interferon gamma inhibit the development of radiation-induced thymic lymphomas in C57BL/Ka mice. Leukemia **3**, 611.

**Bonneau RH**, Brehm MA, Kern AM (1997) The impact of psychological stress on the efficacy of anti-viral adoptive immunotherapy in an immunocompromised host. J Neuroimmunol **78**, 19.

**Bonnez W**, Oakes D, Bailey-Farchione A et al (1995) A randomized, double-blind, placebo-controlled trial of systemically administered interferon-alpha, -beta, or -gamma in combination with cryotherapy for the treatment of condyloma acuminatum. J Infect Dis **171**, 1081.

**Boraschi D**, Censini S, Tagliabue A (1984) Macrophage antitumor activity:impaired responsiveness to interferon-γ of macrophages from genetically defective mice. Eur J Immunol **14**, 1061.

**Borden EC** (1988) Augmented tumor-associated antigen expression by interferons. J Natl Cancer Inst **80**, 148.

**Borecky L** (1989) Interferon after 30 years. Acta Virol **33**, 378.

**Borish L** (1999) Genetics of allergy and asthma. Ann Allergy Asthma Immunol **82**, 413.

**Born J**, Spath-Schwalbe E, Pietrowsky R et al (1989) Neurophysiological effects of recombinant interferon-gamma and alpha in man. Clin Physiol Biochem **7**, 119.

**Bornstein J**, Lahat N, Kinarty A et al (1997) Interferon beta and gamma but not tumor necrosis factor alpha demonstrate immunoregulatory effects on carcinoma cell lines infected with human papillomavirus. Cancer **79**, 924.

**Boshkov LK**, Kelton JG, Halloran PF (1992) HLA-DR expression by platelets in acute idiopathic thrombocytopenic purpura. Br J Haematol **81**, 552.

**Bot A**, Bot S, Bona CA (1998) Protective role of gamma interferon during the recall response to influenza virus. J Virol **72**, 6637.

**Boteva R**, Zlateva TH, Dorovska-Taran, V, et al (1996) Dissocioation equilibrium of human recombinant interferon-γ. Biochemistry **35**, 14825.

**Bottomley JM**, Toy JL (1985) Clinical side effects and toxicities of interferon. Interferon **4**, 155.

**Bottomley WW**, Keczkes K (1991) Treatment of basal cell carcinomas with intralesional recombinant interferon α-2b. J Dermatol Treat **2**, 15.

**Bouillon M**, Audette M (1993) Transduction of retinoic acid and gamma-interferon signal for intercellular adhesion molecule-1 expression on human tumor cell lines: Evidence for the late-acting involvement of protein kinase C inactivation. Cancer Res **53**, 826.

**Bouillon M**, Audette M (1994) Retinoic acid-stimulated intercellular adhesion molecule-1 expression on SK-N-SH cells: Calcium/calmodulin–dependent pathway. Cancer Res **54**, 4144.

**Bouillon M**, Leblank E, Fortier MA, Audette M (1992) Regulation of intracellular adhesion molecule-1 (CD54) expression on tumor cell lines by retinoic acid and interferon-gamma: Evidence of protein kinase c-dependent and independent pathways (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **33**, A2361.

**Bouley DM**, Kanangat S, Wire W, Rouse BT (1995) Characterization of herpes simplex virus type-1 infection and herpetic stromal keratitis development in IFN-gamma knockout mice. J Immunol **155**, 3964.

**Bourgeade MF**, Silbermann F, Thang MN, Besanson F (1988) Reduction of transferrin receptor expression by interferon γ in a human cell line sensitive to its antiproliferative effect. Biochem Biophys Res Commun **153**, 897.

**Bourgeade MF**, Silbermann F, Kuhn L et al (1992) Post-transcriptional regulation of trannsferrin receptor mRNA by IFN-γ. Nucleic Acids Res **20**, 2997.

**Boussiotis VA**, Tsai EY, Yunis EJ et al (2000) IL-10–producing T cells suppress immune responses in anergic tuberculosis patients. J Clin Invest **105**, 1317.

**Bovolenta C**, Lorini AL, Mantelli B et al (1999) A selective defect of IFN-gamma – but not of IFN-alpha–induced JAK/STAT pathway in a subset of U937 clones prevents the antiretroviral effect of IFN-gamma against HIV-1. J Immunol **162**, 323.

**Boyer CM**, Dawson DV, Neal SE et al (1989) Differential induction by interferons of major histocompatibility complex-encoded and non-major histocompatibility complex-encoded antigens in human breast and ovarian carcinoma cell lines. Cancer Res **49**, 2928.

**Branca AA** (1988) Interferon receptors. In Vitro Cell Develop Biol **24**, 155.

**Branca AA**, Baglioni C (1981) Evidence that types I and II interferons have different receptors. Nature **294**, 768.

**Braude IA** (1984) Purification of human γ-interferon to essential homogeneity and its biochemical characterization. Biochemistry **23**, 5603.

**Braun CM**, Huang SK, Bashian GG et al (1997) Corticosteroid modulation of human, antigen-specific Th1 and Th2 responses. J Allergy Clin Immunol **100**, 400.

**Breen EC**, Salazar-Gonzalez JF, Shen LP et al (1997) Circulating CD8 T cells show increased interferon-gamma mRNA expression in HIV infection. Cell Immunol **178**, 91.

**Brinckerhoff CE**, Guyre PM (1985) Increased proliferation of human synovial fibroblasts treated with recombinant immune interferon. J Immunol **134**, 314

**Britton J** (2000) Interferon-gamma-1b therapy for cryptogenic fibrosing alveolitis. Thorax 55 (Suppl 1) S37.

**Brochon JC**, Tauc P, Merola F, Schoot BM (1993) Analysis of a recombinant protein preparation on physical homogeneity and state of aggregation. Anal Chem **65**, 1028.

**Brodowicz T**, Kotz R, Wiltschke C, et al (1997) Isolated limb perfusion with high-dose tumor necrosis factor-alpha in combination with interferon-gamma and melphalan for nonresectable extremity soft tissue sarcomas: a multicenter trial. J Clin Onkol **15**, 2174.

**Broker BJ**, Rosen D, Amsberry J et al (1996) Keloid excision and recurrence prophylaxis via intradermal interferon-gamma injections: a pilot study. Laryngoscope **106**, 1497.

**Brown DA**, Nelson FB, Reinherz EL, Diamond DJ (1991a) The human interferon-γ gene contains an inducible promoter that can be transactivated by tax I and II. Eur J Immunol **21**, 1879.

**Brown TD**, Goodman PJ, Fleming T et al (1991b) Phase II trial of recombinant DNA gamma-interferon in advanced colorectal cancer: A Southwest Oncology Group study. J Immunother **10**, 379.

**Broxmeyer HE**, Williams DE, Lu L et al (1986) The suppressive influences of human tumor necrosis factor on bone marrow hematopietic progenitor cells from normal donors and patients with leukemia: Synergism of tumor necrosis factor and interferon-gamma. J Immunol **136**, 4487.

**Bruch-Gerharz D**, Fehsel K, Suschek C et al (1988) A proinflammatory activity of interleukin 8 in human skin: expression of the inducible nitric oxide synthase in psoriatic lesions and cultured keratinocytes. J Exp Med **184**, 2007.

**Bruchelt G**, Handretinger R, Schibach-Stuckle K et al (1990) The role of interferons in neuroblastoma. Part 1: Antiproliferative effects. Klin Padiatr **202**, 202.

**Brummer E**, Hanson LH, Restrepo A, Stevens DA (1988) In vivo and in vitro activation of pulmonary macrophages by IFN-γ for enhanced killing of *Paracoccidioides brasiliensis* or *Blastomyces dermatitidis.* J Immunol **140**, 2786.

**Brunda MJ**, Davatelis V (1985) Augmentation of natural killer cell activity by recombinant interleukin-2 and recombinant interferons. In: Mechanisms of cytotoxicity by NK cells (Herberman RB and Callewaert DM, eds). Academic Press, New York, p 397.

**Brunda MJ**, Wright RB (1986) Differential antiproliferative effects of combinations of recombinant interferons alpha and gamma on two murine tumor cell lines. Int J Cancer **37**, 287.

**Brunda MJ**, Sulich V, Bellantoni D (1987) The anti-tumor effect of recombinant interferon alpha or gamma is influenced by tumor location. Int J Cancer **40**, 807.

**Bruselle GG**, Kips JC, Peleman RA et al (1997) Role of IFN-gamma in the inhibition of the allergic airway inflammation caused by IL-12. Am J Respir Cell Mol Biol **17**, 767.

**Bryckaert M**, Fontenay M, Liote F et al (1994) Increased mitogenic activity of scleroderma serum: Inhibitory effect of human recombinant interferon-gamma. Ann Rheum Dis **53**, 776.

**Brysk MM**, Newton RC, Fleischmann WR, Fleischmann CM (1983) Different anticellular effects of interferon on non-malignant and malignant epidermal cell lines. J Invest Dermatol **80**, 331.

**Bryson YJ**, Dillon M, Lovett M et al (1983) Treatment of first episodes of genital herpes simplex virus infection with oral acyclovir: a randomized double-blind controlled trial in normal subjects. N Engl J Med **308**, 916.

**Brzoska J**, Obert HJ (1987) Interferon gamma: ein januskopfiger mediator bei entzundungen. Arzneimittel Forsch **37**, 1410.

**Brzoska J**, Deutschle R, Fierlbeck G et al (1989) Phase II clinical trials of interferon beta gel (Fiblaferon$^{R}$ gel) in dermatological diseases. J Interferon Res **9**(Suppl 2), 259.

**Buchmeier NA**, Schreiber RD (1985) Requirement of endogenous interferon-gamma production for resolution of *Listeria monocytogenes* infection. Proc Natl Acad Sci USA **82**, 7404.

**Buechner, StA**. (1991) Intralesional interferon alfa-2b in the treatment of basal cell carcinoma. Therapy **24**, 731.

**Buja LM** (1996) Does atherosclerosis have an infectious etiology? [editorial; comment]. Circulation **94**, 872.

**Bukowski RM**, Rayman P, Molto L et al (1999) Interferon-gamma and CXC chemokine induction by interleukin 12 in renal cell carcinoma. Clin Cancer Res **5**, 2780.

**Bulleid NJ**, Curling E, Freedman RB, Jenkins N (1990) Source of heterogeneity in secreted interferon-γ. Biochem J **268**, 777.

**Burke DC** (1986) Interferon and cell differentiation. Br J Cancer **53**, 301.

**Burke F**, Knowles RG, East N, Balkwill FR (1995) The role of indoleamine 2,3-dioxygenase in the antitumor activity of human interferon-gamma in vivo. Int J Cancer **60**, 115.

**Buschle M**, Campana D, Carding SR et al (1993) Interferon gamma inhibits apoptotic cell death in B cell chronic lymphocytic leukemia. J Exp Med **177**, 213.

**Buszello H** (1995) Antiproliferative effects of four different cytokines on renal cell carcinoma lines. Anticancer Res **15**, 735.

**Byrne G.I**, Schachter J (1992) Interferon-gamma and the control of intracellular *Chlamydia* replication In: Antiinfective application of interfereon gamma (Jaffe HS, Bucalo LR, Sherwin SA, eds). Marcel Dekker, New York, p 251.

**Byron KA**, Varigos GA, Wootton AM (1991) Recombinant interferon-gamma inhibits the expression of IL-4 receptors on human lymphocytes. Clin Exp Immunol **85**, 307.

**Byron PR**, Patton JS (1994) Drug delivery via the respiratory tract. J Aerosol Med Deposition Clear Eff Lung **7**, 49.

## C

**Calderon J**, KCF Sheehan, C Chance, ML Thomas, RD Scheiber (1988) Purification and characterization of the human interferon γ receptor from placenta. Proc Natl Acad Sci USA **85**, 4837.

**Campbell DE**, Hill DJ, Kemp AS (1998) Enhanced IL-4 but normal interferon-gamma production in children with isolated IgE mediated food hypersensitivity. Pediatr Allergy Immunol **9**, 68.

**Campbell DE**, Fryga AS, Bol S, Kemp AS (1999) Intracellular interferon-gamma (IFN-gamma) production in normal children and children with atopic dermatitis. Clin Exp Immunol **115**, 377**.**

**Campbell IL**, Grace HW, Schrader JW, Harrison LC (1985) Interferon-γ enhances the expression of the major histocompatibility class I antigens on mouse pancreatic beta cells. Diabetes **34**, 1205.

**Campbell SC**, Tanabe K, Alexander JP et al (1994) Intercellular adhesion molecule-1 expression by bladder cancer cells. Functional effects. J Urol **151**, 1385.

**Canaris AD**, Caruso A, Licenziati S et al (1998) Lack of polarized type 1 or type 2 cytokine profile in asymptomatic HIV-1–infected patients during a two-year bimonthly follow-up. Scand J Immunol **47**, 146.

**Candore G**, Di Lorenzo G, Melluso M et al (1993) Gamma interferon, interleukin-4 and interleukin-6 in vitro production in old subjects. Autoimmunity **16**, 275.

**Canete JD**, Martinez SE, Farres J et al (2000) Differential Th1/Th2 cytokine patterns in chronic arthritis: interferon gamma is highly expressed in synovium of rheumatoid arthritis compared with seronegative spondyloarthropathies. Ann Rheum Dis **59**, 263.

**Cantin E**, Tanamachi B, Openshaw H (1999a) Role for gamma interferon in control of herpes simplex virus type 1 reactivation. J Virol **73**, 3418.

**Cantin E**, Tanamachi B, Openshaw H et al (1999b) Gamma interferon (IFN-gamma) receptor null-mutant mice are more susceptible to herpes simplex virus type 1 infection than IFN-gamma ligand null-mutant mice. J Virol **73**, 5196.

**Cantin EM**, Hinton DR, Chen J, Openshaw H (1995) Gamma interferon expression during acute and latent nervous system infection by herpes simplex virus type 1. J Virol **69**, 4898.

**Caplen HS**, Gupta SL (1988) Differential regulation of acellular gene by human interferon-γ and interferon-α. J Biol Chem **263**, 332.

**Capron L** (1990) Viruses and atherosclerosis. Rev Prat **40**, 2227.

**Carlo-Stella C**, Cazzola M, Ganser A et al (1988) Synergistic antiproliferative effect of recombinant interferon-gamma with recombinant interferon-alpha on chronic myelogenous leukemia hematopoietic progenitor cells. (CFU-GEMM, CFU-Mk, BFU-E and CFU-GM) Blood **72**, 1293.

**Carlucci S**, Bescin A, Tuosto L et al (1993) Mycobacterium antigen complex A60-specific T-cell repertoire during the course of pulmonary tuberculosis. Infect Immun **61**, 439.

**Carolino J**, Perez J, Popa A (1998) Osteopetrosis. Am Acad Fam Physician March 15, 1998.

**Carrel S**, Schmidt-Kessen A, Giuffre L (1985) Recombinant interferon-γ can induce the expression of HLA-DR and -DC on DR-negative melanoma cells and enhance the expression of HLA-ABC and tumor-associated antigens. Eur J Immunol **15**, 118.

**Carrel S**, Hartmann F, Salvi S et al (1995) Expression of type A and B tumor necrosis factor (TNF) receptors on melanoma cells can be regulated by dbc-AMP and IFN gamma. Int J Cancer **62**, 76.

**Carreno V**, Moreno A, Galiana F, Bartolome FJ (1993) Alpha- and gamma-interferon versus alpha-interferon alone in chronic hepatitis B. A randomized controlled study. J Hepatol **17**, 321.

**Carson WE**, Lindemann MJ, Baiocchi et al (1995) The functional characterization of interleukin 10 receptor expression on human natural kilar cells. Blood **85**, 3577.

**Caruso A**, Licenziati S, Canaris AD et al (1996) Characterization of T cell subsets involved in the production of IFN-gamma in asymptomatic HIV-infected patients AIDS. Res Hum Retroviruses **12**, 135.

**Caselmann WH**, Meyer M, Scholz S et al (1992) Type I interferons inhibit hepatitis B virus replication and induce hepatocellular gene expression in cultured liver cells. J Infect Dis **166**, 966.

**Casscells W** (1991) Smooth muscle cell growth factors. Prog Growth Factor Res **3**, 177.

**Celada A**, Maki RA (1989) The expression of I-A correlates with the uptake of interferon-γ by macrophages. Eur J Immunol **19**, 205.

**Celada A**, Schreiber RD (1987) Internalization and degradation of receptor-bound interferon-γ by murine macrophages; demonstration of receptor recycling. J Immunol **139**, 147.

**Cembrzynska-Nowak M**, Szklarz E, Inglot AD, Teodorczyk-Injeyan A (1993) Elevated release of tumor necrosis factor-alpha and interferon-gamma by bronchoalveolar leukocytes from patients with bronchial asthma. Am Rev Respir Dis **147**, 291.

**Cemerlic D**, Dadey B, Han T, Vaickus L (1991) Cytokine influence on killing of fresh chronic lymphocytic leukemia cells by human leukocytes. Blood **77**, 2707.

**Cendan JC**, Topping DL, Pruitt J et al (1996) Inflammatory mediators stimulate arginine transport and arginine-derived nitric oxide production in a murine breast cancer cell line. J Surg Res **60**, 284.

**Centers for Disease Control** (1983) Condylomata acuminata. MMWR **32**, 306.
**Cesano A**, Clark SC, Santoli D (1993) Cytokine modulation of the susceptibility of acute T-Lymphoblastic leukemia cell lines to LAK activity. Leukemia **7**, 404.
**Cesario TC**, Andrews BS, Martin DA et al (1983). Interferon in synovial fluid and serum of patients with rheumatic disease. J Rheumatol **10**, 647.
**Cesario TC**, Yousefi S, Carandang G et al (1994) Enhanced yields of gamma interferon in prolactin treated human peripheral blood mononuclear cells. Proc Soc Exp Biol Med **205**, 89.
**Chachoua A**, Krigel R, Schiller JH et al (1990) Phase II study of combination interferon beta and gamma in patients with non–small cell lung cancer (NSCLC) an ECOG pilot study (meeting abstract). Proc Annu Meet Am Soc Clin Oncol **9**, A936.
**Chakravarthy A**, Chen LC, Mehta D, Hamburger AW (1991) Modulation of epidermal growth factor receptors by gamma interferon in a breast cancer cell line. Anticancer Res **11**, 347.
**Chang E**, Ridge J, Black R et al (1987) Interferon-γ induces altered oncogene expression and terminal differentiation in A431 cells (42620). Proc Soc Exp Biol Med **186**, 319.
**Chang WC**, Hsiao MH, Pattengale PK (1991) Natural killer cell immunodeficiency in patients with chronic myelogenous leukemia. IV. Interleukin-1 deficiency and the restorative effects of short-term culture in the presence of interleukin-2 on natural killer cytotoxicity, chang killer-target binding and production of natural killer cytotic factor. Nat Immun Cell Growth Regul **10**, 57.
**Chapekar MS**, Glazer RI (1985) Synergistic effect of human immune interferon and double-stranded RNA against human colon carcinoma cells *in vitro.* Cancer Res **45**, 2539.
**Chapekar MS**, Glazer RI (1986) Potentiaion of the cytocidal effect of human immune interferon by different synthetic double-stranded RNAs in the refractory human colon carcinoma cell line BE. Cancer Res **46**, 1698.
**Chatte G**, Panteix G, Perrin-Fayolle M, Pacheco Y (1995) Aerosolized interferon gamma for Mycobacterium avium–complex lung disease. Am J Respir Crit Care Med **152**, 1094.
**Chatterjee SE**, Hunter E, Whitly R (1985) Effect of cloned human interferons on protein synthesis and morphogenesis of herpes simplex virus. J Viral **56**, 419.
**Chen E**, Keystone EC, Fish EN (1993a) Restricted cytokine expression in rheumatoid arthritis. Arthritis Rheum **36**, 901.
**Chen GJ**, Huang-DS, Watzl-B, Watson-RR (1993b) Ethanol modulation of tumor necrosis factor and gamma interferon production by murine splenocytes and macrophages. Life Sci **52**, 1319.
**Chen JT**, Teshima H, Shimizu Y et al (1990) Sizofiran and recombinant interferon-gamma stimulate peritoneal macrophages obtained from patients with gynecologic malignancies. Increased secretion of tumor necrosis factor, IL-1 and interferon-gamma. Jpn J Cancer Chemother **17**, 1365.
**Chen SH**, Arany I, Apisarnthanarax N et al (2000) Response of keratinocytes from normal and psoriatic epidermis to interferon-gamma differs in the expression of zinc-alpha (2)-glycoprotein and cathepsin D. FASEB J **14**, 565.
**Chen SH**, Oakes JE, Lausch RN (1994) Synergistic anti-herpes effect of TNF-alpha and IFN-gamma in human corneal epithelial cells compared with that in corneal fibroblasts. Antiviral Res **25**, 201.
**Chen YM**, Yang WK, Whang-Peng J et al (1996) Elevation of interleukin-10 levels in malignant pleural effusion. Chest **110**, 433.

**Chisari FV**, Ferrari C (1995) Hepatitis B virus immunopathogenesis. Annu Rev Immunol **13**, 29.

**Chizzolini C**, Rezzonico R, Ribbens C (1998) Inhibition of type I collagen production by dermal fibroblasts upon contact with activated T cells: different sensitivity to inhibition between systemic sclerosis and control fibroblasts. Arthritis Rheum **41**, 2039.

**Chodorowska G** (1998) Plasma concentrations of IFN-gamma and TNF-alpha in psoriatic patients before and after local treatment with dithranol ointment. J Eur Acad Dermatol Venereol **10**, 147.

**Chong AS**, Scuderi P, Grimes WJ, Hersh EM (1989) Tumor targets stimulate IL-2 activated killer cells to produce interferon-gamma and tumor necrosis factor. J Immunol **142**, 2133.

**Chougnet C**, Thomas E, Landay AL et al (1998) CD 40 ligand and IFN-gamma synergistically restore IL-12 production in HIV infected patients. Eur J Immunol **28**, 646.

**Chu E**, Casey LC, Harris JE, Braun DP (1993a) Suppression of the development of tumoricidal function in gamma interferon-treated human peripheral blood monocytes by lipopolysaccharide: The role of cyclooxygenase metabolites. J Clin Immunol **13**, 49.

**Chu E**, Koeller DM, Johnston PG et al (1993b) Regulation of thymidylate synthase in human colon cancer cells treated with 5-fluorouracil and interferon-gamma. Mol Pharmacol **43**, 527.

**Cirelli R**, Tyring SK (1994) Interferons in human papillomavirus infections. Antiviral Res **24**, 191.

**Clark JG**, Dedon TF, Wayner EA et al (1989) Effect of interferon-γ on expression of cell surface receptors for collagen and deposition of newly synthesized collagen by cultural human lung fibroblasts. J Clin Invest **83**, 1505.

**Clark S**, McGuckin MA, Hurst T, Ward BG (1994a) Effect of interferon-gamma and TNF-alpha on MUC1 mucin expression in ovarian carcinoma cell lines. Dis Markers **12**, 43.

**Clark S**, McGuikin MA, Hurst T, Ward BG (1994b) Effect of interferon gamma and tumor necrosis factor alpha on sensivity to cisplatin in ovarian carcinoma cell lines. Cancer Immunol Immunother **39**, 100.

**Clarkson B**, Strife A (1993) Cytokinetic considerations relevant to development of a successful therapeutic strategy in chronic myelogenous leukemia (CML). Leuk Lymphoma **11**, 101.

**Cleland JL**, Builder SE, Swartz JR et al (1992) Polyethylene glycol enhanced protein refolding. Biotechnology **10**, 1013.

**Clemens MJ**, McNurlan MA (1985) Regulation of cell proliferation and differentiation by interferons. Biochem J **226**, 345.

**Clement MV**, Stamenkovic I (1996) Superoxide anion is a natural inhibitor of FAS-mediated cell death. EMBO J **15**, 216.

**Clerici M**, Balotta C, Meroni L et al (1996) Type 1 cytokine production and low prevalence of viral isolation correlate with long-term nonprogression in HIV infection. AIDS Res Hum Retroviruses **12**, 1053.

**Clerici M**, Fusi ML, Ruzzante S (1997a) Type 1 and type 2 cytokines in HIV infection — a possible role in apoptosis and disease progression. Ann Med **29**, 185.

**Clerici M**, Merola M, Ferrario E et al (1997b) Cytokine production patterns in cervical intraepithelial neoplasia: association with human papillomavirus infection. J Natl Cancer Inst **89**, 245.

**Codde JP**, Vines JA, Burton MA et al (1990) Enhanced efficacy by regional administration of interferon gamma in the treatment of liver tumours. Reg Cancer Treat **3**, 163.

**Cofano F**, Fassio A, Cavallo G, Landolfo S (1986) Binding of murine 1251-labelled natural interferon gamma to murine cell receptors. J Gen Virol **67**, 1205.

**Cofano F**, Landolfo S, Apella E, Ullrich SJ (1989) Analysis of murine interferon-γ binding to its receptor on intact cells and solubilized membranes. Identification of an 80 kDa receptor. FEBS Lett **2**, 233.

**Coker RK**, Laurent GJ (1998) Pulmonary fibrosis: cytokines in the balance. Eur Respir J **11**, 1218.

**Cole BF**, Gelber RD, Kirkwood-JM et al (1996) A quality-of-life–adjusted survival analysis of interferon alfa-2b (IFNa-2b) adjuvant treatment for high-risk resected cutaneous melanoma: an Eastern Cooperative Oncology Group (ECOG) study (meeting abstract). Proc Annu Meet Am Soc Clin Oncol **15**, A1357

**Colle SPC**, Campigotto BMT, Johnson JG, Elliott B (1991) Differential growth inhibition and enhancement of major histocompatibility complex class I antigen expression by interferons in a small-cell lung cancer cell line and its doxorubicin-selected multidrug-resistant variant. Cancer Immunol Immunother **33**, 274.

**Collette Y**, Chang HL, Cerdan C et al (1996) Specific Th1 cytokine down-regulation associated with primary clinically derived human immunodeficiency virus type 1 Nef.gene-induced expression. J Immunol **156**, 360.

**Colombo N**, Peccatori F, Paganin C et al (1992) Anti-tumor and immunomodulatory activity of intraperitoneal IFN-gamma in ovarian carcinoma patients with minimal residual tumor after chemotherapy. Int J Cancer **51**, 42.

**Condos R**, Rom WN, Schluger NW (1997) Treatment of multidrug-resistant pulmonary tuberculosis with interferon-gamma via aerosol. Lancet **349**, 1513.

**Condylomata International Collaborative Study Group** (1991) A comparison of interferon alfa-2a and podophyllin in the treatment of primary condylomata acuminata. Genitourin Med **67**, 394.

**Condylomata International Collaborative Study Group** (1993) Recurrent condylomata acuminata treated with recombinant interferon alpha-2a. Acta Derm Venereol **73**, 223.

**Constantin A**, Loubet-Lescoulie P, Lambert N et al (1998) Antiinflammatory and immunoregulatory action of methotrexate in the treatment of rheumatoid arthritis: evidence of increased interleukin-4 and interleukin-10 gene expression demonstrated in vitro by competitive reverse transcriptase-polymerase chain reaction. Arthritis Rheum **41**, 48.

**Cooper AM**, Dalton DK, Stewart TA et al (1993) Disseminated tuberculosis in interferon gamma gene-disrupted mice. J Exp Med **178**, 2243.

**Cooper AM**, Roberts AD, Rhoades ER et al (1995) The role of interleukin-12 in acquired immunity to Mycobacterium tuberculosis infection. Immunology **84**, 423.

**Coradini D**, Biffi A, Pirronello E, Di Fronzo G (1994) The effect of alpha, beta and gamma-interferon on the growth of breast cancer cell lines. Anticancer Res **14**, A1779.

**Corey I**, Nahmias AJ, Guinan ME et al (1982) A trial of topical acyclovir in genital herpes simplex virus infection. N Engl J Med **306**, 1313.

**Cornaglia-Ferraris P**, Mariottini GL, Ponzoni M (1992) Interferon and retinoic acid synergize in inhibiting the growth of human neuroblastoma cells in nude mice. Cancer Lett **61**, 215.

**Cornaglia-Ferraris P**, Bocca P, Cara A, et al (1993) Human neuroblastoma: New therapeutic strategies suggested by experimental models. Clin Chem Enzymol Commun 5, 327.

**Cornetta K**, Berebitsky D, Behnia M et al (1994) A retroviral vector expressing human interferon gamma upregulates MHC antigen expression in human breast cancer and leukemia cell lines. Cancer Gene Ther **1**, 91.

**Corrias MV**, Gribaudo G, Guarnaccia F, Ponzoni M (1995) Induction of 2.5 OAS gene expression and activity is not sufficient for IFN-gamma–induced neuroblastoma cell differentiation. Intern J Cancer **62**, 223.

**Cortes JE**, Talpaz M, Kantarjian H (1996) Chronic myelogenous leukemia: A review. Am J Med **100**, 555.

**Coto C**, Varela G, Hernandes V et al (1998) Use of recombinant interferon gamma in pediatric patients with advanced juvenile chronic arthritis. Biotherapy **11**, 15.

**Coughlin CM**, Salhany KE, Gee MS et al (1998) Tumor cell responses to IFNgamma affect tumorigenicity and response to IL-12 therapy and antiangiogenesis. Immunity **9**, 25.

**Coyle AJ**, Tsuyuki S, Bertrand C et al (1996). Mice lacking the IFN-gamma receptor have impaired ability to resolve a lung eosinophilic inflammatory response associated with a prolonged capacity of T cells to exhibit a Th2 cytokine profile. J Immunol **156**, 2680.

**Craig RW**, Buchan HL (1989) Differentiation-inducing and cytotoxic effects of tumor necrosis factor and interferon-gamma in myeloblastic ML-1 cells. J Cell Physiol **141**, 46.

**Creagan ET**, Ahmann DL, Long HJ et al (1987) Phase II study of recombinant interferon-gamma in patients with disseminated malignant melanoma. Cancer Treat Rep **71**, 843.

**Creagan ET**, Schaid DJ, Ahmann DL, Frytak S (1988) Recombinant interferons in the management of advanced malignant melanoma. Am J Clin Oncol **11**, 652.

**Creagan ET**, Schaid DJ, Ahmann DL, Frytak S (1990) Disseminated malignant melanoma and recombinant interferon: Analysis of seven consecutive phase II investigations. J Invest Dermatol **95**, 188.

**Cristoforoni PM**, Lotzova E, Cook KR et al (1994) Oncolytic activity of NK cells against SW-756 squamous cervical carcinoma cell line: Role of interferons alpha and gamma and CD54 adhesion molecule in oncolysis. Gynecol Oncol **52**, 365.

**Croen KD** (1993) Evidence for antiviral effect of nitric oxide. Inhibition of herpes simplex virus type 1 replication. J Clin Invest **91**, 2446.

**Croll AD**, Siggins KW, Morris AG, Pither JM (1987) The induction of IFN-gamma production and mRNAs of interleukin 2 and IFN-gamma by phorbol esters and a calcium jonophore. Biochem Biophys Res Commun **146**, 927.

**Cummins JM**, Tompkins MB, Olsen RG et al (1988) Oral use of human alpha interferon in cats. J Biol Response Modif **7**, 513.

**Curling EMA**, Hayter PM, Baines AJ et al (1990) Recombinant human interferon-γ. Differences in glycosylation and proteolytic processing lead to heterogeneity in batch culture. Biochem J **272**, 333.

**Curnutte JT** (1993) Conventional versus interferon-gamma therapy in chronic granulomatous disease. J Infect Dis. **167**(Suppl 1), S8-S12.

**Czarniecki CW**, Peterson E, De la Maza LM (1986) Interferon-induced inhibition of *Chlamydia trachomatis*: dissociation from other biological activities of interferons. In: Interferons as cell growth inhibitors and antitumor factors. Alan R Liss, New York, p 467.

## D

**D'Acquisto R**, Markman M, Hakes T et al (1988) A phase I trial of intraperitoneal recombinant gamma-interferon in advanced ovarian carcinoma. J Clin Oncol **6**, 689.

**Dai W**, Gupta SL (1990) Regulation of indoleamine 2,3-dioxygenase gene expression in human fibroblasts by interferon-gamma. Upstream control region discriminates between interferon-gamma and interferon-alpha. J Biol Chem **265**, 19871.

**Daireaux M**, Redini F, Loyau G, Pujol JP (1990) Effects of associated cytokines (IL-1, TNF-alpha, IFN-gamma, TGF-beta) on collagen and glycosaminoglycan production by cultured human synovial cells. Int J Tissue React **12**, 21.

**Dalod M**, Sinet M, Deschemin JC et al (1999) Altered ex vivo balance between CD28+ and CD28- cells within HIV-seropositive patients. Eur J Immunol **29**, 38.

**Dalton D**, Buchmeier MJ, Sarvetnick N et al (1997) Interferon-gamma protects against herpes simplex virus type 1-mediated neuronal death. Virology **238**, 189.

**Dalton DK**, Pitts Meek S, Keshav S et al (1993) Multiple defects of immune cell function in mice with disrupted interferon-gamma genes [see comments]. Science **259**, 1739.

**Dani CH**, Mechti N, Piechaczyk M et al (1985) Increased rate of degradation of c-myc mRNA in interferon-treated Daudi cells. Proc Natl Acad Sci USA **82**, 4896.

**Dansky-Ullmann C**, Salgaller M, Adams S et al (1995) Synergistic effects of IL-6 and IFN-gamma on carcinoembryonic antigen (CEA) and HLA expression by human colorectal carcinoma cells: role for endogenous IFN-beta. Cytokine **7**, 118.

**Das G**, Vohra H, Saha B et al (1999) Apoptosis of Th1-like cells in experimental tuberculosis (TB). Clin Exp Immunol **115**, 324.

**Das KM**, Squillante L, Robertson FM (1993) Amplified expression of intercellular adhesion molecule-1 (ICAM-1) and $M_R$ 40K protein by DLD-1 colon tumor cells by interferon-γ. Cell Immunol **147**, 215.

**Daubener W**, Wanagat N, Pilz K et al (1994) A new, simple, bioassay for human IFN-γ. J Immunol Methods **168**, 39.

**Daubener W**, Nockemann S, Gubsche M, Hadding U (1995) Heparin inhibits the antiparasitic and immune modulatory effects of human recombinant interferon-gamma. Eur J Immunol **25**, 688.

**Daubener W**, Gutsche M, Nockemann S et al (1996) Protamine enhances the activity of human recombinant interferon-γ. J Interferon Cytokine Res **16**, 531.

**Davignon JL**, Castanie P, Yorke JA et al (1996) Anti-human cytomegalovirus activity of cytokines produced by CD4+ T-cell clones specifically activated by IE1 peptides in vitro. J Virol **70**, 2162.

**Davis GS**, Pfeiffer LM, Hemenway DR (1999) Expansion of interferon-gamma-producing lung lymphocytes in mouse silicosis. Am J Respir Cell Mol Biol **20**, 813.

**Dayton MA**, Knobloch TJ, Benjamin D (1992) Human B cell lines express the interferon gamma gene. Cytokine **4**, 454.

**De AK**, Kodys KM, Pellegrini J et al (2000) Induction of global anergy rather than inhibitory Th2 lymphokines mediates posttrauma T cell immunodepression. Clin Immunol **96**, 52.

**Dealtry GB**, Naylor MS, Fiers W, Balkwill FR (1987) DNA fragmentation and cytotoxicity caused by tumor necrosis factor is enhanced by interferon gamma. Eur J Immunol **17**, 689.

**De Francesco MA**, Caruso A, Dima F et al (1996) IFN-gamma restores HIV- and non–HIV-specific cell mediated immune response in vitro and its activity is neutralized by antibodies from patients with AIDS. Scand J Immunol **43**, 94.

**De Fries RU**, Golub SH (1988) Characteristics and mechanism of IFN-gamma–induced protection of human tumor cells from lysis by lymphokine-activated killer cells. J Immunol **140**, 3686.

**Degling L**, Stjarnkvist P, Sjoholm I (1993) Interferon-gamma in starch microparticles: nitric oxide-generating activity in vitro and antileishmanial effect in mice. Pharmacol Res **10**, 783.

**De Grado WF**, Wasserman ZR, Chowdhry V (1982) Sequence and structural homologies among type I and type II interferons. Nature **300**, 379.

**Degre M** (1996) Interferons and other cytokines in bacterial infections. J Interferon Cytokine Res **16**, 417.

**Degre M**, Mellbye OJ, Clarkr Jenssen O (1983) Immune interferons in serum and synovial fluid in rheumatoid arthritis and related disorders. Ann Rheum Dis **42**, 672.

**De Jong EC**, Spanhaak S, Martens BP et al (1996) Food allergen (peanut)–specific TH2 clones generated from the peripheral blood of a patient with peanut allergy. J Allergy Clin Immunol **98**, 73.

**De Jong R**, Altare F, Haagen IA et al (1998) Severe mycobacterial and Salmonella infections in interleukin-12 receptor-deficient patients. Science **280**, 1435.

**De Kossado S**, Moore R, Gschmeissner S et al (1995) Changes in endogenous cytokines, adhesion molecules and platelets during cytokine-induced tumor necrosis. Br J Cancer **72**, 1165.

**De Kruyff RH**, Fang Y, Umetsu DT (1998) Corticosteroids enhance the capacity of macrophages to induce Th2 cytokine synthesis in CD4+ lymphocytes by inhibiting IL-12 production. J Immunol **160**, 2231.

**De la Maza LM**, Peterson EM, Fennie CW, Czarniecki CW. (1985) The anti-chlamydial and anti-proliferative activities of recombinant murine interferon-γ are not dependent on tryptophan concentrations. J Immunol **135**, 4198.

**De la Maza LM**, Peterson EM, Burton LE et al (1987a) The antichlamydial, antiviral, and antiproliferative activities of human gamma interferon are dependent on the integrity of the C terminus of the interferon molecule. Infect Immun **55**, 2727.

**De la Maza LM**, Plunkett MJ, Carlson EJ et al (1987b) Ultrastructural analysis of the anti-chlamydial activity of recombinant murine interferon-γ. Exp Mol Pathol **47**, 13.

**Delforge A**, Vandenplas B, Lagneaux L et al (1990) Influence of recombinant alpha and gamma interferons on the *in vitro* proliferation of myeloid and leukemic progenitors. Eur J Haematol **44**, 307.

**Delvenne P**, al-Saleh W, Gilles C et al (1995) Inhibition of growth of normal and human papillomavirus-transformed keratinocytes in monolayer and organotypic cultures by interferon gamma and tumor necrosis factor-alpha. Am J Pathol **146**, 589.

**De Marco F**, Marcante ML (1993) HPV-16 E6-E7 differential transcription induced in Siha cervical cancer cell line by interferons. J Biol Regul Homeostatic Agents **7**, 15.

**De Mulder PH**, Oosterhof G, Bouffioux C et al (1995) EORTC (30885) randomised phase III study with recombinant interferon alpha and recombinant interferon alpha and gamma in patients with advanced renal cell carcinoma. Br J Cancer **71**, 371.

**De Mulder PHM**, Debruyne FM, van Oosterom A, et al (1991) Eortc randomized phase II study of recombinant interferon alpha and recombinant interferon alpha and gamma in advanced renal cell carcinoma (meeting abstract). Proc Annu Meet Am Soc Clin Oncol **10**, A 528.

**Denis M**, Chadee K (1989) Human neutrophils activated by interferon-gamma and tumor necrosis factor-alpha kill Entamoeba histolytica trophozoities in vitro. J Leukoc Biol **46**, 270.

**De Paoli P**, Zanussi S, Simonelli C et al (1997) Effects of subcutaneous interleukin-2 therapy on CD4 subsets and in vitro cytokine production in HIV+ subjects. J Clin Invest **100**, 2737.

**De Sanctis G**, Bottari V, Frezzolini A et al (1992) Ability of recombinant interferon gamma in vitro to restore the defective polymorphonuclear-cell but not lymphocyte-mediated cytotoxic activities in patients with myelodysplastic syndromes. Cancer Immunol Immunother **34**, 414.

**De Stasio PR**, Taylor MW (1990) Specific effect of interferon on the herpes simplex virus type 1 transactivation event. J Virol **64**, 2588.

**Devos R**, Cheroute H, Taya Y et al (1982) Molecular cloning of human immune interferon cDNA and its expression in eukaryotic cells. Nucleic Acids Res **10**, 2487.

**De Waard-Siebinga I**, Creyghton WM, Kool J, Jager MJ (1995) Effects of interferon alpha and gamma on human uveal melanoma cells in vitro. Br J Ophthalmol **79**, 847.

**De Young KL**, Ray ME, Su YA et al (1997) Cloning a novel member of the human interferon-inducible gene family associated with control of tumorigenicity in a model of human melanoma. Oncogene **15**, 453.

**Dhabhar FS**, Satoskar AR, Bluethmann H et al (2000) Stress-induced enhancement of skin immune function: A role for gamma interferon. Proc Natl Acad Sci USA **97**, 2846.

**Dhawan S**, Heredia A, Wahl LM et al (1995a) Interferon-gamma–induced downregulation of CD4 inhibitis the entry of human immunodeficiency virus type-1 in primary monocytes. Pathobiology **63**, 93.

**Dhawan S**, Wahl LM, Heredia A et al (1995b) Interferon-gamma inhibitis HIV-induced invasiveness of monocytes. J Leukoc Biol **58**, 713.

**Dhib-Jalbut S**, Kufta CV, Flerlage M et al (1990) Adult human glial cells can present target antigens to HLA-restricted cytotoxic T-cells. J Neuroimmunol **29**, 203.

**Dhingra K**, Kurzrock R, Kantarijan H et al (1992) Minimal residual disease in interferon-treated chronic myelogenous leukemia: Results and pitfalls of analysis based on polymerase chain reaction. Leukemia **6**, 754.

**Dianzani F** (1985) The biology of the Interferon system. Estratto dalla rivista EOS **5**, 59.

**Diaz-Mitoma F**, Kumar A, Karimi S et al (1995) Expression of IL-10, IL-4 and interferon-gamma in unstimulated and mitogenstimulated peripheral blood lymphocytes from HIV-seropositive patients. Clin Exp Immunol **102**, 31.

**Dick RS**, Hubbell HR (1987) Sensitivities of human glioma cell lines to interferons and double-stranded RNAs individually and in synergistic combinations. J Neurooncol **5**, 331.

**Diez RA**, Mistchenko AS, Falcoff ET (1987) Corticosteroids modulate the binding of recombinant interferons alpha and gamma in namalva cells. Immunopharmacol Immunotoxicol **9**, 115.

**Diezel W**, Washke SR, Sonnichsen N (1983) Detection of interferon in the sera of patients with psoriasis, and its enhancement by PUVA treatment. Br J Dermatol **109**, 549.

**Digel W**, Zahn G, Heinzel G, Porzsolt F (1991) Pharmacokinetics and biological activity in subcutaneous long-term administration of recombinant interferon-gamma in cancer patients. Cancer Immunol Immunother **34**, 169.

**Dighe AS**, Farrar MA, Schreiber RD (1993) Inhibition of cellular responsiveness to interferon-gamma induced by overexpression of inactive forms of of the IFN gamma receptor. J Biol Chem **268**, 10645.

**Dinauer MC**, Orkin SH, Brown R et al (1987) The glycoprotein encoded by the X-linked granulomatous disease locus is a component of the neutrophil cytochrome b complex. Nature **327**, 717.

**Dinauer MC**, Pierce EA, Bruns GA et al (1990) Human neutrophil cytochrome B light chain (P22-PHOX). Gene structure, chromosomal location, and mutations in cytochrome-negative autosomal recessive chronic granulomatous disease. J Clin Invest **86**, 1729.

**Ding AH**, Nathan CF (1987) Trace levels of bacterial polysaccharide prevent interferon-gamma or tumor necrosis factor-alpha from enhancing mouse peritoneal macrophage respiratory burst capacity. J Immunol **139**, 1971.

**Ding AH**, Nathan CF, Stur D (1988) Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritonel macrophages: comparison of activating cytokines and evidence for independent production. J Immunol **141**, 2407.

**Dinney CP**, Utsugi T, Fidler IJ et al (1992) Immunotherapy of murine renal adenocarcinoma by systemic administration of liposomes containing the synthetic macrophage activator CGP 31362 or CGP 19835A in combination with interleukin 2 or gamma-interferon. Cancer Res **52**, 1155.

**Di Piro** JT (1997) Cytokine networks with infection; mycobacterial infections, leishmaniasis, human immunodeficiency virus infection and sepsis. Pharmacotherapy **17**, 205.

**Dlugovitzky D**, Bay ML, Rateni L et al (1999) In vitro synthesis of interferon-gamma, interleukin-4, transforming growth factor-beta and interleukin-1 beta by peripheral blood mononuclear cells from tuberculosis patients: relationship with the severity of pulmonary involvement. Scand J Immunol **49**, 210.

**Doherty GM**, Alexander HR, Merino MJ et al (1996) Role of endogenous interferon gamma in murine tumor growth and tumor necrosis factor alpha antitumor efficacity. Ann Surg Oncol **3**, 198.

**Doi T**, Ando M, Akaike T et al (1993) Resistance to niric oxide in Mycobacterium avium complex and its implication in pathogenesis. Infect Immun **61**, 1980.

**Domke I**, Straub P, Jacobsen H et al (1985) Inhibition of replication of herpes simplex virus in mouse macrophages by interferons. J Gen Virol **66**, 2231.

**Donald PR**, Schoeman JF, Beyers N et al (1995) Concentration of interferon gamma, tumor necrosis factor alpha and interleukin-1 beta in the cerebrospinal fluid of children treated for tuberculous meningitis. Clin Infect Dis **21**, 924.

**Dong R**, Wang LI (1994) Inhibitory effect of interferon on expression of oncogene in over-proliferative smooth muscle cell. Chung Hua I Hsueh Tsa Chih **74**, 335.

**Dong-Ji Z**, Yang X, Shen C et al (2000) Priming with Chlamydia trachomatis major outer membrane protein (MOMP) DNA followed by MOMP ISCOM boosting enhances protection and is associated with increased immunoglobulin A and Th1 cellular immune responses. Infect Immun **68**, 3074.

**Douglas JM**, **Jr**, Eron, LJ, Judson, FN et al (1990) A randomized trial of combination therapy with intralesional interferon α2b and podophyllin versus podophyllin alone for the therapy of anogenital warts. J Infect Dis **162**, 52.

**Douglas MS**, Rix DA, Dark JH et al (1997) Examination of the mechanism by which heparin antagonizes activation of a model endothelium by interferon-gamma (IFN-gamma). Clin Exp Immunol **107**, 578.

**Douillard JY**, Mouren M, Fety R et al (1992) Comparative pharmacokinetics of recombinant human gamma interferon (rh-gamma IFN} after subcutaneous (sc) or intrapleural (ip) injection in malignant pleural effusion (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **33**, A3154.

**Drapier JC**, Wietzerbin J (1991) IFN-γ reduces specific binding of tumor necrosis factor on murine macrophages. J Immunol **146**, 1198.

**Dries DJ**, Walenga JM, Hoppensteadt D, Fareed J (1998) Molecular markers of hemostatic activation and inflammation following major injury: effect of therapy with IFN-gamma. J Interferon Cytokine Res **18**, 327.

**Dubinett SM**, Kurnick JT, Kradin RL (1989) Adoptive immunotherapy of murine pulmonary metastases with interleukin 2 and interferon-gamma. Am J Respir Cell Mol Biol **1**, 361.

**Du Bois RM (**1999) Interferon gamma-1b for the treatment of idiopathic pulmonary fibrosis. N Engl J Med **341**, 1302.

**Dubreuil P**, Courcoul M, Mannoni P (1988) Molecular events induced in the HL60 cell line following treatment with gamma-interferon. Cancer Detect Prev **12**, 15.

**Duff GW** (1993) Cytokines and anti-cytokines. Br J Rheumatol **32**(Suppl 1), 15.

**Dummer R**, Nestle E, Schafer E et al (1990) Influence of soluble interleukin-2 receptors and soluble CD8 proteins on natural killer cell activity and clinical course in cutaneous T-cell lymphoma patients. Arch Dermatol Res **8**, 557.

**Dummer R**, Muller W, Nestle F et al (1991). Formation of neutralizing antibodies against natural interferon-beta, but not against recombinant interferon-gamma during adjuvant therapy for high-risk malignant melanoma patients. Cancer **67**, 2300.

**Dummer R**, Sigg Zemann S, Kalthof K et al (1994) Various cytokines modulate ICAM-1 shedding on melanoma-and CTCL-derived cell lines:inverse regulation of ICAM-1 shedding in a Sezary cell line by interferon-gamma. Dermatology **189**, 120.

**Dumont P**, Nehme A, Cros S, Canal P (1995) Interaction between cisplatin and human recombinant interferon gamma (IFNgamma) in human ovarian cancer xenograft (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **36**, A1746.

**Duncan MR**, Berman B (1985) Gamma interferon is the lymphokine and beta interferon the monokine responsible for inhibition of fibroblast collagen production and late but not early fibroblast proliferation. J Exp Med **162**, 516.

**Duncan MR**, Berman B. (1987) Persistence of a reduced collagen production phenotype in cultured scleroderma fibroblasts after short-term exposure to interferon. J Clin Invest **79**, 1318.

**Du Plessis J**, Egbaria K, Ramachandran C, Weiner N (1992) Topical delivery of liposomally encapsulated gamma interferon. Antiviral Res **18**, 259.

**Dustin ML**, Singer KH, Tuck DT, Springer TA (1988) Adhesion of T lymphoblasts to epidermal keratinocytes is regulated by interferon gamma and is mediated by intercellular adhesion molecule 1 (ICAM-1). J Exp Med **167**, 1323.

## E

**Ealick SE**, Cook WJ, Vijay-Kumar S et al (1991). Three-dimensional structure of recombinant human interferon-γ. Science **252**, 698.

**Edwards L**, Whiting D, Rogers D et al (1990) The effect of intralesional interferon gamma on basal cell carcinomas. J Am Acad Dermatol **22**, 496.

**Edwards P**, Cendan JC, Topping DB et al (1996) Tumor cell nitric oxide inhibits cell growth in vitro, but stimulates tumorigenesis and experimental lung metastasis in vivo. J Surg Res **63**, 49.

**Efferth T**, Fabry U, Osieka R (1996) Anti-Fas/Apo1 monoclonal antibody CH-11 depletes glutathione and kills multidrug-resistant human leukemic cells. Blood Cells Mol Dis **22**, 2.

**Egbaria K**, Ramachandran C, Kittayanond D, Weiner N (1990) Topically delivery of liposomally encapsulated interferon evaluated by in vitro diffusion studies. Antimicrob Agents Chemother **34**, 107.

**Eggermont AM** (1993) Regional infusions and perfusions with cytokines (meeting abstract). Eur J Cancer **29**A(Suppl 6), 15.

**Eggermont AM**, Lienard D, Schraffordt-Koops H et al (1992) Limb salvage by high-dose tumor necrosis factor-alpha (TNF), gamma-interferon (IFN) and melphalan isolated limb perfusion (ILP) in patients with unresectable soft tissue sarcomas (meeting abstract). Proc Annu Meet Am Soc Clin Oncol **11**, A 1444.

**Eggermont AM**, Schraffordt Koops H, Lienard D et al (1996) Isolated limb perfusion with high-dose tumor necrosis factor-alpha in combination with interferon-gamma and melphalan for nonresectable extremity soft tissue sarcomas: a multicenter trial. J Clin Oncol **14**, 2653.

**Egorova ON**, Balabanova RM (1994) The use of interferon in treating systemic lupus erythematosus. Ter Arkh **66**, 45.

**Eibl B**, Schwaighofer H, Nachbaur D et al (1996); Evidence for a graft-versus-tumor effect in a patient treated with marrow ablative chemotherapy and allogeneic bone marrow transplantation for breast cancer. Blood **88**, 1501.

**Einat M**, Resnitzky D, Kimchi A (1985) Close link between reduction of c-myc expression by interferon and $G_0/G_1$ arrest. Nature **313**, 597.

**Einhorn S**, Grander D (1996) Why do so many cancer patients fail to respond to interferon therapy? J Interferon Cytokine Res **16**, 275.

**Einhorn S**, Fernberg JO, Grander D, Lewenohn R (1988) Interferon exerts a cytotoxic effect on primary human myeloma cells. Eur J Cancer Clin Oncol **24**, 1505.

**Eisenberg BL**, Taylor DD, Weese JL (1991) Aerosolized gamma-interferon and lipopolysaccharide enhances cytotoxicity of murine pulmonary alveolar macrophages. J Immunother **10**, 51.

**Eisenthal A**, Marder O, Maymon B et al (1998) The effect of interferon-gamma and tumor necrosis factor-alpha on the expression of ICAM-1 and HLA-DR molecules on cells of a human germ cell neoplasm and their susceptibility to lysis by lymphokine-activated killer cells. Pathobiology **66**, 205.

**Elder JT**, Klein SB, Tavakkol A et al (1990) Growth factor and proto-oncogene expression in psoriasis. J Invest Dermatol. **95**, 7S.

**Elias JA**, Freundlich B, Adams S, Rosenbloom J (1990) Regulation of human lung fibroblast collagen production by recombinant inteleukin-1, tumor necrosis factor, and interferon gamma. Ann NY Acad Sci **580**, 233.

**Ellerhorst J**, Jones E, Kilbourn R et al (1992) Fixed low-dose gamma-interferon (IFN) is active against metastatic renal cell carcinoma (RCC) (meeting abstract). Proc Annu Meet Am Soc Clin Oncol **11**, A693.

**Ellerhorst JA**, Kilbourn RG, Amato RJ et al (1994) Phase II trial of low dose gamma-interferon in metastatic renal cell carcinoma. J Urol **152**, 841.

**Ellis CN**, Stevens SR, Blok BK et al (1999) Interferon-gamma therapy reduces blood leukocyte levels in patients with atopic dermatitis: correlation with clinical improvement. Clin Immunol **92**, 49.

**ElMasry M**, Rich R (1989) Prostaglandin $E_2$ selectively increases interferon gamma receptor expression on human $CD8^+$ lymphocytes. J Clin Invest **83**, 1436.

**Elsasser D**, Stadick H, Stark S et al (1999) Preclinical studies combining bispecific antibodies with cytokine-stimulated effector cells for immunotherapy of renal cell carcinoma. Anticancer Res **19**, 1525.

**Elsasser-Beile U**, Kolble N, Grussenmeyer T et al (1998a) Th1 and Th2 cytokine response patterns in leukocyte cultures of patients with bladder, renal cell and prostate carcinomas. Tumour Biol **19**, 470.

**Elsasser-Beile U**, Kolble N, Grussenmeyer T et al (1998b) Correlation of clinical and immunological parameters of metastatic renal cell carcinoma patients undergoing therapy with interleukin 2, interferon-alpha and retinoic acid. Anticancer Res **18**, 1883.

**Elsasser-Beile U**, von Kleist S, Fischer R, Monting JS (1992) Impaired cytokine production in whole blood cell cultures from patients with colorectal careronrcinomas as compared to benign colorectal tumors and controls. J Clin Lab Anal **6**, 311.

**Emami S**, Chastre E, Nizard S et al (1990) Immortalization and neoplastic transformation of fetal rat intestinal epithelial cells: morphological and cytogenetic analysis, (proto)oncogene expression and effect of gamma-interferon on cell growth. Digestion **46** Suppl 2, 74.

**Empson M**, Bishop GA, Nightingale B, Garsia R (1999) Atopy, anergic status, and cytokine expression in HIV-infected subjects. Allergy Clin Immunol **103**, 833.

**Enk AH**, Jonuleit H, Saloga J, Knop J (1997) Dendritic cells as mediators of tumor-induced tolerance in metastatic melanoma. Int J Cancer **73**, 309.

**Ennen J**, Kurth R (1993) Interferon-γ-activated monocytes impair infectivity of HIV particles by an oxygen metabolite–dependent reaction. Immunology **78**, 171.

**Ennis FA**, Meager A (1981) Immune interferon produced to high levels by antigen stimulation of human lymphocytes with influenza virus. J Exp Med. **154**, 1279.

**Epstein LB** (1982) Interferon-γ: success structure and speculation. Nature **295**, 453.

**Epstein SE**, Speir E, Zhou YF et al (1996) The role of infection in restenosis and atherosclerosis: focus on cytomegalovirus. Lancet **348**(Suppl 1), 13.

**Ernstoff MS**, Nair S, Bahnson R, Gooding W et al (1990) Sequentially combined recombinant interferon (RIFN) gamma and alpha in patients with renal cell carcinoma (RCC) (meeting abstract). Proc Annu Meet Am Soc Clin Oncol **9**, A594.

**Ernstoff MS**, Gooding W, Nair S et al (1992) Immunological effects of treatment with sequential administration of recombinant interferon gamma and alpha in patients with metastatic renal cell carcinoma during a phase I trial. Cancer Res **52**, 851.

**Eron LJ**, Harvey L., Toy C, Santomauro D (1986) Interferon in the prevention of genital herpes recurrence. Antimicrob Agents Chemother **30**, 608.

**Eron LJ**, Toy C, Salsitz B et al (1987) Therapy of genital herpes with topically applied interferon. Antimicrob Agents Chemother **31**, 1137.

**Escudier B**, Farace F, Angevin E et al (1993). Combination of interleukin-2 and gamma interferon in metastatic renal cell carcinoma. Eur J Cancer Part A Gen Top **29**, 724.

**Estauier J**, Idziorek T, Zou W et al (1995) T helper type1/T helper type 2 cytokines and T cell death: preventive effect of interleukin 12 on activation-induced and CD 95 (FAS/APO-1)–mediated apoptosis of CD4+T cells from human immunodeficiency virus–infected persons. J Exp Med **182**, 1759.

**Esteller M**, Garcia A, Martinez-I-Palones JM et al (1995) Detection of c-erbB-2/neu and fibroblast growth factor-3/INT-2 but not epidermal growth factor receptor gene amplification in endometrial cancer by differential polymerase chain reaction. Cancer **75**, 2139.

**Evans A**, Main E, Zier K et al (1989) The effects of gamma interferon on the natural killer and tumor cells of children with neuroblastoma. A preliminary report. Cancer **64**, 1383.

**Evans CH**, Baker PD (1996) Differential modulation of cervical carcinoma cell adhesion molecule expression by interferon-gamma, leukoregulin and tumor necrosis factor-alpha (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **37**, A3063

**Evans CH**, Flugelman AA, DiPaolo JA (1993) Cytokine modulation of immune defenses in cervical cancer. Oncology **50**, 245.

**Evans TG**, Fitzgerald T, Gibbons DC et al (1998) Th1/Th2 cytokine responses following HIV-1 immunization in seronegative volunteers. Clin Exp Immunol **111**, 243.

**Everlien H**, Hockertz S (1999) Combined lyposomal immuno- and chemotherapy of visceral leishmaniasis. Arzneimittelforschung **49**, 954.

**Eymard JC**, Lopez M, Cattan A, Bouche O et al (1996) Phase I/II trial of autologous activated macrophages in advanced colorectal cancer. Eur J Cancer **32A**, 1905.

**Ezekowitz** et al (1991) A controlled trial of interferon gamma to prevent infection in chronic granulomatous disease. N Engl J Med **324**, 509.

**F**

**Faggioli L**, Merola M, Hiscott J et al (1997) Molecular mechanisms regulating induction of interleukin-6 gene transcription by interferon-gamma. Eur J Immunol **27**, 3022.

**Fakoya A**, Matear PM, Filley E et al (1997) HIV infection alters the production of both type 1 and 2 cytokines but does not induce a polarized type 1 or 2 state. AIDS **11**, 1445.

**Faltynek CR**, McCandless S, Chebath J, Baglioni C. (1985) Different mechanisms for activation of gene transcription by interferons α and γ. Virology **144**, 173.

**Fan J**, Li P, Kok TW et al (1997) AZT blocks down-regulation of IL-2 and IFN-gamma gene expression in HIV acutely infected cells. Arch Virol **142**, 1035.

**Fan S**, Fehr HG, Adams D (1991) Activation of macrophages for ADCC in vitro: Effects of IL-4, THF, interferons-alpha/beta, interferon-gamma and GM-CSF. Cell Immunol **135**, 78.

**Fan XD**, Goldberg M, Bloom BR (1988) Interferon-γ–induced transcriptional activation is mediated by protein kinase C. Proc Natl Acad Sci USA **85**, 5122

**Fantuzzi L**, Gessani S, Borghi P et al (1996) Induction of interleukin-12 (IL-12) by recombinant glycoprotein gp 120 of human immunodeficiency virus type 1 in human monocytes/macrophages; requirement of gamma interferon for IL-12 secretion. J Virol **70**, 4121.

**Farace F**, Pallardy M, Angevin E et al (1994) Metastatic renal-cell carcinoma patients treated with interleukin 2 or interleukin 2 plus interferon-gamma: Immunological monitoring. Int J Cancer **57**, 814.

**Farkkila M**, Iivanainen M, Harkonen M et al (1988) Effect of interferon-γ on biogenic amine metabolism, electroencephalographic recordings, and transient potentials. Clin Neuropharmacol **11**, 63.

**Farrar MA**, Schreiber RD (1993) The molecular cell biology of interferon γ and its receptor. Annu Rev Immunol **11**, 571.

**Farrar MA**, Fernandez-Luna J, Schreiber RD (1991) Identification of two regions within the cytoplasmic domain of the human interferon-gamma receptor required for function. J Biol Chem **266**, 19626.

**Farrar MA**, Campbell JD, Schreiber RD (1992) Identification of a functionally important sequence in the C terminus of the interferon-gamma receptor. Proc Natl Acad Sci USA **89**, 11706.

**Fassio A**, Gariglio M, Cofano F et al (1988) Functional characterization of murine cell lines expressing high intermediate or negative levels of surface receptors for interferon-gamma. J Interferon Res **8**, 333.

**Fathallah-Shaykh HM**, Zhao LJ et al (2000) Gene transfer of IFN-gamma into established brain tumors represses growth by antiangiogenesis. J Immunol **164**, 217.

**Fattal-German M**, Ladurie FL, Cerrina J et al (1996). Modulation of ICAM-1 expression in human alveolar macrophages in vitro. Eur Respir J **9**, 463.

**Favre C**, Wijdenes J, Cabrillat H et al (1989) Epitope mapping of recombinant human gamma interferon using monoclonal antibodies. Mol Immunol **26**, 17.

**Favrot M**, Goillot E, Combaret V et al (1991) Growth-promoting activity of TNF on neuroblastoma (NB) cell lines; modulation by insulin and interferon gamma (IFN gamma) (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **32**, A1347.

**Feelders RA**, Vreugdenhil G, Eggermont AM et al (1998) Regulation of iron metabolism in the acute phase response: interferon gamma and tumour necrosis factor alpha induce hypoferraemia, ferritin production and a decrease in circulating transferrin receptors in cancer patients. Eur J Clin Invest **28**, 520.

**Fell AH**, Currier J, Good MF (1994) Inhibition of Plasmodium falciparum erythrocytic growth in vitro by CD4+ and CD8+ T cells from non-exposed donors. Parasite Immunol **16**, 579.

**Fellenberg J**, Mau H, Scheuerpflug C et al (1997). Modulation of resistance to anti–APO-1–induced apoptosis in osteosarcoma cells by cytokines. Int J Cancer **72**, 536.

**Feng CG**, Bean AG, Hooi H et al (1999) Increase in gamma interferon-secreting CD8(+), as well as CD4(+), T cells in lungs following aerosol infection with Mycobacterium tuberculosis. Infect Immun **67**, 3242.

**Feng GS**, Dai W, Gupta SL et al (1991) Analysis of interferon-gamma resistant mutants that are possibly defective in their signaling mechanism. Mol Gen Genet **230**, 91.

**Feng HM**, Walker DH (1993) Interferon-gamma and tumor necrosis factor-alpha exert their antirickettsial effect via induction of synthesis of nitric oxide. Am J Pathol **143**, 1016.

**Ferm M**, Gronberg A, Tally M (1996) IFN-gamma treatment increases insulin binding and MHC class I expression in erythroleukemia cells. Immunol Invest **25**, 37.

**Ferreira A**, Schofield L, Enea V et al (1986) Inhibition of development of exoerythrocytic forms of malaria parasites by γ-interferon. Science **232**, 881.

**Ferrero D**, Carlesso N, Pregno P et al (1992) Self-renewal inhibition of acute myeloid leukemia clonogenic cells by biological inducers of differentiation. Leukemia **6**, 100.

**Ferry B**, Antrobus P, Huzicka I et al (1997) Intracellular cytokine expression in whole blood preparations from normals and patients with atopic dermatitis. Clin Exp Immunol **110**, 410.

**Fidler IJ** (1990) Systemic activation of macrophages by liposomes containing muramyltripeptide phosphatidylethanolamine for therapy of cancer metastasis. J Liposome Res **1**, 451.

**Fidler IJ**, Fogler WE, Kleinerman ES, Saiki I (1985) Abrogation of species specificity for activation of tumoricidal properties in macrophages by recombinant mouse or human interferon-gamma encapsulated in liposomes. J Immunol **135**, 4289.

**Fidler IJ**, Fan D, Ichinose Y (1989) Potent in situ activation of murine lung macrophages and therapy of melanoma metastases by systemic administration of liposomes containing muramyltripeptide, phosphatidylethanolamine and interferon gamma. Invas Metast **9**, 75.

**Fiedler W**, Zeller W, Peimann CJ et al (1991) A phase II combination trial with recombinant human tumor necrosis factor and gamma interferon in patients with colorectal cancer. Klin Wochenschr **69**, 261.

**Fierlbeck G**, Rassner G (1988) Rekombinantes interferon-gamma bei psoriasis arthropathica, progressiv systemischer sklerodermie und morbus Behcet. Medizin Klinik **83**, 659.

**Fierlbeck G**, Rassner G (1990) Treatment of psoriasis and psoriatic arthritis with interferon gamma. J Invest Dermatol **95**, 138.

**Figdor CG**, van Kooyk Y, Keizer GD (1990) On the mode of action LFA-1. Immunol Today **11**, 277.

**Filonzi EL**, Zoellner H, Stanton H, Hamilton JA (1993) Cytokine regulation of granulocyte-macrophage colony stimulating factor and macrophage colony-stimulating factor production in human arterial smooth muscle cells. Atherosclerosis **99**, 241.

**Finbloom DS** (1990) The interferon γ receptor of human monocytes, monocyte-like cell lines and polymorphonuclear leukocytes. (Phagocyte cell surface) Biochem Soc Trans 222.

**Finbloom DS** (1991) Regulation of cell-surface receptors for human interferon-gamma on the human histiocytoc lymphoma cell line U937. Biochem J **274**, 775.

**Fine RL**, Roberson KM, Chang GC, Robertson CN (1994) Fenretinide and gamma interferon display synergistic cytotoxicity against hormone insensitive prostate cancer cells (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **35**, A1644.

**Fiorelli V**, Gendelman R, Sirianni MC et al (1998) Gamma-interferon produced by CD8+ T cells infiltrating Kaposi's sarcoma induces spindle cells with angiogenic phenotype and synergy with human immunodeficiency virus-1 Tat protein: an immune response to human herpesvirus-8 infection? Blood **91**, 956.

**Fiorelli V**, Barillari G, Toschi E et al (1999) IFN-gamma induces endothelial cells to proliferate and to invade the extracellular matrix in response to the HIV-1 Tat protein: Implications for AIDS-Kaposi`s sarcoma pathogenesis. J Immunol **162**, 1165.

**Firestein GS**, Zvaifler NJ (1987) Peripheral blood and synovial fluid monocyte activation in inflammatory arthritis II. Low levels of synovial fluid and synovial tissue interferon suggest that gamma-interferon is not the primary macrophage activating factor. Arthritis Rheum **30**, 864.

**Fischer JR**, Schindel M, Stein N et al (1995) Selective suppression of cytokine secretion patients with small-cell lung cancer. Ann Oncol **6**, 921.

**Fischer T**, Thoma B, Scheurich P et al (1990) Glycosylation of the human interferon-γ receptor. N-linked carbohydrates contribute to structural heterogeneity and are required for ligand binding. J Biol Chem **265**, 1710.

**Fisher PB**, Grant S (1985) Effects of interferon on differentiation of normal and tumor cells. Pharmacol Ther **27**, 143

**Fisk B**, Ioannides CG, Aggarwal S et al (1994) Enhanced expression of HLA-A,B,C and inducibility of TAP-1, TAP-2 and HLA-A,B,C by interferon-gamma in a multidrug-resistant small cell lung cancer line. Lymphokine Cytokine Res **13**, 125.

**Fleischmann WR**, Newton RC, Fleischmann CM et al (1984) Discrimination between nonmalignant and malignant cells by combinations of IFN-γ and IFN-α/β. J Biol Response Modif **3**, 397.

**Fleischmann WR**, Fields EE, Wang JL et al (1991) Modulation of peripheral leukocyte counts in mice by oral administration of interferons. Proc Soc Exp Biol Med **197**, 424.

**Fleischmann WR Jr**, Fleischmann CM (1994) Enhancement of MulFN-gamma antitumor effects by hyperthermia: Sequence dependence and time dependence of hyperthermia. J Biol Regul Homeostatic Agents **8**, 101.

**Flemming CL**, Patel PM, Box G et al (1997) Sarcoma cells engineered to secrete IFN-gamma or IL-2 acquire sensitisation to immune cell killing via different mechanisms. Cytokine **9**, 328.

**Flesch IE**, Hess JH, Huang S et al (1995) Early interleukin 12 production by macrophages in response to mycobacterial infection depends on interferon gamma and tumor necrosis factor alpha. J Exp Med **181**, 5615.

**Fluckiger AC**, Durand I, Banchereau J (1994) Interleukin 10 induces apoptotic cell death of B-chronic lymphocytic leukemia cells. J Exp Med **179**, 91.

**Flynn JL**, Chan J, Triebold K.J. et al (1993) An essential role for interferon gamma in resistance to Mycobacterium tuberculosis infection. J Exp Med **178**, 2249.

**Foon KA** (1989) Biological response modifiers: the new immunothrtapy. Cancer Res **49**, 1621.

**Forbes A**, Johnson PJ, Williams R (1985). Recombinant human gamma-interferon in primary hepatocellular carcinoma. J R Soc Med **78**, 826.

**Foster GR**, Ackrill AM, Goldin RD et al (1991) Expression of the terminal protein region of hepatitis B virus inhibits cellular responses to interferons alpha and gamma and double-stranded RNA [erratum in Proc Natl Acad Sci USA 1995, **92**, 3632]. Proc Natl Acad Sci USA **88**, 2888.

**Fountoulakis M**, Lahm HW, Maris A et al (1991) A 25-kD stretch of the extracellular domain of the human interferon-γ receptor is required for full ligand binding capacity. J Biol Chem **266**, 14970.

**Fountoulakis M**, Zulauf M, Lustig A, Garotta G (1992) Stoichiometry of interaction between interferon γ and its receptor. J Biochem **208**, 781.

**Fountoulakis M**, Juranville JF, Stuber D et al (1990b) Purification and biochemical characterization of a soluble human interferon γ receptor expressed in E. coli. J Biol Chem **265**, 13268.

**Fountoulakis M**, Kania M, Ozmen L et al (1989) Structure and membrane topology of the high-affinity receptor for human IFNγ: requirement for binding IFNγ. J Immunol **143**, 3266.

**Fountoulakis M**, Juranville JF, Maris A et al (1990a) One interferon gamma receptor binds one interferon gamma dimer. J Biol Chem **265**, 19758.

**Fraker DL**, Alexander HR, Andrich M, Rosenberg SA (1996) Treatment of patients with melanoma of the extremity using hyperthermic isolated limb perfusion with melphalan, tumor necrosis factor, and interferon gamma: results of a tumor necrosis factor dose-escalation study. J Clin Oncol **14**, 479.

**Fransson J**, Emilson A, Scheynius A, Hammar H (1995) Proliferation and interferon-gamma receptor expression in psoriatic and healthy keratinocytes are influenced by interactions between keratinocytes and fibroblasts in a skin equivalent model. Arch Dermatol Res **287**, 517.

**Freund M**, Heussner P, Hild F et al (1996) Treatment of chronic myelogenous leukemia with interferon alpha. A decade of experience. Medizin Klinik **91**(Suppl 3), 18.

**Friedman-Kien AE**, Klein RJ, Glaser RD, Cselusniak SM (1986) Treatment of recurrent genital herpes with topical alpha interferon gel combined with nonoxynol-9. J Am Acad Dermatol **15**, 989.

**Friese MA**, Hellwage J, Jokiranta TS, Meri S et al (2000) Different regulation of factor H and FHL-1/reconectin by inflammatory mediators and expression of the two proteins in rheumatoid arthritis. Clin Exp Immunol **121**, 406.

**Fruh K**, Ahn K, Djaballah H et al (1995) A viral inhibitor of peptide transporters for antigen presentation. Nature **375**, 415.

**Fu X**, Chang G, Wang Y(1995) Systemic and local administration of interferon-gamma impairs wound healing. Chung Hua Cheng Hsing Shao Shang Wai Ko Tsa Chih **11**, 209.

**Fu YK**, Arkins S, Li YM et al (1994) Reduction in superoxide anion secretion and bactericidal activity of neutrophils from aged rats: reversal by the combination of gamma interferon and growth hormone. Infect Immun **62**, 1.

**Fuith LC**, Marth C, Holzner M et al (1991) Enhancement of CA 125 expression by interferon-gamma in ovarian carcinoma xenografts. J Tumor Marker Oncol **6**, 85.

**Fujii A**, Yui-En K, Ono Y et al (1999) Preliminary results of the alternating administration of natural interferon-alpha and recombinant interferon-gamma for metastatic renal cell carcinoma. Br J Urol Int **84**, 399.

**Fujisaki T**, Nagafuchi S, Okamura T (1993) Gamma-interferon for severe chronic active Epstein-Barr virus. Ann Intern Med **118**, 474.

**Fujishima H**, Saito I, Takeuchi T et al (1997) Characterization of cytokine mRNA transcripts in conjunctival cells in patients with allergic conjunctivitis. Invest Ophthalmol Vis Sci **38**, 1350.

**Fujiwara H**, Hamaoka T (1996) Antitumor and antimetastatic effects of interleukin 12. Cancer Chemother Pharmacol Suppl **38**, S22.

**Fukusato T**, Gerber MA, Thung SN et al (1986) Expression of HLA class I antigens on hepatocytes in liver disease. Am J Pathol **123**, 264.

**Funauchi M**, Minoda M, Horiuchi A (1991a) Relationship between serum interleukin-4 or interferon-gamma level and B cell abnormality in patients with collagen vascular diseases. Ryumachi **31**, 493.

**Funauchi M**, Sugishima H, Minoda M, Horiuchi A (1991b) Effect of interferon-gamma on B lymphocytes of patients with systemic lupus erythematosus. J Rheumatol **18**, 368.

**Furue H**, Uroshizaki I, Wakui A et al (1987) Phase I study of natural interferon-gamma. Jpn J Cancer Chemother **14**, 2537.

**Furukawa A**, Miyamoto T, Aki M et al (1996) Induction of expressions of HLA class I and class II antigens by interferon on human renal cell carcinoma or bladder cancer cell lines. Shikoku Acta Med **52**, 25.

## G

**Gabrielsson S**, Paulie S, Rak S et al (1997) Specific induction of interleukin-4-producing cells in response to in vitro allergen stimulation in atopic individuals. Clin Exp Allergy **27**, 808.

**Galli G**, Annunziato F, Mavilia C et al (1998) Enhanced HIV expression during Th2-oriented responses explained by the opposite regulatory effect of IL-4 and IFN-gamma of fusin/CXCR4. Eur J Immunol **28**, 3280.

**Galligioni E**, Santarosa M, Favaro D et al (1994) In vitro synergic effect of interferon gamma combined with liposomes containing muramyl tripeptide on human monocyte cytotoxicity against fresh allogeneic and autologous tumor cells. Tumori **80**, 385.

**Gallin JI**, Malech HL, Melnick DA et al (1991) A controlled trial of interferon gamma to prevent infection in chronic granulomatous disease. N Engl J Med **324**, 509.

**Gallin JI**, Arber JM, Holland SM, Nutman TV (1995) Interferon-gamma in the management of infectious diseases. Ann Intern Med **123**, 216

**Garbe C**, Krasagakis K (1993) Effects of interferons and cytokines on melanoma cells. J Invest Dermatol **100**, 239.

**Garbe C**, Krasagakis K, Zouboulis CC et al (1990) Antitumor activities of interferon alpha, beta, and gamma and their combinations on human melanoma cells in vitro: Changes of proliferation, melanin synthesis, and immunophenotype. J Invest Dermatol **95**, 231.

**Garnick MB**, Reich SD, Maxwell B et al (1988) Phase I/II study of recombinant interferon gamma in advanced renal cell carcinoma. J Urol **139**, 251.

**Garotta G**, Ozmen L, Fountoulakis M et al (1990) Human interferon-γ receptor. Mapping of epitopes recognized by neutralizing antibodies using native and recombinant receptor proteins. J Biol Chem **265**, 6908.

**Gaspar ML**, Alvarez Mon M, Gutierrez C (1988) Role of interleukin 2 in inducing normalization of natural killer activity in systemic lupus erythematosus. Clin Immunol Immunopathol **49**, 204.

**Gastl G**, Ebert T, Finstad CL et al (1996) Major histocompatibility complex class I and class II expression in renal cell carcinoma and modulation by interferon gamma. J Urol **155**, 361.

**Gastl G**, Huber C (1988) The biology of interferon actions. Blut **56**, 193.

**Gastl G**, Marth C, Leiter E et al (1985) Effects of human recombinant α2 arg-interferon and γ-interferon on human breast cancer cell lines: Dissociation of antiproliferative activity and induction of HLA-DR antigen expression. Cancer Res **45**, 2957.

**Gatanaga T**, Hwang C, Kohr W et al (1990) Purification and characterization of an inhibitor (soluble tumor necrosis factor receptor) fot tumor necrosis factor and lymphotoxin obtained from the serum ultrafiltrates of human cancer patients. Proc Natl Acad Sci USA **87**, 8781

**Gately MK**, Mulqueen MJ (1996) Interleukin-12; potential clinical applications in the treatment and prevention of infectious diseases. Drugs Suppl **2**, 18.

**Geary SM**, Ashman LK (1996) HL-60 myeloid leukemia cells acquire immunostimulatory capability upon treatment with retinoic acid: Analysis of the responding population and mechanism of cytotoxic lymphocyte activation. Immunology **88**, 428.

**Geertsma MF**, Nibbering PH, Pos O, Van Furth R (1990) Interferon-gamma–activated human granulocytes kill ingested Mycobacterium fortuitum more efficiently than normal granulocytes. Eur J Immunol **20**, 869.

**Gehrz RC** (1992) The role of interferon in Cytomegalovirus infection. In: Antiinfective application of interferon gamma (Jaffe HS, Bucalo LR, Sherwin SA, eds). Marcel Dekker, New York, p 201.

**Geiger KD**, Gurushanthaiah D, Howes EL et al (1995a) Cytokine-mediated survival from lethal herpes simplex virus infection: role of programmed neuronal death. Proc Natl Acad Sci USA **92**, 3411.

**Geiger KD**, Lee MS, Baugh C, Sarvetnick NE (1995b) Protective effects of interferon-gamma in intraocular herpes simplex type 1 infection do not depend on major histocompatibility complex class I or class II expression. J Neurovirol **1**, 405.

**Geiger KD**, Nash TC, Sawyer S et al (1997) Interferon-gamma protects against herpes simplex virus type 1–mediated neuronal death. Virology **238**, 189.

**Geissler K**, Tricot G, Leemhuis T et al (1989) Differentiation-inducing effect of recombinant human tumor necrosis factor alpha and gamma-interferon in vitro on blast cells from patients with acute myeloid leukemia and myeloid blast crisis of chronic myeloid leukemia. Cancer Res **49**, 3057.

**Geng YJ**, Hellstrand K, Wennmalm A, Hansson GK (1996) Apoptotic death of human leukemic cells induced by vascular cells expressing nitric oxide synthase in response to gamma-interferon and tumor necrosis factor-alpha. Cancer Res **56**, 866.

**Geras-Raaka E**, Varma A, Ho H et al (1998) Human interferon-gamma–inducible protein 10 (IP-10) inhibits constitutive signaling of Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor. J Exp Med **188**, 405.

**Gergert VIa**, Kosmiadi, GA and Abramova, ZP (1995) Cytokine in immunopathogenesis of pulmonal tuberculosis. Probl Tuberk **2**, 32.

**Gerrard TL**, Dyer DR, Enterline JC, Zoon KC (1989) Products of stimulated monocytes enhance the activity of interferon-γ. J Interferon Res **9**, 115.

**Ghadirian E**, Salimi A (1993) In vitro effect of recombinant interferon gamma in combination with LPS on amoebocidal activity of murine Kupffer cells. Immunobiology **188**, 203.

**Ghosh P**, Komschlies KL, Cippitelli M et al (1995) Gradual loss of T-helper 1 populations in spleen of mice during progressive tumor growth. J Natl Cancer Inst **87**, 1478.

**Ghyka G**, Alecu M, Calugaru A, Coman G (1989) Interferon efficiency in the treatment of herpetic dermatites. II. Comparison between human leukocyte interferon (Ginterferon) and a recombinant interferon (Roferon). Med Interne **27**, 229.

**Giacomini P**, Aguzzi A, Ferrone S (1986) Differential susceptibility to modulation by recombinant immune interferon of HLA-DR and -DQ antigens synthesized by melanoma COLO 38 cells. Hybridoma **5**, 277.

**Giacomini P**, Gambari R, Barbieri R et al (1990) Recombinant immune interferon down-regulates Ha-ras-1 proto-oncogene products in a human melanoma cell line. Anticancer Res **10**, 629.

**Gianni M**, Zanotta S, Terao M, et al (1996) Interferons induce normal and aberrant retinoic-acid receptors type alpha in acute promyelocytic leukemia cells: Potentiation of the induction of retinoid-dependent differentiation markers. Int J Cancer **68**, 75.

**Giavazzi R**, Nicoletti MJ, Chirivi RGS et al (1994) Soluble intercellular adhesion molecule-1 (ICAM-1) is released into the serum and ascites of human ovarian carcinoma patients and in nude mice bearing tumor xenografts. Eur J Cancer Part A Gen Top **30**, 1865.

**Giese NA**, Gabriele L, Doherty TM et al (1997) Interferon (IFN) consensus sequence-binding protein, a transcription factor of the IFN regulatory factor family, regulates immune responses in vivo through control of interleukin 12 expression. J Exp Med **186**, 1535.

**Gillery P**, Serpier H, Polette M et al (1992). Gamma interferon inhibits extracellular matrix synthesis and remodeling in collagen lattice cultures of normal and scleroderma skin fibroblasts. Eur J Cell Biol **57**, 244.

**Giovarelli M**, Cofano F, Vecchi A et al (1986) Interferon-activated tumor inhibition in vivo. Small amounts of interferon-gamma inhibit tumor growth by eliciting host systemic immunoreactivity. Int J Cancer **37**, 141.

**Giovarelli M**, Musiani P, Modesti A et al (1995) Local release of IL-10 by transfected mouse mammary adenocarcinoma cells does not suppress but enhances antitumor reaction and elicits a strong cytotoxic lymphocyte and antibody-dependent immune memory. J Immunol **155**, 3112.

**Giri SN**, Hyde DM, Marafino BJ (1986) Ameliorating effect of murine interferon-gamma on Bleomycin-induced lung collagen fibrosis in mice. Biochem Med Metabol Biol **36**, 194.

**Gleave ME**, Elhilali M, Fradet Y, Davis I et al (1998) Interferon gamma-1b compared with placebo in metastatic renal-cell carcinoma. Canadian Urologic Oncology Group. N Engl J Med **338**, 1265.

**Glezerman M**, Cohen V, Movshovitz M et al (1988) Plazebo-kontrollierte Studie uber die topische Interferon-Anwendung bei Herpes labialis und genitalis. Lancet (Deuttsche Ausgabe) **2**, 293.

**Gluszko P**, Undas A, Kwasny B et al. (1998) Treatment with interferon gamma enhances fibrinolysis in systemic sclerosis. Thromb Res **91**, 303.

**Goebel A**, Kavanagh E, Lyons A et al (2000) Injury induces deficient interleukin-12 production, but interleukin-12 therapy after injury restores resistance to infection. Ann Surg **231**, 253.

**Gohji K**, Fidler IJ, Tsan R et al (1994). Human recombinant interferons-beta and gamma decrease gelatinase production and invasion by human kg-2 renal-carcinoma cells. Int J Cancer **58**, 380.

**Gohji K**, Nomi M, Hara I et al (1998) Influence of cytokines and growth factors on matrix metalloproteinase-2 production and invasion of human renal cancer. Urol Res **26**, 33.

**Goldbach P**, Dumont S, Kessler R et al (1996) In situ activation of mouse alveolar macrophages by aerosolized liposomal IFN-gamma and muramyl tripeptide. Am J Physiol **270**, 429.

**Goldman MH**, James DC, Ison AP, Bull AT (1997) Monitoring proteolysis of recombinant human interferon-gamma during batch culture of Chinese hamster ovary cells. Cytotechnology **23**, 103.

**Goldring MB**, Fukuo K, Birkhead JR et al (1994) Transcriptional suppression by interleukin-1 and interferon-gamma of type II collagen gene expression in human chondrocytes. J Cell Biochem **54**, 85.

**Goldstein D**, Bushmeyer SM, Witt PL et al (1989) Effects of type I and II interferons on cultured human breast cells: Interaction with estrogen receptors and with tamoxifen. Cancer Res **49**, 2698.

**Gomes MS**, Florido M, Pais TF, Appelberg R (1999) Improved clearance of Mycobacterium avium upon disruption of the inducible nitric oxide synthase gene. J Immunol **162**, 6734.

**Gomi K**, Morimoto M, Nakamizo N (1985) Characteristics of antiviral and anticellular activities of human recombinant interferon-gamma. Jpn J Cancer Res **76**, 224.

**Gomi K**, Kimura H, Okabe M et al (1986) Synergistic anticellular and antiviral activities of human recombinant interferon-γ and -β. J Pharmacobiol Dyn **9**, 871.

**Gomi T**, Shiohara T, MunakataT et al (1991) Interleukin 1 alpha, tumor necrosis factor alpha and interferon gamma in psoriasis [comments]. Arch Dermatol **127**, 827.

**Gooch JL**, Herrera RE, Yee D (2000) The role of p21 in interferon gamma-mediated growth inhibition of human breast cancer cells. Cell Growth Differ **11**, 335.

**Gottlieb DJ**, Prentice HG, Mehta AB et al (1990) Malignant plasma cells are sensitive to LAK cell lysis: Preclinical and clinical studies of interleukin 2 in the treatment of multiple myeloma. Br J Haematol **75**, 499.

**Gowen M**, Nedwin GE, Mundy GR (1986) Preferential inhibition of cytokine-stimulated bone resorption by recombinant interferon-gamma. J Bone Mineral Res **1**, 469.

**Gracie JA**, Forsey RJ, Chan WL et al (1999) A proinflammatory role for IL-18 in rheumatoid arthritis. J Clin Invest **104**, 1393.

**Graham GM**, Guarini L, Moulton TA et al (1991). Potentiation of growth suppression and modulation of the antigenic phenotype in human melanoma cells by the combination of recombinant human fibroblast and immune interferons. Cancel Immunol Immunother **32**, 382.

**Graham SD Jr**. (1994) Immunotherapy of renal cell carcinoma. Prob Urol **8**, 575.

**Grander D**, Xu B, Wang P, Einhorn S (1994) Gamma IFN induced cell adhesion in chronic myelogenous leukemia cells. Leukemia **8**, 299.

**Granstein RD**, Flotte TJ, Amento EP (1990) Interferons and collagen production. J Invest Dermatol **95**, 6 Suppl., 75.

**Grassegger A**, Rollinger-Holzinger I, Zelger BW et al (1997) Spontaneous or interferon-gamma–induced T-cell infiltration, HLA-DR and ICAM-1 expression in genitoanal warts are associated with TH1 or mixed TH1/TH2 cytokine mRNA expression profiles. Arch Dermatol Res **289**, 243.

**Grasso G**, Muscettola M, Stecconi R et al (1992) Restorative effect of thymomodulin and zinc on interferon-gamma production in aged mice. Ann NY Acad Sci **673**, 256.

**Gray PW**, Goeddel DV (1982a) Structure of the human immune interferon gene. Nature **298**, 859.

**Gray PW**, Leung DW, Pennica D et al (1982b) Expression of human immune interferon cDNA in E. coli and monkey cells. Nature **295**, 503.

**Grawunder U**, Melchers F, Rolink A (1993) Interferon-gamma arrests proliferation and causes apoptosis in stromal cell/interleukin-7–dependent normal murine pre-B cell lines and clones in vitro, but does not induce differentiation to surface immunoglobulin-positive B cells. Eur J Immunol **23**, 544.

**Green JD**, Terrell TG (1992) Utilization of homologous proteins to evaluate the safety of recombinant human proteins-case study: Recombinant human interferon-gamma (rhIFN-γ). Toxicol Lett **64/65**, 321.

**Green SJ**, Scheller LF, Marletta MA et al (1994) Nitric oxide: cytokine regulation of nitric oxide in host resistance to intracellular pathogens. Immunol Lett **43**, 87.

**Greenberg PL** (1991) Treatment of myelodysplastic syndromes. Blood Rev **5**, 42.

**Greenlund AC**, Schreiber RD, Goeddel DV, Pennica D (1993) Interferon-gamma induces receptor dimerization in solution and on cells. J Biol Chem **268**, 18103.

**Gregory SH**, Wing EJ (1993) IFN-γ inhibits the replication of *Listeria monocytogenes* in hepatocytes. J Immunol **151**, 1401.

**Greiner JW**, Guadagni F, Roselli M et al (1994) Improved experimental radioimmunotherapy of colon xenografts by combining (131)I-CC49 and interferon-gamma. Dis Colon Rectum **37** Suppl. S100.

**Greiner JW**, Guadagni F, Roselli M, Nieroda CA (1996) Novel approaches to tumor detection and therapy using a combination of monoclonal antibody and cytokine. Anticancer Res **16**, 2129.

**Grem JL**, McAtee N, Murphy RF et al. (1997) A pilot study of gamma-1b-interferon in combination with fluorouracil, leucovorin, and alpha-2a-interferon. Clin Cancer Res **3**, 1125.

**Gresser I** (1990) Biological effects of interferons. J Invest Dermatol **95**, 66.

**Gridley DS**, Slater JM, Stickney DR (1989). Effects of monoclonal antibody, recombinant interferon-γ, and tumor necrosis factor-α in the treatment of human melanoma xenografts. J Biol Response Modif **8**, 593.

**Griffiths CEM**, Voorhees JJ, Nickoloff BJ (1989a) Characterization of intercellular adhesion molecule-1 and HLA-DR expression in normal and inflamed skin: Modulation by recombinant gamma interferon and tumor necrosis factor. J Am Acad Dermatol **20**, 617.

**Griffiths CEM**, Voorhees JJ, Nickoloff BJ (1989b) Gamma interferon induces different keratinocyte cellular patterns of expression of HLA-DR and DQ and intercellular adhesion molecule-1 (ICAM-1) antigens. Br J Dermatol **120**, 1.

**Grob JJ**, Dreno B, Delaunay M et al (1996) Results of the French multicenter trial on adjuvant therapy with interferon alfa-2a in resected primary melanoma (larger than 1.5 mm) (meeting abstract). Proc Annu Meet Am Soc Clin Oncol **15**, A1359.

**Gronberg A**, Kiessling R, Fiers W (1985) Interferon-gamma is a strong modulator of NK susceptibility and expression of beta 2 microglobulin but not of transferrin receptors of K562 cells. Cell Immunol **95**, 195.

**Gronberg A**, Ferm M, Tsai L, Kiessling R (1989a) Interferon is able to reduce tumor cell susceptibility to human lymphokine-activated killer (LAK) cells. Cell Immunol **118**, 10.

**Gronberg A**, Ng J, Ferm MT et al (1989b) Analysis of the mechanisms of interferon-induced resistance to NK cell-mediated lysis. IFN-induced NK resistance. In: Natural Killer Cells and Host Defense (Ades EW, Lopez C eds). Karger, Basel, p 188.

**Gross G**, Degen KW, Hilgarth M et al (1989a) Recombinant interferon gamma in genital warts: Results of a multicenter placebo-controlled clinical trial. J Invest Dermatol **93**, 553.

**Gross N**, Beck D, Favre S (1987) In vitro antigenic modulation of human neuroblastoma cells induced by IFN-gamma, retinoic acid and dibutyryl cyclic AMP. Int J Cancer **39**, 521.

**Gross N**, Beck D, Portoukalian J et al (1989b) New anti-GD2 monoclonal antibodies produced from gamma-interferon–treated neuroblastoma cells. Int J Cancer **43**, 665.

**Gross N**, Beck D, Favre S (1990) In vitro modulation and relationship between N-myc and HLA class I RNA steady-state levels in human neuroblastoma cells. Cancer Res **50**, 7532.

**Groves RW**, Ross EL, Barker JN, MacDonald DM (1993) Vascular cell adhesion molecule-1: expression in normal and diseased skin and regulation in vivo by interferon-gamma. J Am Acad Dermatol **29**, 67.

**Gruner S**, Liebenthal C, Heusser C et al (1990) The influence of interferon-gamma and interleukin-4 on IgE production in B lymphocytes of patients with atopic dermatitis. Acta Derm Venereol **71**, 484.

**Grups JW**, Frohmuller HG (1988) Antiproliferative effects of interferons against human bladder carcinoma cell lines in vitro. Urol Int **43**, 265.

**Grups JW**, Frohmuller HG (1989) Cyclic interferon-gamma treatment of patients with metastatic renal carcinoma. Br J Urol **64**, 218.

**Grups JW**, Frohmuller HG (1990) Influence of cyclic interferon-gamma on lymphocytes and their subpopulations in patients with renal carcinoma. Urology **35**, 111.

**Guadagni F**, Witt PL, Robbins PF et al (1990) Regulation of carcinoembryonic antigen expression in different human colorectal tumor cells by interferon-gamma. Cancer Res **50**, 6248.

**Guckel B**, Moebius U, Meuer S et al(1996) Development of immunogenic breast and ovarian tumour cells for vaccination: Improved immunogenicity and induction of Cytolytic T-lymphocytes after CD80-transfection. Geburtshilfe-Frauenheilkunde **56**, 574.

**Guha A**, Dainiak N, Freedman M et al (1993) Regulatory effect of interferon-gamma and phorbol esters on the surface expression and biosynthesis of MHC class I antigens by human leukemia cells. Cell Immunol **151**, 404.

**Guidotti LG**, Chisari FV (1996) To kill or to cure: options in host defense against viral infection. Curr Opinion Immunol **8**, 478.

**Guillou PJ**, Sedman PC, Ramsden CW (1989). Inhibition of lymphokine-activated killer cell generation by cultured tumor cell lines in vitro. Cancer Immunol Immunother **28**, 43.

**Gullberg U**, Nilsson E, Einhorn S, Olsson I (1985) Combinations of interferon-gamma and retinoic acid or 1 alpha, 25-dihydroxycholecalciferol induce differentiation of the human monoblast leukemia cell line U-937. Exp Hematol **13**, 675.

**Gumina RJ**, Freire-Moar J, De Young L et al (1991) Transduction of the IFN-gamma signal for HLA-DR expression in the promonocytic line THP-1 involves a late-acting PKC activity. Cell Immunol **138**, 265.

**Gunter HH**, Lelle RL, Luck HJ et al (1995) Therapy of cervical intraepithelial neoplasis (CIN) and of cervix carcinoma with cytokines. Tumor Diagn Ther **16**, 213.

**Gunther G**, Otto B (1993) Interferon. An overview of the state of basic research with special regard to interferon-gamma. Arzneimittelforsch **43**, 182.

**Guo FH**, Comhair SA, Zheng S et al (2000) Molecular mechanisms of increased nitric oxide (NO) in asthma: evidence for transcriptional and post-translational regulation of NO synthesis. J Immunol **164**, 5970.

**Gupta S**, Vayuvegula B (1991) A comprehensive immunological analysis in chronic fatigue syndrome. Scand J Immunol **33**, 319.

**Gurujeyalakshmi G**, Giri SN Molecular mechanisms of antifibrotic effect of interferon gamma in bleomycin-mouse model of lung fibrosis: Downregulation of TGF-beta and procollagen I and III gene expression. Exp Lung Res **21**, 791.

**Gutierrez P**, Delgado MD, Richard C et al (1997) Interferon induces up-regulation of Spi-1/PU.1 in human leukemia K 562 cells. Biochem Biophys Res Commun **240**, 862.

**Gutterman JU**, Rosenblum MG, Rios A et al (1984) Harmacokinetic study of partially pure gamma-interferon in cancer patients. Cancer Res **44**, 164.

## H

**Haas H**, Falcone FH, Holland MJ et al (1999) Early interleukin-4: its role in the switch towards a Th2 response and IgE-mediated allergy. Int Arch Allergy Immunol **119**, 86.

**Haase KD**, Lange OF, Scheef W (1987) Interferon-gamma treatment of metastasized malignant melanoma. Anticancer Res **7**, 335.

**Haczku A**, Macary P, Haddad EB et al (1996) Expression of Th-2 cytokines interleukin-4 and –5 and of Th-1 cytokine interferon-gamma in ovalbumin-exposed sensitized Brown-Norway rats. Immunology **88**, 247.

**Haddad A**, Bienvenu J, Miossec P (1998) Increased production of a Th2 cytokine profile by activated whole blood cells from rheumatoid arthritis patients. J Clin Immunol **18**, 399.

**Haelewyn J**, Michiels L, Verhaert P et al (1997) Interaction of truncated human interferon gamma variants with the interferon gamma receptor: crucial importance of Arg-129. Biochem J **324**, 591.

**Hagiwara E**, Gourley MF, Lee S, Klinman DK (1996a) Disease severity in patients with systemic lupus erythematosus correlates with an increased ratio of interleukin-10: Interferon-gamma–secreting cells in the peripheral blood. Arthritis Rheum **39**, 379.

**Hagiwara E**, Sacks T, Leitman-Klinman SF et al (1996b) Effect of HIV infection on the frequency of cytokine-secreting cells in human peripheral blood. AIDS Res Hum Retroviruses **12**, 127.

**Hahne M**, Rimoldi D, Schroter M et al (1996) Melanoma cell expression of Fas (Apo-1/CD95)ligand: Implications for tumor immune escape. Science **274**, 1363.

**Hakonarson H**, Maskeri N, Carter C, Grunstein MM (1999) Regulation of TH1- and TH2-type cytokine expression and action in atopic asthmatic sensitized airway smooth muscles. J Clin Invest **103**, 1077.

**Haku T**, Yanagawa H, Nabioullin R et al (1997) Interleukin-12–mediated killer activity in lung cancer patients. Cytokine **9**, 846.

**Halford WP**, Gebhardt BM, Carr DJ (1996) Persistent cytokine expression in trigeminal ganglion latently infected with herpes simplex virus type 1. J Immunol **157**, 3542.

**Hall S**, Hsieh TC, Ng CY et al (1996) Control of renal carcinoma TC-1 growth, cyclin/kinase and nm23 expression by IFN-gamma and TGF-beta. Anticancer Res **16**, 1755.

**Halme M**, Maasilta PK, Pyrhonen SO, Mattson KV (1994a) Interferons combined with chemotherapy in the treatment of stage III-IV non–small cell lung cancer — a randomised study. Eur J Cancer **30A**, 11.

**Halme M**, Maasilta P, Repo H et al (1994b) Subcutaneously administered recombinant interferon-gamma in humans: Pharmacokinetics and effects on chemiluminescence responses of alveolar macrophages, blood neutrophils, and monocytes. J Immunother Emphasis Tumor Immunol **15**, 283.

**Halme M**, Maasita P, Repo H et al (1995) Inhaled recombinant interferon gamma in patients with lung cancer: Pharmacokinetics and effects on chemiluminescence responses of alveolar macrophages and peripheral blood neutrophils and monocytes. Int J Radiat Oncol Biol Phys **31**, 93.

**Hamburger AW**, Gayatri P (1991) Interferon induced increases in c-myc expression in a human breast carcinoma cell line. Anticancer Res 11, 1891.

**Hamburger AW**, Gayatri P (1993) Interferon-induced enhancement of transforming growth factor-alpha expression in human breast cancer cell line. Proc Soc Exp Biol Med **202**, 64.

**Hanano R**, Kaufmann SH (1995) Nitric oxide production and mycobacterial growth inhibition by murine alveolar macrophages: the sequence of rIFN-gamma stimulation and Mycobacterium bovis BCG infection determines macrophage activation. Immunol Lett **45**, 23.

**Hand A**, Pelin K, Halme M et al (1993) Interferon-alpha and interferon-gamma combined with chemotherapy: In vitro sensitivity studies in non–small cell lung cancer cell lines. Anticancer Drugs **4**, 365.

**Handgretinger R**, Kimmig A, Lang P et al (1989) Interferon-gamma upregulates the susceptibility of human neuroblastoma cells to interleukin 2–activated natural killer cells. Nat Immun Cell Growth Regul **8**, 189.

**Handretinger R**, Bruchelt G, Daurer B et al (1990a) The role of interferons in neuroblastoma: Part 2: Immunolaboratory effects. Klin Padiatr **202**, 206.

**Handretinger R**, Bruchelt G, Kimming A et al (1990b) In vitro and in vivo effect of interleukin-2 on the 2′,5′-oligodenylate synthease activity of peripheral mononuclear blood cells. J Interferon Res **10**, 75.

**Hanifin JM**, Schneider LC, Leung DYM et al (1993) Recombinant interferon gamma therapy for atopic dermatitis. J Am Acad Dermatol **28**, 189.

**Hannigan GE**, Fish EN, Williams BR (1984) Modulation of human interferon-alpha receptor expression by human interferon-gamma. J Biol Chem **259**, 8084.

**Hansen AB**, Bouchelouche PN, Giese BN, Andersen CB (1993) Role of protein kinase C during interferon-γ and phorbol ester-stimulated immunocytochemical expression of ICAM-1 in human renal carcinoma cells. APMIS **101**, 437.

**Hansen ER** (1996) Immunoregulatory events in the skin of patients with cutaneous T-cell lymphoma. Arch Dermatol **132**, 554.

**Hansson GK** (1994) Immunological control mechanisms in plaque formation. Basic Res Cardiol. **89**(Suppl 1), 41.

**Hansson GK**, Helistrand M, Rymo L et al (1989) Interferon γ inhibits both proliferation and expression of differentiation-specific α-smooth muskle actin in arterial smooth muskle cells. J Exp Med **170**, 1595.

**Hansson GK**, Holm J, Holm S et al (1991) T lymphocytes inhibit the vascular response to injury. Proc Natl Acad Sci USA **88**, 10530.

**Hansz J**, Kozlowska-Skrzypczak M (1992) Interferon-gamma–mediated suppression of erythroid progenitor growth by a HLA-DR and CD4-positive subset of T lymphocytes in acute myeloid leukemia. Immunobiology **186**, 327.

**Hara M** (1989) Fundamental studies on intravesical instillation of interferons for treatment of bladder cancer. Jpn J Urol **80**, 158.

**Harel-Bellan A**, Brini AT, Farrar WL (1988) IFN-gamma inhibits c-myc gene expression by impairing the splicing process in a colony-stimulating factor dependent murine myeloid cell line. J Immunol **141**, 1012.

**Harms G**, Zwingenberger K, Sandkamp B et al (1993) Immunochemotherapy of visceral leishmaniasis: A pilot trial of sequential treatment with recombinant interferon-γ and pentavalent antimony. J Interferon Res **13**, 39.

**Harris DT**, Matyas GR, Gomella LG et al (1999) Immunologic approaches to the treatment of prostate cancer. Semin Oncol **26**, 439.

**Harris N**, Buller RM, Karupiah G (1995) Gamma interferon-induced, nitric oxide-mediated inhibition of vaccinia virus replication. J Virol **69**, 910.

**Harris PE**, Ralph P, Gabrilove J et al (1985) Distinct differentiation-inducing activities of γ-interferon and cytokine factors acting on the human promyelocytic leukemia cell line HL-60. Cancer Res **45**, 3090.

**Harrison TS**, Levitz SM (1997) Priming with IFN-gamma restores deficient IL-12 production by peripheral blood mononuclear cells from HIV-serpositive donors. J Immunol **158**, 459.

**Harrop AR**, Ghahary A, Scott PG et al (1995) Regulation of collagen synthesis and mRNA expression in normal and hypertrophic scar fibroblasts in vitro by interferon-gamma. J Surg Res **58**, 471.

**Harvat BL**, Seth P, Jetten AM (1997) The role of $p27^{Kip1}$ in gamma interferon-mediated growth arrest of mammary epithelial cells and related defects in mammary carcinoma cells. Oncogene **14**, 2111.

**Hashimura T**, Hiura M, Yoshida O et al (1993) Cancer immunotherapy by murine bladder cancer cells transfected with mouse interferon-gamma gene. Acta Urol Jpn **39**, 1205.

**Hasler F**, Bluestein HG, Zvaifler NJ, Epstein LB (1983) Analysis of the defects responsible for impaired regulation of the EBV-induced B-cells proliferation by rheumatoid arthritis lymphocytes. I. Diminished gamma interferon production in response to autologous stimulation. J Exp Med **157**, 173.

**Hassan HT**, Grell S, Bormann-Danso U, Freund M (1993) Recombinant human interferon-alpha and interferon-beta/interleukin-6 but not interferon gamma induce megakaryocytic differentiation of acute megakaryoblastic leukemia blast cells (meeting abstract). Molecular Biology of Hematopoiesis, 8th Symposium, July 9–13, Basel, Switzerland, p 120.

**Hassan HT**, Grell S, Bormann-Danso U, Freund M (1995) Effect of recombinant human interferons in inducing differentiation of acute megakaryoblastic leukaemia blast cells. Leukemia Lymphoma **16**, 329.

**Hauer AC**, Breese EJ, Walker-Smith JA, MacDonald TT (1997) The frequency of cells secreting interferon-gamma and interleukin-4, -5, and -10 in the blood and duodenal mucosa of children with cow's milk hypersensitivity. Pediatr Res **42**, 629.

**Hawkyard S**, James K, Prescott S et al (1991) The effects of recombinant human interferon-gamma on a panel of human bladder cancer cell lines. J Urol **145**, 1078.

**Hawkyard SJ**, Jackson AM, Hawkins RA et al (1992a). Expression of interferon-gamma receptors on bladder cancer cells: Does it correlate with biological response? Urol Res **20**, 229.

**Hawkyard SJ**, Jackson AM, James K et al (1992b) The inhibitory effects of interferon gamma on the growth of bladder cancer cells. J Urol **147**,1399.

**Hawkyard SJ**, Jackson AM, Prescott S et al (1993) The effect of recombinant cytokines on bladder cancer cells in vitro. J Urol **150**, 514.

**Hayashi H**, Tatebe S, Osaki M et al (1997) Expression of Fas antigen and its mediation of apoptosis in human gastric cancer cell lines. Jpn J Cancer Res **88**, 49.

**Hayden C**, Pereira E, Rye P et al (1997) Mutation screening of interferon-gamma (IFNgamma) as a candidate gene for asthma. Clin Exp Allergy **27**, 1412.

**He J**, Ichimura H, Iida T et al (1999) Kinetics of cytokine production in the cornea and trigeminal ganglion of C57BL/6 mice after corneal HSV-1 infection. J Interferon Cytokine Res **19**, 609.

**Heck DE**, Laskin JD (1994) Potential role of G proteins in the regulation of interferon-gamma-induced apoptosis in transformed keratinocytes (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **35**,A11

**Heck DE**, DeGeorge GL, Laskin JD (1993) Post-transcriptional regulation of nitric oxide synthase by gamma interferon in mouse keratinocytes (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **34**, A2640

**Heil G**, Krauter J, Beyer-Johannboke E et al (1994) CD 54 expression and its role in homotypic aggregation of the blasts of acute myeloblastic leukemias (AML). Int J Hematol **60**, 39.

**Heise MT**, Virgin HW (1995) The T-cell-independent role of gamma interferon and tumor necrosis factor alpha in macrophage activation during murine cytomegalovirus and herpes simplex virus infections. J Virol **69**, 904.

**Hemmi H**, Breitman TR (1987) Combinations of recombinant human interferons and retinoic acid synergistically induce differentiation of the human promyelocytic leukemia cell line HL-60. Blood **69**, 501.

**Hendricks RL** (1997) An immunologist's view of herpes simplex keratitis. Cornea **16**, 503.

**Hendrix MG**, Salimans MM, van-Boven CP, Bruggeman CA (1990) High prevalence of latently present cytomegalovirus in arterial walls of patients suffering from grade III atherosclerosis. Am J Pathol **136**, 23.

**Hennemann B**, Beckmann G, Eichelmann A et al (1998) Phase I trial of adoptive immunotherapy of cancer patients using monocyte-derived macrophages activated with interferon gamma and lipopolysaccharide. Cancer Immunol Immunother **45**, 250.

**Heo Y**, Lee WT, Lawrence DA (1998) Differential effects of lead and cAMP on development and activities of Th1- and Th2-lymphocytes. Toxicol Sci **43**, 172.

**Hercend T** (1991) Immunotherapy of renal cell carcinoma (meeting abstract). 7th Int Symp Cancer Res. 12–14 June, 1991, Budapest, Hungary.

**Heremans H**, Billiau A (1989) The potential role of interferons and interferon antagonists in inflammatory disease. Drugs **38**, 957.

**Hernandes-Pando R**, Orozkoe H, Sampieri A et al (1996) Correlation between the kinetics of Th1, Th2 cells and pathology in a murine model of experimental pulmonary tuberculosis. Immunology **89**, 26.

**Herrman F**, Bambach T, Bonifer R et al (1988) The suppressive effects of recombinant human tumor necrosis factor-alpha on normal and malignant myelopoiesis: Synergism with interferon-gamma. Int J Cell Cloning **6**, 241.

**Herrman F**, Jonas D, Helfrich SG et al (1990) Interferon alpha-2c in chronic myelogenous leukemia (CML): Hematologic, cytogenetic and molecular-genetic response of patients with chronic phase CML previously resistant to therapy with interferon-gamma. Blut **61**, 226.

**Hershman MJ**, Sonnenfeld G, Logan WA et al (1988) Effect of interferon-γ treatment on the course of a burn wound infection. J Interferon Res **8**, 367.

**Hershman MJ**, Apel SH, Wellhausen SR et al (1989) Interferon-gamma treatment increases HLA-DR expression on monocytes in severely injured patients. Clin Exp Immunol **77**, 67.

**Herzog PL**, Emery P, Cheetham BF et al (1988). Interferons in rheumatoid arthritis: Alterations in production and response related to disease activity. Clin Immunol Immunopathol **48**, 192.

**Heslop HE**, Gottlieb DJ, Bianchi ACM et al (1989) In vivo induction of gamma interferon and tumor necrosis factor by interleukin-2 infusion following intensive chemotherapy or autologous marrow transplantation. Blood **74**, 1374.

**Hess AD** (1995) Antitumor effect of syngeneic/autologous graft-vs-host disease (meeting abstract). Bone Marrow Transplantation in the 90s: Into the 21st Century. NY Acad Sci A18.

**Heuer JG**, Tucker-McClung C, Hock RA (1999) Neuroblastoma cells expressing mature IL-18 but not proIL-18 induce a strong and immediate antitumor immune response. J Immunother **22**, 324.

**Heyman MR** (1991) Recent advances in biology and treatment of myelodysplasia. Curr Opin Oncol **3**, 44.

**Hibino Y**, Mariano TM, Kumar CS et al (1991) Expression and reconstitution of a biologically active mouse interferon-gamma receptor in hamster cells: Chromosomal location of an accessory factor. J Biol Chem **266**, 6948.

**Hibino Y**, Kumar CS, Mariano TM et al (1992) Chimeric Interferon-γ receptors demonstrate that an accessory factor required for activity interacts with the extracellular domain. J Biol Chem **267**, 3741.

**Higashihara J**, Saito T, Berens ME, Welander CE (1988) Effects of scheduling and ascites-associated macrophages on combined antiproliferative activity of alpha-2b interferon and gamma-interferon in a clonogenic assay. Cancer Chemother Pharmacol **22**, 215.

**Higuchi T**, Tanaka A Hiratani H et al (1989) Variant human neuroblastoma cell lines resistant to the differentiation inducing effect of interferon gamma. Cell Struct Func **14**, 439.

**Higuchi T**, Tanaka A, Watanabe H et al (1990) Rapid phosphorylation of cellular proteins during differentiation of neuroblastoma cells induced by recombinant human interferon-gamma. J Interferon Res **10**, 413.

**Hilkens C**, Snijders A, Vermeulen-H et al (1996a) Accessory cell-derived interleukin-12 and prostaglandin E2 determine the level of interferon-gamma produced by activated human CD4+ T cells. Ann NY Acad Sci. 795, 349.

**Hilkens CM**, Messer G, Tesselaar K et al (1996b) Lack of IL-12 signaling in human allergen-specific Th2 cells. J Immunol **157**, 4316.

**Hill A**, Jugovic P, York I et al (1995) Herpes simplex virus turns off the TAP to evade host immunity. Nature **375**, 411.

**Hill HR** (1993) Modulation of host defenses with interferon-γ in pediatrics. J Infect Dis **167**, 23.

**Hillman GG**, Younes E, Visscher D et al (1997) Inhibition of murine renal carcinoma pulmonary metastases by systemic administration of interferon gamma: mechanism of action and potential for combination with interleukin 4. Clin Cancer Res **3**, 1799.

**Hinoda Y**, Saito T, Takahashi H et al (1997) Induction of non-specific cross-reacting antigen mRNA by interferon-gamma and anti-fibronectin receptor antibody in colon cancer cells. J Gastroenterol **32**, 200.

**Hirano N**, Takahashi T, Ohtake S et al (1996a). Expression of costimulatory molecules in human leukemias. Leukemia **10**, 1168.

**Hirano T**, Hizuta A, Tanaka N, Orita K (1996b) Enhancement of experimental metastasis by gamma-interferon in a murine adenocarcinoma. Acta Med Okayama **50**, 11.

**Hirofuji H**, Kakumu S, Fuji A et al (1987) Natural killer and activated killer activities in chronic liver disease and hepatocellular carcinoma: Evidence for a decreased lymphokine-induced activity of effector cells. Clin Exp Immunol **68**, 348.

**Hirose K**, Hanazawa S, Amano S et al (1989). Recombinant interferon gamma is a potent inhibitor of osteoblastic cell functions. Meikai Daigaku Shigaku Zasshi **18**, 296.

**Hirsch CS**, Toossi Z, Othieno C et al (1999a) Depressed T-cell interferon-gamma responses in pulmonary tuberculosis: analysis of underlying mechanisms and modulation with therapy. J Infect Dis **180**, 2069.

**Hirsch CS**, Toossi Z, Vanham G et al (1999b) Apoptosis and T cell hyporesponsiveness in pulmonary tuberculosis. J Infect Dis **179**, 945.

**Hirschman SZ**, Chen CW (1996) Peptide nucleic acids stimulate gamma interferon and inhibit the replication of the human immunodeficiency virus. J Invest Med **44**, 347.

**Hobarth K**, Szabo N, Hallas A et al (1994) Serum neopterin as a parameter for monitoring the course of renal cell carcinoma during interferon-gamma therapy. Clin Immunol Immunopathol **70**, 241.

**Hobarth K**, Hallas A, Steiner G et al (1996) Circulating immune markers in advanced renal cell carcinoma during immunotherapy with interferon-gamma.Urol Res **24**, 101.

**Hobeika AC**, Etienne W, Cruz PE et al (1998) IFNgamma induction of p21WAF1 in prostate cancer cells: Role in cell cycle, alteration of phenotype and invasive potential. Int J Cancer **77**, 138.

**Hober D**, Benyoucef S, Chehadeh W et al (1998) Production of interleukin-4, interferon (IFN)-gamma and IFN-alpha in human immunodeficiency virus-1 infection: an imbalance of type 1 and type 2 cytokines may reduce the synthesis of IFN-alpha. Scand J Immunol **48**, 436.

**Hoekstra MO**, Hoekstra Y, De Reus D et al (1997) Interleukin-4, interferon-gamma and interleukin-5 in peripheral blood of children with moderate atopic asthma. Clin Exp Allergy **27**, 1254.

**Hofmockel G**, Wirth MP, Heimbach D, Frohmuller HG (1993) Low dose cyclic interferon gamma treatment in metastatic renal cell carcinoma. Urologie Ausg A **32**, 290.

**Hofstra CL**, Van Ark I, Hofman G et al (1998) Differential effects of endogenous and exogenous interferon-gamma on immunoglobulin E, cellular infiltration, and airway responsiveness in a murine model of allergic asthma. Am J Respir Cell Mol Biol **19**, 826.

**Hoft DF**, Kemp EB, Marinaro M et al (1999) A double-blind, placebo-contolled study of Mycobacterium-specific human immune responses induced by intradermal bacille Calmette-Guerin vaccination. J Lab Clin Med **134**, 244**.**

**Hogrefe HH**, McPhie P, Bekisz JB et al (1989) Amino terminus is essential to the structural integrity of recombinant human interferon-gamma. J Biol Chem **264**, 12179.

**Holland MJ**, Bailey RL, Conway DJ et al (1996) T helper type-1 (Th1)/Th2 profiles of peripheral blood mononuclear cells (PBMC); responses to antigens of Chlamydia trachomatis in subjects with severe trachomatous scarring. Clin Exp Immunol **105**, 429.

**Holland SM**, Eisenstein EM, Kuhns DB et al (1994) Tretment of refractory disseminated nontuberculous mycobacterial infection with interferon gamma. A preliminary report. N Engl J Med **330**, 1348.

**Hong L**, Johansen L, Krueger JM (1991) Modulation of human leukocyte antigen (DR) expression in glioblastoma cells by interferon gamma and other cytokines. J Neuroimmunol **35**, 139.

**Honma S**, Nakamura M, Maruhashi T et al (1991) Effect of local injection of interferon gamma on cell-mediated immunity of patients with uterine cervical carcinomas. Serono Symp Publ. Raven Press **82**, 227.

**Hooks JJ**, Moutsopoulos HM, Geis SA (1979). Immune interferon in the circulation of patients with autoimmune disease. N Engl J Med **301**, 5.

**Hooks JJ**, Chader G, Evans CH, Detrick B (1990) Interferon-gamma enhances the expression of retinal S-antigen, a specific neuronal cell marker. J Neuroimmunol **26**, 245.

**Hopkins SJ**, Meager A (1988). Cytokine in synovial fluid: II. The presence of tumor necrosis factor and interferon. Clin Exp Immunol **73**, 88.

**Horie T**, Dobashi K, Yoshii A et al (1999) Interferon-gamma rescues TNF-alpha-induced apoptosis mediated by up-regulation of TNFR2 on EoL-1 cells. Exp Hematol **27**, 512

**Horikoshi T**, Fukuzawa K, Hanada N et al (1995) In vitro comparative study of the antitumor effects of human interferon-alpha, beta and gamma on the growth and invasive potential of human melanoma cells. J Dermatol **22**, 631.

**Horisberger MA**, Staritzky K (1985) Sensivity of inluenza A viruses to human interferons in human diploid cells. FEMS Microbiol Lett **29**, 207.

**Horisberger MA**, Zeller H (1987) The action of human interferons on influenza virus-host cell interactions. In: The biology of negative strand viruses (Mahy and Kolakofsky, eds.), Elsevier Science Publisher BV (Biomedical Division), p. 129.

**Horowitz BJ** (1989) Interferon therapy for condylomatous vulvitis. Obstet Gynecol **73**, 446.

**Horwitz MA** (1992) Influence of interferon-gamma on *Legionella pneumophila* mononuclear phagocyte interaction. In: Antiinfective application of interferon gamma (Jaffe HS, Bucalo LR, Sherwin SA, eds). Marcel Dekker, New York, p 265.

**Hoshino T**, Mikura Y, Shimidzu H et al (1987). Reduction in antiviral activity of human interferon γ in acidic media with reference to structural change. Biochim Biophys Acta **916**, 245.

**Hosoi T**, Ozawa K, Ohta M et al (1985) Recombinant immune interferon inhibits leukemic cell growth by a monocyte-macrophage-mediated mechanism. Exp Hematol **13**, 597.

**Howard AD**, Zwilling BS (1999) Reactivation of tuberculosis is associated with a shift from type 1 to type 2 cytokines. Clin Exp Immunol **115**, 428.

**Howel AL**, Stukel TA, Bloomfield CD et al (1990) Induction of differentiation in blast cells and leukemia colony-forming cells from patients with acute myeloid leukemia. Blood **75**, 721.

**Howel AL**, Groveman DS, Wallace PK et al (1997) HIV-1 infected monocytes and monocyte-derived macrophages are impaired in their ability to produce superoxide radicals. Int J Clin Lab Res **27**, 111.

**Hsia J**, Sarin N, Oliver JH, Goldstein AL (1989) Aspirin and thymosin increase interkeukun-2 and interferon-γ production by human peripheral blood lymphocytes. Immunopharmacology **17**, 167.

**Hsu YR**, Arakawa T (1985) Structural studies on acid unfolding and refolding of recombinant human interferon γ. Biochemistry **24**, 7959.

**Hsu YR**, Ferguson B, Narachi M et al (1986) Structure and activity of recombinant human interferon-γ analogs. J Interferon Res **6**, 663.

**Huang YW**, Vitetta ES (1995) Immunotherapy of multiple myeloma. Stem Cells **13**, 123.

**Hujanen ES**, Turpeenniemi HT (1991). Recombinant interferon alpha and gamma modulate the invasive potential of human melanoma in vitro. Int J Cancer **47**, 576.

**Hunninghake GW**, Hemken C, Brady M, Monick M (1986) Immune interferon is a growth factor for human lung fibroblasts. Am Rev Respir Dis **134**, 1025.

**Hunt JP**, Hunter CT, Brownstein MR et al (1998) The effector component of the cytotoxic T-lymphocyte response has a biphasic pattern after burn injury. J Surg Res **80**, 243.

**Husby G**, Williams RC Jr (1985). Immunohistochemical studies of interleukin-2 and γ-interferon in rheumatoid arthritis. Arthritis Rheum **28**, 174.

**Hutchinson V**, Cummins JM (1987) Low-dose oral interferon in patient with AIDS. Lancet 2,1530.

**Huygen K**, Van Vooren JP, Turneer M et al (1988). Specific lymphoproliferation. Gamma interferon production and serum immunoglobulin G directed against a purified 32kDa mycobacterial protein antigen (P32) in patients with active tuberculosis. Scand J Immunol **27**, 187.

## I

**Iacobelli S**, Natoli C, Arno E et al (1986) An antiestrogenic action of interferons in human breast cancer cells. Anticancer Res **6**, 1391.

**Ikic D**, Brnobic A, Jurkovic-Vukelic V et al (1975) Therapeutical effect of human leukocyte interferon incorporated into ointment and cream on condylomata acuminata. In: Proceedings of a Symposium on the Clinical Use of Interferon. Zagreb, Yugoslavia, Acad Sci Arts, p 235.

**Imami N**, Antonopoulos C, Hardy GA et al (1999) Assessment of type 1 and type 2 cytokines in HIV type 1-infected individuals: Impact of highly active antiretroviral therapy. AIDS Res Hum Retroviruses **15**, 1499.

**Imanishi J**, Watanabe H, Hiratani H et al (1987) Morphological changes of human neuroblastoma cells induced by human gamma interferon. C R Seances Soc Biol Fil **181**, 94.

**Imbert-Marcille BM**, Thedrez P, Sai Maurel C et al (1994) Modulation of associated ovarian carcinoma antigens by 5 cytokines used as single agents or in combination. Int J Cancer **57**, 392.

**Imbert-Marcille BM**, Berreur M, Thedrez P et al (1995) Effects of interferon gamma (IFN gamma) and tumor necrosis factor alpha (TNF alpha) association in intraperitoneal radioimmunotherapy: Studies on an in vitro micrometastasis model. Anti Cancer Drug Des **10**, 491.

**Inaba T**, Gotoda T, Harada K et al (1995) Induction of sustained expression of protooncogene c-fms by platelet- derived growth factor, epidermal growth factor, and basic fibroblast growth factor, and its suppression by interferon- gamma, and macrophage colony- stimulating factor in human aortic medial smooth muscle cells. J Clin Invest **95**, 1133.

**Inbal B**, Cohen O, Polak-Charcon S et al (1997) DAP kinase links the control of apoptosis to metastasis. Nature **390**, 180.

**Isaacs A** and Lindenmann J (1957) Virus interference. I. The interferon. Proc R Soc Lond B**147**, 258**.**

**Ishida H** (1994) Clinical implication of IL-10 in patients with immune and inflammatory diseases. Rinsho Byori **42**, 843.

**Ishida H**, Muchamuel T, Sakaguchi S et al (1994) Continuous administration of anti-interleukin 10 antibodies delays onset of autoimmunity in NZB/W F1 mice. J Exp Med **179**, 305.

**Ishigami S**, Hokita S, Iwashige H et al (1996) The effect of EALs (effusion associating lymphocytes) against ascitic tumor cells in patients with gastric cancer after IFN-gamma. Biotherapy **10**, 781.

**Ishihara H**, Hara T, Aramaki Y et al (1990) Preparation of asialofetuin-labelled liposomes with encapsulated human interferon-gamma and their uptake by isolated rat hepatocytes. Pharm Res **7**, 542.

**Ishihara H**, Hayashi Y, Hara T et al (1991) Specific uptake of asialofetuin-tacked liposomes encapsulating interferon-gamma by human hepatoma cells and its inhibitory effect on hepatitis B virus replication. Biochem Biophys Res Commun **174**, 839.

**Ishii H**, Gouchi A, Orita K (1994) The enhancement of cell surface ICAM-I and HLA class I antigens in human gastric cancer cell lines by IFN-gamma. Acta Med Okayama **48**, 73.

**Ishii Y**, Tsukagoshi S (1989) The synergistic effect of human recombinant interferon-alpha (2a) in combination with interferon-gamma and the induction of interferon-alpha (2a) receptor by interferon-gamma. J Pharmacobiol Dyn **12**, 299.

**Isomaki P**, Luukkainen R, Lassila O et al (1999) Synovial fluid T cells from patients with rheumatoid arthritis are refractory to the T helper type 2 differentiation-inducing effects of interleukin-4. Immunology **96**, 358.

**Itoh K**, Shiba K, Shimizu Y et al (1985) Generation of activated killer (ak) cells by recombinant interleukin 2(rIL2) in collaboration with interferon-γ (IFN-γ). J Immunol **134**, 3124.

**Itoh K**, Platsoucas CD, Balch CM et al (1987) Monocyte and natural killer cell-mediated spontaneous cytotoxicity against human noncultured solid tumor cells. Cell Immunol **108**, 495.

**Iwagaki H**, Hizuta A, Mori K et al (1995) Endogenous gamma-interferon activates thymidine phosphorylase in colorectal cancer tissue. Res Commun Mol Pathol Pharmacol **87**, 345.

**Iwamoto I**, Nakajima H, Endo H, Yoshida S (1993) Interferon gamma regulates antigen-induced eosinophil recruitment into the mouse airways by inhibiting the infiltration of CD4+ T cells. J Exp Med **177**, 573.

**Iwamoto I**, Kumano K, Kasai M et al (1996) Interleukin-12 prevents antigen-induced eosinophil recruitment into mouse airways. Am J Respir Crit Care Med **154**, 1257.

**Iwanami M**, Takeda K, Konno K (1990) Enhancement of interferon-gamma–induced differentiation of human monoblastic leukemia U-937 cells by cAMP-inducing agents. J Interferon Res **10**, 477.

**Iwasaka T**, Tsugitomi H, Ohkuma Y et al (1987) Antitumor activities of human recombinant interferon-γ against cervical tumor transplanted into nude mice. Acta Obset Gynecol Scand **66**, 501.

**Iyer S**, Zaheer S, Vandre D et al (1986) Characterization of human interferon species using gel extraction and monoclonal antibodies: Implications on clinical use of interferon preparations. J Biol Response Modif **5**, 548.

**Izu AE**, Jaffe HS, Curnitte JT, Ezckowitz RAB (1992) Interferon-Gamma treatment of Chronic Granulomatous Disease. In: Antiinfective application of interferon gamma (Jaffe HS, Bucalo LR, Sherwin SA, eds.). Marcel Dekker, New York, p. 11.

## J

**Jabbar SA**, Twentyman PR (1990) Relationship between growth inhibition by cytokines (interferons and tumor necrosis factor) and receptor binding in human lung cancer cell lines (meeting abstract). Br J Cancer **62**, 500.

**Jabrane-Ferrat N**, Calvo F, Faille A et al (1990a) Recombinant gamma interferon provokes resistance of human breast cancer cells to spontaneous and IL-2 activated non-MHC restricted cytotoxicity. Br J Cancer **61**, 558.

**Jabrane-Ferrat N**, Faille A, Loiseau P et al (1990b). Effect of gamma interferon on HLA class I and II transcription and protein expression in human breast adenocarcinoma cell lines. Int J Cancer **45**, 1169.

**Jacqmin D** (1994) Renal cancer. Curr Opin Urol **4**, 242.

**Jackson AM**, Alexandrov AB, Prescott S et al (1992a) Role of adhesion molecules in lymphokine-activated killer cell of bladder cancer cells: Further evidence for a third ligand for leucocytes function-associated antigen-1. Immunology **76**, 286.

**Jackson AM**, Alexandrov AB, Prescott S et al (1992b) Expression of adhesion molecules by bladder cancer cells: Modulation by interferon-gamma and tumor necrosis factor alpha. J Urol **148**, 1583.

**Jackson AM**, Alexandrov AB, Gribben SC et al (1993) Expression and shedding of ICAM-1 in bladder cancer and its immunotherapy. Int J Cancer **55**, 921.

**Jackson AM**, Alexandrov AB, Fleming D et al (1994) Bacillus Calmette-Guerin (BCG) organisms directly alter the growth of bladder tumour cells. Int J Oncol **5**, 697.

**Jadeski LC**, Lala PK (1999) Nitric oxide synthase inhibition by N(G)-nitro-L-arginine methyl ester inhibits tumor-induced angiogenesis in mammary tumors. Am J Pathol **155**, 1381.

**Jaffe HA**, Gao Z, Mori Y et al (1999) Selective inhibition of collagen gene expression in fibroblasts by an interferon-gamma transgene. Exp Lung Res **25**, 199.

**Jaffe HA**, Buhl R, Mastrangeli A et al (1991) Organ specific cytokine therapy. Local activation of mononuclear phagocytes by delivery of an aerosol of recombinant interferon-gamma to the human lung. J Clin Invest **88**, 297.

**Jaffe HS**, Chen AB, Kramer S, Sherwin SA (1987) The absence of interferon antibody formation in patients receiving recombinant human interferon-gamma. J Biol Response Modif **6**, 576.

**Jahnke A**, Johnson JP (1995) Intercellular adhesion molecule 1 (ICAM-1) is synergistically activated by TNF-alpha and IFN-gamma responsive sites. Immunobiology **193**, 305.

**Jakse G**, Marth C, Zechner J, Daxenbichler G (1988) Antiproliferative effect of Hu-interferon-gamma in 674V and J82 bladder carcinoma cell lines. Urol Res **16**, 403.

**James DC**, Goldman MH, Hoare M et al (1996) Posttranslational processing of recombinant human interferon-gamma in animal expression systems. Protein Sci **5**, 331.

**Jang IG**, Yang JK, Lee HJ et al (2000) Clinical improvement and immunohistochemical findings in severe atopic dermatitis treated with interferon gamma. J Am Acad Dermatol **42**, 1033.

**Jay E**, Mac Knight D, Lutze-Wallace C et al (1984) Chemical synthesis of a biologically active gene for human immune interferon γ. Prospect for site-specific mutagenesis and structure-function studies. J Biol Chem **10**, 6311.

**Jernberg-Wiklund** H, Petterson M, Nilsson K (1991) Recombinant interferon-gamma inhibits the growth of IL-6 dependent human multiple myeloma cell lines in vitro. Eur J Haematol **46**, 231.

**Jett JR**, Maksymiuk AW, Su JQ et al (1994) Phase III trial of recombinant interferon gamma in complete responders with small-cell lung cancer. J Clin Oncol **12**, 2321.

**Jia SF**, Kleinerman ES (1991) Antitumor activity of TNF-alpha, IL-1, and IFN-gamma against three human osteosarcoma cell lines. Lymphokine Cytokine Res **10**, 281.

**Jiang X**, Baldwin CL (1993) Effects of cytokines on intracellular growth of Brucella abortus. Infect Immun **61**, 124.

**Jinquan T**, Frydenberg J, Mukaida N et al (1995) Recombinant human growth–regulated oncogene-alpha induces T lymphocyte 1996 chemotaxis. A process regulated via IL-8 receptors by IFN-gamma, TNF-alpha, IL-4, IL-10 and IL-13. J Immunol **155**, 5359.

**John M**, Lim S, Seybold J et al (1998) Inhaled corticosteroids increase interleukin-10 but reduce macrophage inflammatory protein-1 alpha, granulocyte-macrophage colony-stimulating factor, and interferon-gamma release from alveolar macrophages in asthma. Am J Respir Crit Care Med **157**, 256.

**Johnson BJ**, Estrada I, Shen Z et al (1998) Differential gene expression in response to adjunctive recombinant human interleukin-2 immunotherapy in multidrug-resistant tuberculosis patients. Infect Immun **66**, 2426.

**Johnson HM**, Torres BA (1985) Peptide growth factors PDGF, EGF and FGF regulate interferon-γ production. J Immunol **134**, 2824.

**Johnson HM**, Torres BA, Green MM et al (1998) Cytokine-receptor complexes as chaperones for nuclear translocation of signal transducers. Biochem Biophys Res Commun **244**, 607.

**Johnston MD**, Finter NB, Young PA (1981) Dye uptake method for assay of interferon activity. Methods Enzymol **78**, A394.

**Joklik WK** (1985) Interferons. In: Virology (Fields BN et al, eds). Raven Press, New York, p. 281.

**Jouanguy E**, Altare F, Lamhamedi S et al (1996) Interferon-gamma-receptor–deficiency in an infant with fatal bacille Calmette-Guerin infection. N Engl J Med **335**, 1956.

**Jung T**, Moessner R, Dieckhoff K et al (1999a) Mechanisms of deficient interferon-gamma production in atopic diseases. Clin Exp Allergy **29**, 912.

**Jung T**, Witzak K, Dieckhoff K et al (1999b) IFN-gamma is only partially restored by co-stimulation with IL-12, IL-2, IL-15, IL-18 or engagement of CD28. Clin Exp Allergy **29**, 207.

**Jung V**, Rashidbaigi A, Jones C et al (1987) Human chromosomes 6 and 21 are required for sensitivity to human interferon γ (receptor/class I major histocompatibility complex antigens). Proc Natl Acad Sci USA **84**, 4151.

## K

**Kafka M**, Nathan I, Dvilansky A (1989) New combination of 5-fluorouracil and interferon-γ effective against human myeloid leukemia in vitro. Anticancer Res **9**, 1037.

**Kafka M**, Dvilansky A, Nathan I (1990) Mechanism of interaction between interferon-gamma and antineoplastic agent on the differentiation of HL-60 promyelocytic cells. Exp Hematol **18**, 153.

**Kagaya K**, Watanabe K, Fukazawa Y (1989) Capacity of recombinant gamma interferon to activate macrophages for salmonella-killing activity. Interferon Immun **57**, 609.

**Kageshita T**, Ono T, Hirai S et al (1992) Ganglioside, adhesion molecule, and HLA antigen expression in basal cell carcinoma lesions. Cancer Res **52**, 3201.

**Kahan A**, Amor B, Menkes, CJ, Strauch G (1989) Recombinant interferon-gamma in the treatment of systemic sclerosis. Am J Med **87**, 273.

**Kahari VM**, Chen YQ, Su MW et al (1990) Tumor necrosis factor-alpha and interferon gamma suppress the activation of human type I collagen gene expression by transforming growth factor-beta1. Evidence for two distinct mechanisms of inhibition at the transcriptional and posttranscriptional levels. J Clin Invest **86**, 1489.

**Kakizaki Y**, Kraft N, Atkins RC (1993) Interferon-gamma stimulates the secretion of IL-1, but not of IL-6 by glomerular mesangial cells. Clin Exp Immunol **91**, 521.

**Kalina U**, Ozmen L, Di Padova K et al (1993) The human gamma-interferon receptor accessory factor encoded by chromosome 21 transduces the signal for the induction of 2′,5′-oligodenylate-synthetase, resistance to virus cytopathic effect and major histocompatibility complex class I antigens. J Virol **67**, 1702.

**Kallfelz M**, Jung D, Hilmes C et al (1999) Induction of immunogenicity of a human renal-cell carcinoma cell line by Tap1-gene transfer. Int J Cancer **81**, 125.

**Kamamura Y**, Takahashi K, Komaki K, Monden Y (1998) Effects of interferon-alpha and gamma on development of LAK activity from mononuclear cells in breast cancer patients. J Med Invest **45**, 71.

**Kameyama K**, Takezaki S, Kanzaki T, Nishiyama Sh (1986) HLA-DR and melanoma-associated antigen (p97) expression during the cell cycle in human melanoma cell lines, and the effects of recombinant gamma-interferon: two-color flow cytometric analysis. J Invest Dermatol **87**, 313.

**Kameyama K**, Tone T, Eto H et al (1987) Recombinant gamma interferon induces HLA-DR expression on squamous cell carcinoma, trichilemmoma, adenocarcinoma cell lines, and cultured human keratinocytes. Arch Dermatol Res **279**, 161.

**Kamijo R**, Le J, Shapiro D et al (1993) Mice that lack that interferon-γ receptor have profoundly altered responses to infection with bacillus Calmette-Guerin and subsequent challenge with lipopolysaccharide. J Exp Med **178**, 1435.

**Kamijo R**, Shapiro D, Gerecitano J et al (1994) Biological functions of IFN-gamma and IFN-alpha/beta: lessons from studies in gene knockout mice. Hokkaido Igaku Zasshi **69**, 1332.

**Kanaoka E**, Nagata S, Hirano K (1999) Stabilization of aerosolized IFN-gamma by liposomes. Int J Pharm **188**, 165.

**Kanda Y**, Futatsugi K, Saifuku K et al (1988) Antitumoral effect of gamma-interferon in patients with hepatocellular carcinoma. Gan No Rinsho **34**, 1073.

**Kane P**, Mayotte J, Levitt ML (1992) Induction of squamous differentiation in a human non–small cell lung cancer (NSCLC) cell line by gamma-interferon (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **33**, A178.

**Kanik KS**, Hagiwara E, Yarboro CH (1998) Distinct patterns of cytokine secretion characterize new onset synovitis versus chronic rheumatoid arthritis. J Rheumatol **25**, 16.

**Kanno H**, Hattori S, Sato H et al (1999) Experimental gene therapy against subcutaneously implanted glioma with a herpes simplex virus–defective vector expressing interferon-gamma. Cancer Gene Ther **6**, 147.

**Kantarjian HM**, Talpaz M, Gutterman J (1987a) Biologic therapy of chronic myelogenous leukemia. Onkology Huntingt **1**, 35, 48, 52.

**Kantarjian HM**, Talpaz M, Kurzrock R et al (1987b) Intensive combination chemotherapy and interferons in the management of chronic myelogenous leukemia. Acta Haematol **78**(Suppl 1), 70.

**Kaplan DH**, Shankaran V, Dighe AS et al (1998) Demonstration of an interferon gamma-dependent tumor surveillance system in immunocompetent mice. Proc Natl Acad Sci USA **95**, 7556.

**Kaplan EH**, Rosen ST, Norris DB et al (1990) Phase II study of recombinant human interferon gamma for treatment of cutaneous T-cell lymphoma. J Natl Cancer Inst **82**, 208.

**Kaplan G**, Nathan CF, Gandhi R et al (1986) Effect of recombinant interferon-γ on hydrogen peroxide-releasing capacity of monocyte-derived macrophages from patients with lepromatous leprosy. J Immunol **137**, 983.

**Kapsenberg ML**, Hilkens CM. Jansen HM et al (1996) Production and modulation of T-cell cytokines in atopic allergy. Int Arch Allergy Immunol **110**, 107.

**Karagyozov I**, Dundarov S, Makaveeva V, Dimova R (1989) Human papillomavirus-induced genital infections. Medicina i Fizkultura, Sofia.

**Karavodin LM**, Robbins J, Chong K et al (1998) Generation of a systemic antitumor response with regional intratumoral injections of interferon gamma retroviral vector. Hum Gene Ther **9**, 2231.

**Karimine N**, Arinaga S, Inoue H et al (1994) Lymphokine-activated killer cell function of peripheral blood mononuclear cells, spleen cells and regional lymph node cells in gastric cancer patients. Clin Exp Immunol **96**, 484.

**Karp SE**, Chan HL (1994) Expression of T(H)2 cytokines in human breast cancers (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **35**, A1512.

**Karupiah G**, Xie QW, Buller RM et al (1993) Inhibition of viral replication by interferon-gamma-induced nitric oxide synthase. Science **261**, 445.

**Katano M**, Yamamoto H, Sato S et al (1987) A case of gastric cancer with multiple liver metastasis responding to a combination therapy of recombinant interferon gamma (KW2202) and 5-FU. Jpn J Cancer Chemother **14**, 3351.

**Kato I**, Kimura S, Furuhashi T et al (1990) Effects of recombinant murine interferon-γ on pregnant mice and their fetuses. Fund Appl Toxicol **14**, 658.

**Kaufmann SH**, Lade CH, Flesch IE (1995) T cells and cytokines in intracellular bacterial infections: experiences with Mycobacterium bovis BCG. Ciba Found Symp [discussion 123], **195**, 123.

**Kaufmann Y**, Davidsohn J, LevAnon M et al (1991) Lymphokine-activated killer (LAK) cells: Interferon-gamma synergizes with interleukin-2 to induce LAK cytotoxicity in homogeneous leukemic preparations. Clin Immunol Immunopathol **58**, 278.

**Kavanagh EK**, Kell MR, Goebel A et al (1999) Interleukin 10 is not essential for survival or for modulating T-cell function after injury. Surgery **126**, 456.

**Kavoussi LR**, Ruesing RA, Hudson MA et al (1989) Effect of tumor necrosis factor and interferon gamma on human renal carcinoma cell line growth. J Urol **142**, 875.

**Kawade Y** (1990) Cytokine network in analogy to language — A general view of interferon research from distance. J Interferon Res **10**, 101.

**Kawaguchi H**, Baba M, Shigeta SH (1986) Synergistic inhibitory effect of acyclovir and human native beta-interferon on the growth of herpes simplex virus type 2 in human embryo fibroblast cell cultures. Microbiol Immunol **30**, 593.

**Kawaguchi Y**, Kitani A, Hara M et al (1992) Cytokine regulation of prolyl 4-xydroxylase production in skin fibroblast cultures from patients with systemic sclerosis: contribution to collagen synthesis and fibrosis. J Rheumatol **19**, 1195.

**Kawakami M**, Yoneyama T, Komatsu H, Komiyama I (1993) Complete remission of brain metastasis from renal cell carcinoma with combined therapy of radiation and interferon-gamma: A case report. Nishinihon J Urol **55**,1733.

**Kawata N**, Hirakata H, Igarashi T et al (1996) A case study of metastatic renal cell carcinoma in lungs showing complete response to continuous subcutaneous injection of interferon alpha and gamma. Hinyokika Kiyo **42**, 509.

**Kawata N**, Ono M, Endo M, et al (1993) Immunological effect of recombinant interferon gamma in renal cell carcinoma. Acta Urol Jpn **39**, 511.

**Kawata N**, Takimoto Y, Hirakata H et al (1994) Clinical trial of inhalant recombinant interferon-gamma in patients with pulmonary metastasis from renal tumor. Hinyokika Kiyo **40**, 773.

**Kawata N**, Ono M, Kodama M et al (1995) A study of effective administration of recombinant interferon-gamma in renal cell carcinoma regarding immunological influence on peripheral blood lymphocytes. Hinyokika Kiyo **41**, 21.

**Kawata N**, Takimoto Y, Hirano D et al (1996) Immunological effect of recombinant interferon-gamma on tumor infiltrating lymphocytes of renal cell carcinoma — Relationship with clinical stage. Hinyokika Kiyo **42**, 1.

**Kawatsu H**, Hasegawa Y, Takagi E, Shimokata K (1991) Human alveolar macrophages of anergic patients with lung cancer lack the responsiveness to recombinant interferon gamma. Chest **100**, 1277.

**Keane MM**, Ettenberg SA, Russell E, Lipkowitz S (1996) Fas expression and function in normal and malignant breast epithelial cells (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **37**, A145.

**Keane MP**, Arenberg DA, Lynch JP III et al (1997) The CXC chemokines, IL-8 and IP-10, regulate angiogenic activity in idiopathic pulmonary fibrosis. J Immunol **159**, 1437**.**

**Keane MP**, Belperio JA, Arenberg DA et al (1999) IFN-gamma–inducible protein-10 attenuates bleomycin-induced pulmonary fibrosis via inhibition of angiogenesis. J Immunol **163**, 5686.

**Kelker HC**, Le J, Rubin BY, Yip YK et al (1983a) Three molecular weight forms of natural human interferon-γ revealed by immunoprecipitation with monoclonal antibody. J Biol Chem **259**, 4301.

**Kelker HC**, Yip YK, Anderson P, Vilcek J (1983b) Effects of glycosidase treatment on the physicochemical properties and biological activity of human interferon-γ. J Biol Chem **258**, 8010.

**Kelly JL**, O`Suilleabhain CB, Soberg CC et al (1999) Severe injury triggers antigen-specific T-helper cell dysfunction. Shock **12**, 39.

**Kelly JM**, Gilbert CS, Stark GS (1985) Differential regulation of interferon-induced mRNAs and c-myc mRNA by alpha and gamma interferons. Eur. J. Biochem **153**, 367.

**Kelsey SM**, Coldwell RD, Makin HLJ et al (1994) Neopterin release by myeloid leukaemic cells can be synergistically augmented by 1,25-dihydroxyvitamin D3 in combination with gamma interferon or granulocyte-macrophage colony stimulating factor. J Steroid Biochem Mol Biol **48**, 95.

**Kendrick BS**, Cleland JL, Lam X et al (1998) Aggregation of recombinant human interferon gamma: Kinetics and structural transitions. J Pharmaceut Sci **87**, 1069.

**Kennedy MJ**, Jones RL (1993) Autologous graft-versus-host disease: Immunotherapy of breast cancer after bone marrow transplantation. Breast Cancer Res Treat **26**(Suppl), S31.

**Kennedy MJ**, Vogeslang GB, Jones RL et al (1994) Phase I trial of interferon-gamma to potentiate cyclosporine-induced graft-versus-host disease in women undergoing autologous bone marrow transplantation for breast cancer. J Clin Oncol **12**, 249.

**Kennedy MJ**, Hess AD, Passos Coelho JL et al (1995) Peripheral blood progenitor cell (PBPC) infusions do not inhibit the induction of autologous graft vs host disease by cyclosporine A (CSA) following high dose chemotherapy (meeting abstract). Proc Annu Meet Am Soc Clin Oncol **14**, A915.

**Kennedy MJ**, Hess AD, Passos Coelho JL et al (1996) Autologous graft vs host disease (AGVHD) as immune therapy after high dose chemotherapy (HDC) for metastatic breast cancer (MBC): medium-term follow-up and comparison with historical controls (meeting abstract). Proc Annu Meet Am Soc Clin Oncol **15**, A965.

**Kent SJ**, Zhao A, Dale CJ et al (2000) A recombinant avipoxvirus HIV-1 vaccine expressing interferon-gamma is safe and immunogenic in macaques. Vaccine **18**, 2250.

**Kessler R**, Dumont S, Bartholeyns J et al (1994) Antitumoral potential of aerosolized interferon-gamma in mice bearing lung metastases. Am J Respir Cell Mol Biol **10**, 202.

**Key LL Jr**, Rodriguiz RM, Willi SM et al (1995) Long-term treatment of Osteopetrosis with recombinant human interferon gamma. N Engl J Med **332**, 1594.

**Kiderlen AF**, Kaufmann SHE, Lohmann-Matthes ML (1984) Protection of mice against the intracellular bacterium *Listeria monocytogenes* by recombinant immune interferon. Eur J Immunol **14**, 964.

**Kikuchi H**, Fujinawa T, Kuribayashi F et al (1994) Induction of essential components of the superoxide generating system in human monoblastic leukemia U937 cells. J Biochem **116**, 742.

**Kikuchi K**, Kadono T, Takehara K (1995) Effects of various growth factors and histamine on cultured keloid fibroblasts. Dermatology **190**, 4.

**Kim CJ**, Kim HO, Choe YJ et al (1995) Bcl-2 expression in neuroblastoma is differentially regulated by differentiation inducers. Anticancer Res. **15/**5 B, 1997.

**Kim CJ**, Taubenberger JK, Simonis TB et al (1996) Combination therapy with interferon-gamma and interleukin-2 for the treatment of metastatic melanoma. J Immunother Emphasis Tumor Immunol **19**, 50.

**Kim W**, Min S, Cho M et al (2000) The role of Il-12 in inflammatory activity of patients with rheumatoid arthritis. Clin Exp Immunol **119**, 175.

**Kim WU**, Cho ML, Kim SI et al (2000) Divergent effect of cyclosporine on Th1/Th2 type cytokines in patients with severe, refractory rheumatoid arthritis. J Rheumatol **27**, 324**.**

**Kimball ES**, Kovacs E, Glark MC, Schneider CR (1995) Activation of cytokine production and adhesion molecule expression on THP-1 myelomonocytic cells by macrophage colony-stimulating factor in combination with interferon-gamma. J Leukoc Biol **58,** 585.

**Kimoto Y** et al (1986) Antitumor effect of interferons with chemoterapeutic agents. Jpn J Cancer Chemother **13**, 293.

**Kimura M**, Tsuruta S, Yoshida T (1999) Differences in cytokine production by peripheral blood mononuclear cells (PBMC) between patients with atopic dermatitis and bronchial asthma. Clin Exp Immunol **118**, 192.

**King CL**, Gallin JI, Malech HL et al (1989) Regulation of immunoglobulin production in hyperimmunoglobulin E recurrent-infection syndrome by interferon γ. Proc Natl Acad Sci USA **86**, 10085.

**King CL**, Low CC, Nutman TB (1993) IgE production in human helminth infection. Reciprocal interrelationship between IL-4 and IFN-γ. J Immunol **150**, 1873.

**King MA**, Nelson DS (1989) Tumor cell heterogenety in multiple myeloma: Antigenic morphologic and functional studies of cells from blood and bone marrow. Blood **73**, 1925.

**King TE Jr** (2000) Interferon gamma-1b for the treatment of idiopathic pulmonary fibrosis. N Engl J Med **342**, 974.

**Kirby P**, Corey L (1992) Interferon-gamma for human papillomavirus infection. In: Antiinfective application of interfereon gamma (Jaffe HS, Bucalo LR, Sherwin SA, eds). Marcel Dekker, New York, p 16.

**Kirchner H** (1986) Das Interferonsystem unter besonderer Berucksichtigung des Gamma-interferons. Dtsch Med Wochenschr **2**, 64.

**Kirkwood J**, Bryant J, Schiller J et al (1991). Determination of phenotypic and functional correlates of interferon gamma (IFN gamma) therapy of metastatic melanoma in a multiinstitutional phase IB trial (ECOG 4987/4687) (meeting abstract). Proc Annu Meet Am Soc Clin Oncol **10**, A733.

**Kirkwood J**, Bryant J, Schiller J (1997) Immunomodulatory function of interferon-gamma in patients with metastatic melanoma: results of a phase II-B trial in subjects with metastatic melanoma, ECOG study E 4987. Eastern Cooperative Oncology Group. J Immunother **20**, 146.

**Kirkwood JM**, Strawderman MH, Ernstoff MS et al (1996) Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: The Eastern cooperative Oncology Group trial EST 1684. J Clin Oncol **14**, 7.

**Kirsch M**, Fischer H, Schackert G (1994) Activated monocytes kill malignant brain tumor cells in vitro. J Neurol Oncol **20**, 35.

**Kishikawa H**, Shimokama T, Watanabe T (1993) Localization of T lymphocytes and macrophages expressing IL-1, IL-2 receptor, IL-6 and TNF in human aortic intima. Role of cell-mediated immunity in human atherogenesis. Virchows Arch A Pathol Anat Histopathol **423**, 433.

**Kita M**, Nakagawa S, Watanabe H et al (1990) Interferon-producing capacity in patients with prostate cancer: prognosis value. Seances Soc Biol Fil **184**, 181.

**Kitagaki H**, Ono N, Hayakawa K et al (1997) Repeated elicitation of contact hypersensitivity induces a shift in cutaneous cytokine milieu from a T helper cell type 1 to a T helper cell type 2 profile. J Immunol **159**, 2484.

**Kitagawa R**, Fujime M, Sakamoto Y et al (1992) Intralesional and transarterial administration of interferon for renal cell carcinoma. Jpn J Cancer Chemother **19**, 1462.

**Kizaki M**, Koeffler HP (1992) Differentiation-inducing agents in the treatment of myelodysplastic syndromes. Semin Oncol **19**, 95.

**Klein HO** (1988). Therapie der rheumatoiden Arthritis mit rekombinantem Interferon-gamma. Fortschr Medizin **106**, 721.

**Klein HO**, Golbach G, Voigt P et al (1991a) Combination of interferons and cytostatic drugs for treatment of advanced colorectal cancer. J Cancer Res Clin Oncol **117**(Suppl 4), 214.

**Klein JB**, McLeish KR, Sonnenfeld G, Dean W (1987) Potential mechanisms of cytosolic calcium modulation in interferon-γ treated U937 cells. Biochem Biophys Res Commun **145**, 1295.

**Klein JB**, Scherzer JA, McLeish KR (1991b) Interferon-γ enhances superoxide production by HL-60 cells stimulated with multiple agonists. Interferon Res **11**, 69.

**Klein SA**, Dobmeyer JM, Dobmeyer TS et al (1996) TNF-alpha mediated apoptosis of CD4 positive T-lymphocytes. A model of T-cell depletion in HIV infected individuals. Eur J Med Res **1**, 249.

**Klein SA**, Dobmeyer JM, Dobmeyer TS et al (1997) Demonstration of the Th1 to Th2 cytokine shift during the course of HIV-1 infection using cytoplasmic cytokine detection on single cell level by flow cytometry. AIDS **11**, 1111.

**Kleinerman ES**, Fogler WE, Fidler IJ, Knight et al (1985) Intracellular activation of human and rodent macrophages by human lymphokines encapsulated in liposomes. J Leukoc Biol **37**, 571.

**Kleinerman ES**, Kurzock R, Wyatt D et al (1986). Activation or suppression of the tumorocidal properties of monocytes from cancer patients following treatment with human gamma interferon. Cancer Res **46**, 5401.

**Klieman RL**, Green JA, Spruance SL (1985) Immunostimulatory function of herpes simplex virus isolates from patients with frequent herpes labialis and a deficiency in immune-specific interferon production. J Med Virol **16**, 289.

**Klingemann HG** (1996) Role of postinduction immunotherapy in acute myeloid leukemia. Leukemia **10**, 21.

**Kloke O**, May D, Wandl U et al (1990) Treatment of chronic myelogenous leukemia with interferons alpha and gamma. Blut **61**, 45.

**Klotzbucher A**, Mittnacht S, Kirchner H, Jacobsen H (1990) Different effects of IFN gamma and IFN alpha/beta on "immediate early" gene expression of HSV-1. Virology **179**, 487.

**Klouche M**, Kirchner H, Holzel F (1995) Antiproliferative and recovery effects during treatment of breast and ovarian carcinoma cell cultures with interferon gamma. J Interferon Cytokine Res **15**, 285.

**Knight E**, Anton ED, Fahey O et al (1985) Interferon regulates *c-myc* gene expression in Daudi cells at the post transcriptional level. Proc Natl Acad Sci USA **82**, 1151.

**Kobayashi S**, Nagaura T, Kimura I, Kimura M (1994) Interferon-gamma–activated macrophages enhance angiogenesis from endothelial cells of rat aorta. Immunopharmacology **27**, 23.

**Kobayashi Y**, Urabe T (1988) Gamma interferon therapy of cancer patients. Gan To Kagaku Ryoho **15**, 804.

**Kochi**, **A**. (1991) The global tuberculosis situation and the new control strategy of the World Health Organization. Tubercule **72**, 1.

**Koech DH**, Obel AO, Minowada J et al (1990) Low dose oral alpha-interferon therapy for patients seropositive for the human immunodeficiency virus type-1 (HIV-1). Molec Biother **2**, 91.

**Koeffler HP**, Ranyard J,Yelton L et al (1984) Gamma-interferon induces expression of the HLA-D antigens on normal and leukemic human myeloid cells. Proc Natl Acad Sci USA **81**, 4080.

**Koestler TP**, Johnson WJ, Rieman D et al (1987) Differential expression of murine macrophage-mediated tumor cytotoxicity induced by interferons. Cancer Res **47**, 2804.

**Koff WC**, Fidler IJ, Showalter SD et al (1984) Human monocytes activated by immunomodulators in liposomes lyse herpes virus-infected but not normal cells. Science **224**, 1007.

**Koff WC**, Fogler WE, Gutterman J, Fidler IJ, Korizu et al (1994) Efficient activation of human blood monocytes to a tumoricidal state by liposomes containing human recombinant gamma interferon. Cancer Immunol Immunother **19**, 85.

**Koga S**, Nishikido M, Matsuya F et al (1999) Subcutaneous administration of interferon alpha and gamma in patients with metastatic renal cell carcinoma. Anticancer Res **19**, 5547.

**Kogut MH**, Lange K (1989a). Interferon gamma mediated inhibition of the development of *Eimeria tenella* in cultured cells. J Parasitol **75**, 313.

**Kogut MH**, Lange K (1989b) Recombinant interferon γ inhibits cell invasion by *Eimeria tenella*. J Interferon Res **9**, 67.

**Kohl S**, Loo LS, Drath DB, Cox P (1989) Interleukin-2 protects neonatal mice from lethal herpes simplex virus infection: a macrophage-mediated, gamma interferon–induced mechanism. J Infect Dis **159**, 239.

**Koide Y**, Yoshida A (1994) The signal transduction mechanism responsible for gamma interferon-induced indoleamine 2,3-dioxygenase gene expression. Infect Immun **62**, 948.

**Koide Y**, Ina Y, Nezu N, Yoshida TO (1988) Calcium influx and the CA2+- calmodulin complex are involved in interferon-gamma–induced expression of HLA class II molecules on HL-60 cells. Proc Natl Acad Sci USA **85**, 3120.

**Kolb SA**, Sporer B, Lahrtz F et al (1999) Identification of a T cell chemotactic factor in the cerebrospinal fluid of HIV-1-infected individuals as interferon-gamma inducible protein 10. J Neuroimmunol **93**, 172.

**Kolbeck C** (1993) Treatment of congenital chronic granulomatosis. Higher life-expectancy due to gamma interferon therapy. TW Pediatr **6**, 212.

**Kole L**, Das L, Das PK (1999) Synergistic effect of interferon-gamma and mannosylated lyposome-incorporated doxorubicin in the therapy of experimental visceral leishmaniasis. J Infect Dis **180**, 811.

**Kolecki P**, Baumgarten E, Smid H et al (1994) An attempt to use low-dose interleukin-2 and interferon-gamma as a maintenance treatment in children with high risk acute leukemia and non-Hodgkin lymphoma. Pol J Immunol **19**, 127.

**Komatsu T**, Bi Z, Reiss CS (1996) Interferon-gamma induced type I nitric oxide synthase activity inhibits viral replication in neurons. J Neuroimmunol **68**, 101.

**Kominsky SL**, Hobeika AC, Lake FA et al (2000) Down-regulation of neu/HER-2 by interferon-gamma in prostate cancer cells. Cancer Res **60**, 3904.

**Koning H**, Neijens HJ, Baert MR et al (1997) T cell subsets and cytokines in allergic and non-allergic children. I. Analysis of IL-4, IFN-gamma and IL-13 mRNA expression and protein production. Cytokine **9**, 416.

**Kontsek P**, Waschutza G, Kontsekova E, Otto B (2000) Engineered acid-stabile human interferon gamma. Cytokine **12**, 708.

**Kooy AJ**, Tank B, Vuzevski VD et al (1998) Expression of interferon-gamma receptors and interferon-gamma–induced up-regulation of intercellular adhesion molecule-1 in basal cell carcinoma; decreased expression of IFN-gamma R and shedding of ICAM-1 as a means to escape immune surveillance. J Pathol **184**,169.

**Korber B**, Mermod N, Hood L, Stroynowski I (1988) Regulation of gene expression by interferons: Control of H-2 promoter responses. Science **239**, 1302.

**Kosaka C**, Masuda J, Shimokado K et al (1992) Interferon-gamma suppresses PDGF production from THP-1 cells and blood monocyte-derived macrophages. Atherosclerosis **97**, 75.

**Koshiji M**, Adachi Y, Taketani S et al (1997) Mechanisms underlying apoptosis induced by combination of 5-fluorouracil and interferon-gamma. Biochem Biophys Res Commun **240**, 376.

**Koshiji M**, Adachi Y, Sogo S et al (1998) Apoptosis of colorectal adenocarcinoma (COLO 201) by tumor necrosis factor-alpha (TNF-alpha) and/or interferon-gamma (IFN-gamma), resulting from down-modulation of Bcl-2 expression. Clin Exp Immunol **111**, 211.

**Kotenko S**, Izotova L, Pollack B et al (1995) Interaction between the components of the interferon γ receptor components. J Biol Chem **270**, 20915.

**Kowalzick L**, Weyer U, Lange P, Breibart EW (1990) Systemic therapy of advanced metastatic malignant melanoma with a combination of fibroblast interferon-beta and recombinant interferon-gamma. Dermatologia **181**, 298.

**Koyano-Nakagawa N**, Arai K (1996) Specific versus cooperative regulatory mechanisms of the cytokine genes that are clustered on the same chromosome. J Allergy Clin Immunol. **98** (Pt 2) S174.

**Krahenbuhl JL**, Sibley LD, Chae GT (1990) Gamma interferon in experimental leprosy. Diagn Microbiol Infect Dis **13**, 405.

**Krainick U**, Kantarjian H, Broussard S, Talpaz M (1998) Local cutaneous necrotizing lesions associated with interferon injections. J Interferon Cytokine Res **18**, 823.

**Krajcik E**, Mitchel RE, Kwok CS. (1993) Recent advances in human interferon gamma (IFN gamma) radiosensitization (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **34**, A2153.

**Kramer SM** (1992) Leishmaniasis and interferon-gamma. In: Antiinfective application of interferon gamma (Jaffe HS, Bucalo LR, Sherwin SA, eds). Marcel Dekker, New York, p 123.

**Krasagakis K**, Garbe C, Orfanos CE (1993) Cytokines in human melanoma cells: Synthesis, autocrine stimulation and regulatory functions. An overview. Melanoma Res **3**, 425.

**Kronberger P**, Weiss G, Tschmelitsch J et al (1995) Predictive value of urinary neopterin in patients with lung cancer. Eur J Clin Chem Clin Biochem **33**, 831.

**Kudo S**, Kawano K (1999) Association and dissociation properties of natural human interferon gamma. J Chromatogr B Biomed Sci Appl **723**, 25.

**Kudo T**, Souma H, Kudo S et al (1990), Clinical effects and immunological changes of interferon therapy for advanced renal cell carcinoma. J Jpn Soc Cancer Ther **25**, 40.

**Kuebler JP**, Oberley TD, Meisner LF et al (1987) Effect of interferon alpha, interferon beta and interferon gamma on the *in vitro* growth of human renal adenocarcinoma cells. Invest New Drugs **5**, 21.

**Kuga S**, Otsuka T, Niiro H et al (1996) Suppression of superoxide anion production by interleukin-10 is accompanied by a downregulation of the genes for subunit proteins of NADPH oxidase. Exp Hematol **24**, 151.

**Kuhls TL**, Sacher J, Pineda E et al (1986) Suppression of recurrent genital herpes simplex virus infection with recombinant $\alpha 2$ interferon. J Infect Dis **154**, 437.

**Kumar R**, Korutla L (1995) Growth inhibition of human acute promyelocytic leukemia NB-4 cells by interferons and all-trans retinoic acid: Trans-modulation of inducible gene expression pathways. Anticancer Res **15**, 353.

**Kundu N**, Beaty TL, Jackson MJ, Fulton AM (1996) Antimetastatic and antitumor activities of interleukin 10 in a murine model of breast cancer. J Natl Cancer Inst **88**, 536.

**Kundu SK**, Merigan TC (1997) Heterogeneity of cytokine functions in HIV infection. Immunology **91**, 234.

**Kuo CC**, Grayston JT, Campbell LA et al (1995) Chlamydia pneumoniae (TWAR) in coronary arteries of young adults (15–34 years old). Proc Natl Acad Sci USA **92**, 6911.

**Kuppner MC**, Van-Meir E, Hamou MF, De-Tribolet N (1990) Cytokine regulation of intercellular adhesion molecule-1 (ICAM-1) expression on human glioblastoma cells. Clin Exp Immunol **81**, 142.

**Kurisu H**, Matsuyama H, Ohmoto Y et al (1994) Cytokine-mediated antitumor effect of bacillus Calmette-Guerin on tumor cells in vitro. Cancer Immunol Immunother **39**, 249.

**Kuroki S**, Ohta A, Sueoka N et al (1995) Determination of various cytokines and type III procollagen aminopeptide levels in bronchoalveolar lavage fluid of the patients with pulmonary fibrosis: inverse correlation between type III procollagen aminopeptide and interferon-gamma in progressive patients. Br J Rheumatol **34**, 31.

**Kurosawa S**, Harada M, Matsuzaki G et al (1995) Early-appearing tumor-infiltrating natural killer cells play a crucial role in the generation of anti-tumor T lymphocytes. Immunology **85**, 338.

**Kurtz RS**, Young KM, Czuprynski CJ (1989) Separate and combined effects of recombinant interleukin-1$\alpha$ and gamma interferon on antibacterial resistance. Infect Immunity **57**, 553.

**Kurzrock HM**, Talpaz M, Kantarjian H et al (1987). Therapy of chronic myelogenous leukemia with recombinant interferon-gamma. Blood **70**, 943.

**Kurzrock R**, Rosenblum MG, Sherwin SA et al (1985) Pharmacokinetics, single-dose tolerance, and biological activity of recombinant γ-interferon in cancer patients. Cancer Res **45**, 2866.

**Kurzrock R**, Quesada JR, Rosenblum MG et al (1986) Phase I study of iv administered recombinant gamma interferon in cancer patients. Cancer Treat Rep **70**, 1357.

**Kusaba M**, Honda J, Fukuda T, Oizumi K (1998) Analysis of type 1 and type 2 T cells in synovial fluid and peripheral blood of patients with rheumatoid arthritis. J Rheumatol **25**, 1466.

**Kushnaryov VM**, MacDonald HS, Sedmak JJ, Grossberg SE (1988) The cellular internalization of recombinant gamma interferon differs from that of natural interferon gamma. Biochem Biophys Res Commun **1**, 109.

**Kusugami K**, Matsuura T, Kawase T et al (1990) Interleukin 2 and interferon gamma activities of mononuclear cells from regional lymph nodes in patients with gastric cancer. Gastroenterol Jpn **25**, 306.

**Kuwahara Y**, Kusugami K, Morise K, Shimokata K (1987) Effect of recombinant gamma interferon on natural killer cell activity in patients with gastric cancer. Gastroenterology Jpn **22**, 428.

**Kwiatkowski D** (2000) Genetic dissection of the molecular pathogenesis of severe infection. Intensive Care Med **26**(Suppl 1), 89.

**Kwok CS**, Cooper C, Mac Gregor WD (1990) Sensitization of human cells to acute or low dose rate radiation by recombinant human interferon-gamma. Proc Annu Meet Am Assoc Cancer Res **31**, A2324.

**Kwok CS**, Lee S, Mitchel RE et al (1991). Parameters of sensitization of human cells to low dose rate radiation by recombinant human interferon-gamma (rhIFN-gamma) (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **32**, A2312.

## L

**Laan MP**, Baert MR, Vredendaal AE, Savelkoul HF (1998) Differential mRNA expression and Reports; 70; production of interleukin-4 and interferon-gamma in stimulated peripheral blood mononuclear cells of house-dust mite-allergic patients. Eur Cytokine Netw **9**, 75.

**Lachman LB**, Shih LC, Rao XM et al (1995) Cytokine-containing liposomes as adjuvants for HIV subunit vaccines. AIDS Res Hum Retroviruses **11**, 921.

**Lack G**, Renz H, Saloga J et al (1994) Nebulized but not parenteral IFN-gamma decreases IgE production and normalizes airways function in a murine model of allergen sensitization. J Immunol **152**, 2546.

**Lack G**, Nelson HS, Amran D et al (1997) Rush immunotherapy results in allergen-specific alterations in lymphocyte function and interferon-gamma production in CD4+ T cells. J Allergy Clin Immunol **99**, 530.

**Lagging E**, van Hage-Hamsten M, Gronneberg R et al (1998) Cytokine production in PBMC from allergics and non-allergics following in vitro allergen stimulation. Immunol Lett **60**, 45.

**Lai CK**, Ho S, Cham CH et al (1997) Cytokine gene expression profile of circulating CD4+ T cells in active pulmonary tuberculosis. Chest **111**, 606.

**Lalvani A**, Brookes R, Wilkinson RJ et al (1998) Human cytolytic and interferon-gamma–secreting CD+ T lymphocytes specific for Mycobacterium tuberculosis. Proc Acad Sci USA **95**, 270.

**Lampson LA**, Fisher CA (1984) Weak HLA and beta-2-microglobulin expression of neuronal cell lines can be modulated by interferon. Proc Natl Acad Sci USA **81**, 6476.

**Lampson LA**, George DL (1986) Interferon mediated induction of class I MHC products in human neuronal cell lines: Analysis of HLA and beta 2-m RNA and HLA-A and HLA-B proteins and polymorphic specificities. J Interferon Res **6**, 257.

**Lanciotti M**, Cornaglia-Ferraris P, Ponzoni M. (1989) Phosphatidylinositol turnover is not a general regulator of neuroblastoma cell differentiation: comparison between two differentiating agents, retinoic acid and gamma-interferon. FEBS Lett **243**, 285.

**Lanciotti M**, Montaldo PG, Longone P et al (1990) Differentiation related biochemical changes in human neuroblastoma cells (meeting abstract). FASEB J **4**, A1218.

**Lanciotti M**, Montaldo PG, Folghera S et al (1992) A combined evaluation of biochemical and morphological changes during human neuroblastoma cell differentiation. Cell Mol Neurobiol **12**, 225.

**Landar A**, Curry B, Parker MH et al (2000) Design, characterization, and structure of a biologically active single-chain mutant of human IFN-gamma. J Mol Biol **299**, 169.

**Landolfo S**, Cofano F, Gandino L et al (1985)Monoclonal antibodies against mouse gamma-interferon inhibit tumoricidal macrophage activation by T lymphocytes. Exp Cell Biol **53**, 265.

**Landthaler M**, Braun FO (1987) Adjuvant therapy of patients with melanoma with gamma-interferon. Hautarzt **38**, 479.

**Langer JA**, Pestka S (1988) Interferon receptors. Immunol Today **12**, 393.

**Langer JA**, Rashidbaigi A, Garotta G, Kempner E (1994) Radiation inactivation of human gamma-interferon: cellular activation requires two dimers. Proc Natl Acad Sci USA **91**, 5818.

**Lanza L**, Peirano L, Bosco O et al (1995) Interferons up-regulate with different potency HLA class I antigen expression in M14 human melanoma cell line. Possible interaction with glucocorticoid hormones. Cancer Immunol Immunother **41**, 23.

**Lappin DF**, Birne GD, Whaley K (1990) Interferon-mediated transcriptional and post-transcriptional modulation of complement gene expression in human monocytes. Eur J Biochem **194**, 177.

**Larner AC**, Petricoin EF, Nakagawa Y, Finbloom DS (1993) Il-4 attenuates the transcriptional activation of both IFN-$\alpha$ and IFN-$\gamma$ induced cellular gene expression in monocytes and monocytic cell lines. J Immunol **150**, 1944.

**Lasek W**, Wankowicz A, Kuc K et al (1995) Potentiation of antitumor effects of tumor necrosis factor alpha and interferon gamma by macrophage-colony-stimulating factor in a MmB16 melanoma model in mice. Cancer Immunol Immunother **40**, 315.

**Lasek W**, Wankowicz A, Kuc K et al (1996) Augmentation of antitumor efficacy by the combination of actinomycin D with tumor necrosis factor-alpha and interferon-gamma on a melanoma model in mice. Oncology **53**, 31.

**Laskay T**, Kiessling R (1986) Interferon and butyrate treatment leads to a decreased sensitivity of NK target cells to lysis by homologous but not by heterologous effector cells. Nat Immun **5**, 211.

**Lassus A**, Bergelin I, Paloranta A et al (1987) Efficacy of interferon and placebo in the treatment of recurrent genital herpes. Sex Transm Dis **14**, 185.

**Latina MA**, Belmonte SJ, Park C, Crean E (1991) Gamma-interferon effects on human fibroblasts from tenon's capsule. Invest Ophthalmol Vis Sci **32**, 2806.

**Lau AS**, Sigaroudinia M, Yeung MC et al (1996) Interleukin-12 induces interferon-gamma expression and natural killer cytoxicity in cord blood mononuclear cells. Pediatr Res **39**, 150.

**Lau JY**, Morris AG, Alexander GJ, Williams R (1992) Interferon gamma receptor expression in chronic hepatitis B virus infection. J. Hepatol **14**, 294.

**Lauren SL**, Arakawa T, Stoney K, Rohde M (1993) Covalent dimerization of recombinant human interferon-γ. Arch Biochem Biophys **306**, 350.

**Lautersztain J**, Kleinerman JE, Fidler J et al (1991) Phase I trial of the combination of gamma interferon (IFN) and liposomal MTP-PE (L-MTPPE) in patients (PTS) with disseminated cancer (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **32**, A1204.

**Lavine JE**, Ganem D (1993) Inhibition of duck hepatitis B virus replication by interferon-gamma. J Med Virol **40**, 59.

**Lavnikova N**, Drapier JC, Laskin DL (1993) A single exogenous stimulus activates resident rat macrophages for nitric oxide production and tumor cytotoxicity. J Leukoc Biol **54**, 322.

**Lazutka JR**, Jarmalaite S, Lekevicius R (1990) Modifying effects of interferon and puromycin on cytogenetic damage induced by alkylating drug phopurine in human lymphocytes. Acta Biol Hung **44**, 137.

**Lebendiker MA**, Tal Ch, Sayar D et al (1987) Superinduction of human gene encoding immune interferon. EMBO J **6**, 585.

**Lecchi M**, Lovisone E, Genetta C et al (1989) Gamma IFN induces a differential expression of HLA-DR, DQ and DP antigens on peripheral blood myeloid leukemic blasts at various stages of differentiation. Leukoc Res **13**, 221.

**Ledru E**, Lecoeur H, Garcia S et al (1998) Differential susceptibility to activation-induced apoptosis among peripheral Th 1 subsets: Correlation with Bcl-2 expression and consequences for AIDS pathogenesis. J Immunol **160**, 3194.

**Lee BN**, Lu JG, Kline MW et al (1996) Type 1 and type 2 cytokine profiles in children exposed to or infected with vertically transmitted human immunodeficiency virus. Clin Diagn Lab Immunol **3**, 493.

**Lee CH**, Rhee CS, Oh SH et al (1997a) Increase in expression of IL-4 and IL-5 mRNA in the nasal mucosa of patients with perennial allergic rhinitis during natural allergen exposure. Ann Otol Rhinol Laryngol **106**, 215.

**Lee HM**, Timme TL, Thompson TC (2000a) Resistance to lysis by cytotoxic T cells: a dominant effect in metastatic mouse prostate cancer cells. Cancer Res **60**, 1927.

**Lee JK**, Sayers TJ, Brooks AD et al (2000b) IFN-gamma–dependent delay of in vivo tumor progression by Fas overexpression on murine renal cancer cells. J Immunol **164**, 231.

**Lee MW**, Carroll R, Amelse R (1992) Influence of sequental vs simultaneous treatment with alpha or gamma interferon (IFN) and interleukin-2 (IL-2) on the lytic activity of murine lak cells against B16BL6 (B16) melanoma cells in vitro (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **33**, A1954.

**Lee PJ**, Annunziato P (1998) Curent management of herpes zoster. Infect Med **15**, 709.

**Lee YC**, Park MH, Baek NH (1991) Effect of gamma interferon on fibroblast proliferation and collagen synthesis after glaucoma filtering surgery in white rabbits. Korean J Ophthalmol **5**, 59.

**Lee YJ**, Han Y, Lu HT et al (1997b) TGF-beta suppresses IFN-gamma induction of class II MHC gene expression by inhibiting class II transactivator messenger RNA expression. J Immunol **158**, 2065.

**Lefkowitz EJ**, Fleischmann WR (1985a) An inhibitor of interferon action: I. Physical association of the inhibitor with interferon-gamma. J Interferon Res **5**, 85.

**Lefkowitz EJ**, Fleischmann WR (1985b) An inhibitor of interferon action: II. Biological properties of the IFN-γ–associated inhibitor of interferon action. J Interferon Res **5**, 101.

**Leib DA**, Harrison TE, Laslo KM et al (1999) Interferons regulate the phenotype of wild-type and mutant herpes simplex viruses in vivo. J Exp Med **189**, 663.

**Leigh JE**, Steele C, Wormley FL Jr et al (1998) Th1/Th2 cytokine expression in saliva of HIV-positive and HIV-negative individuals: A pilot study in HIV-positive individuals with oropharyngeal candidiasis. J Acquir Immune Defic Syndr Hum Retrovirol **19**, 373.

**Leinikki PO**, Calderon J, Luquette MH, Schreiber RD (1987) Reduced receptor binding by a human interferon-gamma fragment lacking 11 carboxyl-terminal amino acids. J Immunol **139**, 3360.

**Leissner P**, Calenda V, Marigliano M et al (1998) In vitro and in vivo inhibition of HIV1 replication by retroviral transfer of interferon alpha, beta, or gamma genes: application to gene therapy of AIDS. Ann Biol Clin (Paris) **56**, 167.

**Lejeune F**, Lienard D, Eggermont A et al (1995) Efficacy of tumor necrosis factor-alpha (rTNF-alpha) associated to interferon-gamma (IFN-gamma) and to chemotherapy in isolated limb perfusion for inoperable malignant melanoma, soft tissue sarcoma and epidermoid carcinoma: A 4 year experience. Bull Cancer **82**, 561.

**Lekstrome-Himes JA**, Holland SM, DeCarlo ES et al (1994) Treatment with intralesional granulocyte instillation and interferon gamma for a patient with chronic granulomatous disease and multiple hepatic abscesses. Clin Infect Dis **19**, 770.

**Lekutis C**, Shiver JW, Lui MA et al (1997) HIV-1 env DNA vaccine administered to rhesus monkeys elicits MHC class II–restricted CD4+T helper cells that secrete IFN-gamma and TNF-alpha. J Immunol **158**, 4471.

**Lemire JM**, Archer DC, Beck L, Spiegelberg HL (1995) Immunosuppressive actions of 1.25-dihydroxyvitamin D3: Preferential inhibition of Th1 functions. J Nutr **125**(Suppl), 1708.

**Lemmel EM**, Brackertz D, Franke M et al (1988a). Results of a multicenter placebo-controlled double-blind randomized phase III clinical study of treatment of rheumatoid arthritis with recombinant interferon-gamma. Rheumatol Int **8**, 87.

**Lemmel EM**, Obert HJ, Hofschneider PH (1988b). Erfolgreiche Therapie der rheumatoiden. Arthritis mit niedrig dosiertem IFN-gamma. Lancet (Deutche Ausgabe) **2**, 415.

**Lemmel EM**, Franke M, Gaus W et al (1987) Results of a phase-II clinical trial on treatment of rheumatoid arthritis with recombinant interferon-gamma. Rheumatol Int **7**, 127.

**Leonard C**, Tormey V, Burke C, Poulter LW (1997) Allergen-induced cytokine production in atopic disease and its relationship to disease severity. Am J Respir Cell Mol Biol **17**, 368.

**Leonardi A**, DeFranchis G, Zancanaro F et al (1999) Identification of local Th2 and Th0 lymphocytes in vernal conjunctivitis by cytokine flow cytometry. Invest Ophthalmol Vis Sci **40**, 3036.

**Le Page C**, Sanceau J, Drapier JC, Wietzerbin J (1996) Differential expression of inducible NO synthase in two murine macrophage cell lines. Immunology **89**, 274.

**Le Roy F**, Brown KA, Greaves MW et al (1991a) Blood mononucler cells from patients with psoriasis exhibit an enhanced adherence to cultured vascular endothelium. J Invest Dermatol **97**, 511.

**Le Roy X**, Escot C, Brouillet JP et al (1991) Decrease of c-erbB-2 and c-myc RNA levels in tamoxifen-treated breast cancer. Oncogene **6**, 431.

**Leslie KO** (1994) Gamma-interferon in the treatment of fibrocontractive disorders of the skin. Plast Reconstr Surg **94**, 1100.

**Leung BS**, Stout LE, Shaskan EG, Thompson RM (1992) Differential induction of indoleamine-2,3-dioxygenase (IDO) by interferon-gamma in human gynecologic cancer cells. Cancer Lett **66**, 77.

**Leung DY** (1997) Immunologic basis of chronic allergic diseases: clinical messages from the laboratory bench. Pediatr Res **42**, 559.

**Leung DY** (1998) Molecular basis of allergic diseases. Mol Genet Metab **63**, 157.

**Levin M**, Newport MJ, D'Souza S et al (1995) Familial disseminated atypical mycobacterial infection in childhood: a human mycobacterial susceptibility gene? [comments]. Lancet **345**, 79.

**Levin S** (1984) Interferon α therapy in severe viral disease. Proceedings of the Meeting European Group Immunodeficiencies, Elsevier, Amsterdam.

**Levin S**, Hahn T, Rosenberg H, Bino T (1982) Treatment of life-threatening viral infections with interferon α: pharmacokinetic studies in a clinical trial. Israel J Med Sci **18**,439.

**Levy-Strumpf N**, Deiss LP, Berissi H, Kimchi A (1997) DAP-5, a novel homolog of eukaryotic translation initiation factor 4G isolated as a putative modulator of gamma interferon-induced programmed cell death. Mol Cell Biol **17**,1615.

**Lewandowski G**, Hobbs M, Geller A (1998) Evidence that deficient IFN-gamma production is a biological basis of herpes simplex virus type-2 neurovirulence. J Neuroimmunol **81**, 66.

**Li J**, Liu YL, Sha JY (1994) Anti-proliferative effects of tumor necrosis factor and gamma interferon on human ovarian cancer cell lines. Chung Hua Fu Chan Ko Tsa Chih **29**, 670, 700.

**Li J**, Hui N, Zhu L (1995) Synergistic effects of tumor necrosis factor interferon-gamma and chemotherapeutic drugs on human ovarian cancer cell lines in vitro. Chin J Omcol **17**, 429.

**Li L**, Kilbourn RG, Adams J, Fidler IJ (1991) Role of nitric oxide in lysis of tumor cells by cytokine-activated endothelial cells. Cancer Res **51**, 2531.

**Liang CM**, Heren S, Fountoulakis M et al (1985) Analysis of antibody response in patients receiving recombinant *Escherichia coli*–derived human interferon-gamma. Clin Immunol Immunopathol **36**, 95.

**Libby P**, Aikawa M (1998) New insights into plaque stabilisation by lipid lowering. Drugs **56**, 9.

**Libby P**, Schoenbeck U, Mach F et al (1998) Current concepts in cardiovascular pathology: the role of LDL cholesterol in plaque rupture and stabilization. Am J Med **104**, 14.

**Liebert M**, Stein J, Wedemeyer G, Grossman HB (1991) Changes in integrin expression and function on the UM-UC-9 human bladder cancer cell line after treatment with biologic response modifiers (meeting abstract) Proc Annu Meet Am Assoc Cancer Res **32**, A366.

**Lienard D**, Ewalenko P, Delmotte JJ et al (1992a) High-dose recombinant tumor necrosis factor alpha in combination with interferon gamma and melphalan in isolation perfusion of the limbs for melanoma and sarcoma. J Clin Oncol **10**, 52.

**Lienard D**, Lejeune FJ, Ewalenko P (1992b) In transit metastases of malignant melanoma treated by high dose rTNF alpha in combination with interferon-gamma and melphalan in isolation perfusion. World J Surg **16**, 234.

**Lienard D**, Eggemont AMM, Schraffordt Koops H et al (1994) Isolated perfusion of the limb with high-dose tumor necrosis factor-alpha (TNF-alpha), interferon-gamma (IFN-gamma) and melphalan for melanoma stage III. Results of a multicentre pilot study. Melanoma Res **4**(Suppl 1), 21.

**Littman SJ**, Devos R, Baglioni C (1985) Binding of unglycosylated and glycosylated human recombinant interferon γ to cellular receptors. J Interferon Res **5**, 471.

**Liu X**, Xing Z (1997) A study on rIL-4 and IFN-gamma regulating IgE synthesis in bronchial asthma patients. Chin Med J **110**, 760.

**Livingston DH**, Malangoni MA (1988) Interferon-γ restores immune competence after hemorrhagic shock. J Surg Res **45**, 37.

**Livingston DH**, Appel SH, Sonnenfeld G, Malangoni MA (1989) The effect of tumor necrosis factor-α and interferon-γ on neutrophil function. J Surg Res **46**, 322.

**Loh JE**, Schindler C, Ziemiecki A et al (1994) Mutant cell lines unresponsive to alpha/beta and gamma interferon are defective in tyrosine phosphorylation of ISGF-3 alpha components. Mol Cell Biol **14**, 2170.

**Lollini PL**, De Giovanni C, Nicoletti G et al (1990) Enhancement of experimental metastatic ability by tumor necrosis factor-alpha alone or in combination with interferon-gamma. Clin Expl Metastasis **8**, 215.

**Lollini PL**, Bosco MC, Cavallo F et al (1993) Inhibition of tumor growth and enhancement of metastasis after transfection of the gamma-interferon gene. Int J Cancer **55**, 320.

**Lord SC**, McMullen GL, Marzuki S, Cheetham BF (1989) Functional domains of human interferon gamma probed with antipeptide antibodies**.** Mol Immunol **26**, 637.

**Lorenzen J**, Lewis CE, McCracken D et al (1991) Human tumor-associated NK cells secrete increased amounts of interferon-γ and interleukin-4. Br J Cancer **64**, 457.

**Lotz M**, Tsoukas CD, Fong S et al (1986). Release of lymphokines following infection with Epstein-Barr virus *in vitro.* II. A monocyte dependent inhibitor of interleukin-1 downregulates the production of interleukin-2 and gamma interferon in reumathoid arthritis. J Immunol **136**, 3643.

**Lowes MA**, Bishop GA, Crotty K et al (1997) T helper 1 cytokine mRNA is increased in spontaneously regressing primary melanomas. J Invest Dermatol **108**, 914.

**Lu CZ**, Jensen MA, Arnason BG (1993) Interferon gamma- and interleukin-4–secreting cells in multiple sclerosis. J Neuroimmunol **46**, 123.

**Lucin P**, Jonjic S, Messerle M et al (1994) Late phase inhibition of murine cytomegalovirus replication by synergistic action of interferon-gamma and tumour necrosis factor. J Gen Virol **75**, 101.

**Luk KS**, Jay E, Jay FT (1990) Structure function analysis of the human interferon γ. The COOH terminus is not essential for functional activity. J Biol Chem **265**, 13314.

**Lukina GV**, Sigidin IA, Skurkovich SV, Skurkovich BS (1998) New approaches to biological immunomodulation therapy of rheumatoid arthritis: neutralization of basic cytokines. Ter Arkh **70**, 32.

**Lummen G**, Goepel M, Mollhoff S et al (1996) Phase II study of interferon-gamma versus interleukin-2 and interferon-alpha 2b in metastatic renal cell carcinoma. J Urol **155**, 455.

**Lundell D**, Lunn C, Dalgarno D et al (1991) The carboxyl-terminal region of human interferon gamma is important for biological activity: Mutagenic and NMR analysis. Protein Eng **4**, 335.

**Lunn CA**, Davies L, Dalgarno D et al (1992a) An active covalently linked dimer of human interferon-γ. Subunit orientation in the native protein. J Biol Chem **267**, 17920.

**Lunn CA**, Fossetta J, Dalgarno D et al (1992b) A point mutation of human interferon gamma abolishes receptor recognition. Protein Eng **5**, 253.

**Luster AD**, Leder P (1993) IP-10, a -C-X-C- chemokine, elicits a potent thymus-dependent antitumor response in vivo. J Exp Med **178**, 1057.

**Luty AJ**, Lell B, Schmidt-Ott R et al (1999) Interferon-gamma responses are associated with resistance to reinfection with Plasmodium falciparum in young African children. J Infect Dis **179**, 980.

**Luzzati AL**, Boirivant M, Giacomini E et al (1996) Interferon gamma (IFN-gamma) can counteract the in vitro inhibitory effect of an HIV p24 immunosuppressive heptapeptide. Clin Exp Immunol **105**, 403.

**Lygidakis NJ**, Kosmidis P, Ziras N et al (1995) Combined transarterial targeting locoregional immunotherapy-chemotherapy for patients with unresectable hepatocellular carcinoma; A new alternative for an old problem. J Interferon Cytokine Res **15**, 467.

**Lyons A**, Goebel A, Mannick JA, Lederer JA (1999) Protective effects of early interleukin 10 antagonism on injury-induced immune dysfunction. Arch Surg **134**, 1317.

**Lyons AB**, Ashman LK (1987) The effect of recombinant cytokines on the proliferative potential and phenotype of cells of the human myelomonocytic leukaemia line, RC-2A. Leukoc Res **12**, 659.

## M

**Maas IW**, Boven E, Pinedo HM et al (1991) The effects of gamma-interferon combined with 5-fluorouracil or 5-fluoro-2′-deoxyuridine on proliferation and antigen expression in a panel of human colorectal cancer cell lines. Int J Cancer **48**, 749.

**Macaleff MJ**, Yoshida, K, Kawai S et al (1997) In vivo antitumor effects of murine interferon gamma–inducing factor/interleukin-18 in mice bearing syngenic Meth A sarcoma malignant ascites. Cancer Immunol Immunother **43**, 361.

**Machida T**, Koiso K, Takaku F, Ogawa M (1987) Phase II study of recombinant Human Interferon Gamma (S-6810) in renal cell carcinoma. Urological cooperative study group of recombinant human interferon gamma (S 6810). Gan To Kagaaku Ryoho **14**, 440.

**MacKenzie CR**, Hadding U, Daubener W (1998) Interferon-gamma–induced activation of indoleamine 2,3-dioxygenase in cord blood monocyte-derived macrophages inhibits the growth of group B streptococci. J Infect Dis **178**, 875.

**Madariaga L**, Amurrio C, Martin G et al (1998) Detection of anti–interferon-gamma autoantibodies in subjects infected by Mycobacterium tuberculosis. Int J Tuberc Lung Dis **2**, 62.

**Magazine HI**, Carter JM, Russel JK et al (1988) Use of synthetic peptides to identify a N-terminal epitope on mouse gamma interferon that may be involved in function. Proc Natl Acad Sci USA **85**, 1237.

**Maggi E**, Manetti R, Annunziato F, Romagnani S (1995) CD8+ T lymphocytes producing Th2-type cytokines (Tc2) in HIV-infected individuals. J Biol Regul Homeost Agents **9**, 78.

**Maggi E**, Manetti R, Annunziato F et al (1997) Functional characterization and modulation of cytokine production by CD8+ T cells from human immunodeficiency virus–infected individuals. Blood **89**, 3672.

**Magnan AO**, Mely LG, Camilla CA et al (2000) Assessment of the Th1/Th2 paradigm in whole blood in atopy and asthma. Increased IFN-gamma–producing CD8(+) T cells in asthma. Am J Respir Crit Care Med **161**, 1790.

**Mahaley MS Jr**, Bertsch L, Cush S, Gillespie GY (1988) Systemic gamma-interferon therapy for recurrent gliomas. J Neurosurg **69**, 826.

**Maheshwari RK** (1990) The role of cytokines in malaria infection. Bull WHO **68**(Suppl), 138.

**Mahmoud HH**, Pui CH, Kennedy W et al (1992) Phase I study of recombinant human interferon gamma in children with relapsed acute leukemia. Leukemia **6**, 1181.

**Mahrle G**, Schulze HJ (1990) Recombinant interferon-gamma (rIFN-gamma) in dermatology. J Invest Dermatol **95**, 132.

**Main EK**, Monos DS, Lampson LA (1988) IFN treated neuroblastoma cell lines remain resistant to T cell mediated allo-killing and susceptible to non-MHC restricted cytotoxicity. J Immunol **141**, 2943.

**Maiolo AT**, Cortelezzi A, Calori R, Polli EE (1990) Recombinant γ-interferon as first line therapy for high risk myelodysplastic syndromes. Leukemia **4**, 480.

**Majewski S**, Hunzelmann N, Schirren CG et al (1992) Increased adhesion of fibroblasts from patients with scleroderma to extracellular matrix components: In vitro modulation by IFN-gamma but not by TGF-beta. J Invest Dermatol **98**, 86.

**Makgoba MW**, Sanders ME, Ginter-Luce GE et al (1988a) Functional evidence that intercellular adhesion molecule-1 (ICAM-1) is a ligand for LFA-1–dependent adhesion in T cell–mediated cytoxicity. Eur J Immunol **18**, 637.

**Makgoba MW**, Sanders ME, Ginther-Luce GE et al (1988b) ICAM-1: A ligand for LFA-1-dependent adhesion of B, T and myeloid cells. Nature **331**, 86.

**Malaise MG**, Franchimont P (1987) Defective *in vitro* γ-interferon production in rheumatoid arthritis. Arthritis Rheum **30**, 230.

**Maleci A**, Ausiello C, Gagliardi FM et al (1988) The response of peripheral blood mononuclear cells of glioma-bearing patents to stimulation with microbial antigen and IL-2: Proliferation and IFN-gamma production. Acta Neurochir Wien **43**(Suppl), 130.

**Mallat Z**, Besnard S, Duriez M et al (1999) Protective role of interleukin-10 in atherosclerosis. Circ Res **85**, 17.

**Malmvall BE**, Follin P (1993) Successful interferon-γ therapy in a chronic granulomatous disease (CGD) patient suffering from staphylococcus aureus hepatic abscess and invasive candida albicans infection. Scand J Infect Dis **25**, 61.

**Maluish AE**, Urba WJ, Longo DL et al (1988) The determination of an immunologically active dose of interferon-gamma in patients with melanoma. J Clin Oncol **6**, 434.

**Mamus SW**, Beck-Schroeder S, Zanjani ED (1985) Suppression of normal human erythropoiesis by gamma interferon in vitro. Role of monocytes and T-lymphocytes. J Clin Invest **75**, 1496.

**Mandelson J**, Clecner B, Eiley SH (1986) Effect of interferon α2 on clinical course of first episode genital herpes infection and subsequent recurrences Genitourin Med **62**, 97.

**Manetta A**, Podczaski E, Zaino RJ, Satyaswaroop PG (1989) Therapeutic effect of recombinant human tumor necrosis factor in ovarian carcinoma xenograft in nude mice. Gynecol Oncol **34**, 360.

**Mani S**, Poo WJ (1996). Single institution experience with recombinant gamma-interferon in the treatment of patients with metastatic renal cell carcinoma. Am J Clin Oncol **19**, 149.

**Mao C**, Aguet M, Merlin G (1989). Molecular characterization of the human interferon γ receptor: Analysis of polymorphism and glycosylation. J Interferon Res **9**, 659.

**Mao XW**, Kettering JD, Gridley DS (1995) Immunotherapy with low-dose interleukin-2 and interferon-gamma in a murine tumor model. J. Interferon Cytokine Res **15**, 1017.

**Marcucci F**, Waller M, Kirchner H, Krammer P (1981) Production of immune interferon by murine T cell clones from long-term cultures. Nature **291**, 79.

**Margolin KA**, Doroshow JH, Akman SA et al (1992) Phase I trial of interleukin-2 plus gamma-interferon. J Immunother **11**, 50.

**Margolis S** (1982) Therapy for condyloma acuminatum: a review. Rev Infect Dis **4**(Suppl), S829.

**Marlin SD**, Springer TA (1987) Purified intercellular adhesion molecule-I (ICAM-I) is a ligand for lymphocyte function–associated antigen-I (LFA-I). Cell **51**, 813.

**Martens MF**, Huyben CM, Hendriks T (1992) Collagen synthesis in fibroblasts from human colon: Regulatory aspects and differences with skin fibroblasts. Gut **33**, 1664.

**Marth C**, Daxenbichler G, Dapunt O (1986) Synergistic antiproliferative effect of human recombinant interferons and retinoic acid in cultured breast cancer cells. J Natl Cancer Inst **77**, 1197.

**Marth C**, Gastl G, Zech J et al (1987a) Characterization of an interferon-resistant mutant of the human breast cancer cell line BT-20. J Interferon Res **7**, 195.

**Marth C**, Zech J, Bock G et al (1987b). Effects of retinoids and interferon-gamma on cultured breast cancer cells in comparison with tumor necrosis factor alpha. Int J Cancer **40**, 840.

**Marth C**, Fuith LC, Bock G et al (1989a) Modulation of ovarian carcinoma tumor marker CA-125 by gamma-interferon. Cancer Res **49**, 6538.

**Marth C**, Helmberg M, Mayer I et al (1989b) Effects of biological response modifiers on ovarian carcinoma cell lines. Anticancer Res **9**, 461.

**Marth C**, Kirchebner P, Daxenbichler G (1989c). The role of polyamines in interferon and retinoic acid mediated synergistic antiproliferative action. Cancer Lett **44**, 55.

**Marth C**, Muller Holzner E, Greiter E et al (1990). Gamma-interferon reduces expression of the protooncogene c-erbB-2 in human ovarian carcinoma cells. Cancer Res **50**, 7037.

**Marth C**, Cronauer MV, Doppler W et al (1992) Effects of interferons on the expression of the proto-oncogene HER-2 in human ovarian carcinoma cells. Int J Cancer **50**, 64.

**Marth C**, Widschwendter M, Daxenbichler G (1993) Mechanism of synergistic action of all-trans- or 9-cis-retinoic acid and interferons in breast cancer cells. J Steroid Biochem Mol Biol **47**, 123.

**Marth C**, Zeimet AG, Herold M et al (1996) Different effects of interferons, interleukin-1 beta and tumor necrosis factor-alpha in normal (OSE) and malignant human ovarian epithelial cells. Int J Cancer **67**, 826.

**Martin DM**, Carlson RO, Feldman EL (1993a) Interferon-gamma inhibits DNA synthesis and insulin-like growth factor-II expression in human neuroblastoma cells. J Neurosci Res **34**, 489.

**Martin E**, Nathan C, Xie QW (1994) Role of interferon regulatory factor 1 in induction of nitric oxide synthase. J Exp Med **180**, 977.

**Martin RJ**, Boguniewicz M, Henson JE et al (1993b) The effects of inhaled interferon gamma in normal human airways. Am Rev Respir Dis **148**, 1677.

**Martinon F**, Michelet C, Peguillet I et al (1999) Persistent alterations in T-cell repertoire, cytokine and chemokine receptor gene expression after 1 year of highly active antiretroviral therapy. AIDS **13**, 185.

**Marubayashi M**, Solana R, Ramirez R et al (1991) Sera from patients with colon, breast and lung cancer induce resistance to lysis mediated by NK cytotoxic factors (NKCF). Br J Cancer **63**, 893.

**Massad LS**, Mutch DG, Powal CB et al (1990) Expression of a resistance mechanism in ovarian and cervical carcinoma cells prevents their lysis by gamma-interferon. Cancer Res **50**, 4923.

**Masumori N**, Tsukamoto T, Kumamoto Y (1995) Interferon-gamma and interleukin-2 suppress the experimental metastasis of mouse renal adenocarcinoma. Int J Urol **2**, 6.

**Mathe G**, Colasante U, Morette C et al (1996) Will killing the last HIV1 particle cure AIDS patients? II: Second part. Decrease of viral load and of T-suppressor cells, and increase of the cytotoxic cells, without effect on CD4, after the use of 10 virostatics applied in 3 or 4 drug combinations of different sequences. The time for CD4 immunotherapy? Biomed Pharmacother **50**, 473.

**Matory YL**, Chen M, Dorfman DM et al (1995) Antitumor activity of three mouse mammary cancer cell lines after interferon-gamma gene transfection. Surgery **118**, 251.

**Matsui T**, Takahashi R, Mihara K et al (1985) Cooperative regulation of c-myc expression in differentiation of human promyelocytic leukemia induced by recombinant gamma-interferon and 1.25-dihydroxyvitamin D-3. Cancer Res **45**, 4366.

**Matsui T**, Nakao Y, Koizumi T et al (1988). Effect of prostaglandin E-2 on gamma-interferon and 1.25 (OH)-2D–3 vitamin D-3-induced c-myc reduction during HL-60 cell differentiation. Leukoc Res **12**, 597.

**Matsumoto T** (1991) Ongoing IgE synthesis by atopic B cells is enhanced by interleukin-3 and suppressed directly by interferon-gamma in vitro. Int Arch Allergy Appl Immunol **95**, 48.

**Matsumura H**, Onozuka K, Terada Y et al (1990) Effect of murine recombinant interferon-γ in the protection of mice against *Salmonella*. Int J Immunopharmacol **12**, 49.

**Matsuo S**, Takano S, Yamashita J, Ogawa M (1992) Synergistic cytotoxic effects of tumor necrosis factor, interferon-gamma and tamoxifen on breast cancer cell lines. Anticancer Res **12**, 1575.

**Matsushita K**, Takenouchi T, Kobayashi S et al (1996) HLA-DR antigen expression in colorectal carcinomas: influence of expression by IFN-gamma in situ and its association with tumour progression. Br J Cancer **73**, 644.

**Mattila P**, Hayry P, Renkonen R (1989) Protein kinase C is crucial in signal transduction during IFN-γ induction in endothelial cells. FEBS Lett **250**, 362.

**Mattson K**, Niiranen A, Pyrhonen S et al (1991) Recombinant interferon gamma treatment in non-small cell lung cancer. Antitumour effect and cardiotoxicity. Acta Oncol **30**, 607.

**Maurice MM**, van der Graaff WL, Leow A et al (1999) Treatment with monoclonal anti-tumor necrosis factor alpha antibody results in an accumulation of Th1 CD4+ T cells in the peripheral blood of patients with rheumatoid arthritis. Arthritis Rheum **42**, 2166.

**Mayer A**, Gelderblom H, Kumel G, Jungwirth C (1992) Interferon-gamma-induced assembly block in the replication cycle of adenovirus 2: Augmentation by tumor necrosis factor-alpha. Virology **187**, 372.

**Maziarsz RT**, Mentzer SJ, Burakoff SJ, Faller DV (1990) Distinct effects of interferon γ and MHC class I surface antigen levels on resistance of the K562 tumor cell line to natural killer-mediated lysis. Cell Immunol **130**, 329.

**McAveney KM**, Gomella LG, Lattime EC (1994) Cytokine regulation of antitumor immunity following localized immunotherapy of blader cancer (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **35**, A2863.

**McCachren SS Jr**, Salehi Z, Weinberg JB, Niedel JE (1988) Transcription interruption may be a common mechanism of c-myc regulation during HL-60 differentiation. Biochem Biophys Res Commun **151**, 574.

**McCauley RL**, Chopra V, Li YY et al (1992) Altered cytokine production in black patients with keloids. J Clin Immunol **12**, 300.

**McDonald CF**, Atkins RC (1990) Defective cytostatic activity of pulmonary alveolar macrophages in primary lung cancer. Chest **98**, 881.

**McDyer JF**, Hackley MN, Walsh TE et al (1997) Patient with multidrug-resistant tuberculosis with low CD4+T cell counts have impaired Th1 responses. J Immunol **158**, 492.

**McFarland EJ**, Harding PA, MaWhinney S et al (1998) In vitro effects of IL-12 on HIV-1–specific CTL lines from HIV-1–infected children. J Immunol **161**, 513.

**McGlave P**, Arthur D, Miller WJ et al (1990) Autologous transplantation for CML using marrow treated ex vivo with recombinant human interferon gamma. Bone Marrow Transplant **6**, 115.

**McGlave P**, Mamus S, Vilen B, Dewald G (1987) Effect of recombinant gamma interferon on chronic myelogenous leukemia bone marrow progenitors. Exp Hematol **15**, 331.

**McGlave P**, Verfaillie C, Miller J (1993) Chronic myelogenous leukemia: In search of the benign hematopoietic stem cell. Stem Cells **11**, 10.

**McKimm-Breschkin JL**, Mottram PL, Thomas WR, Miller JFAP (1982) Antigen specific production of immune interferon by T cell lines. J Exp Med **155**, 1204.

**McKolanis JR**, Ragab AH, Schmid DS (1991) In vitro enhancement of natural killer cell activity against herpes virus-infected targets in patients with acute lymphocytic leukemia. Arch Virol **117**, 17.

**Means RT Jr**, Krantz SB, Luna J, Marsters SA, Ashkenazi A (1994) Inhibition of murine erythroid colony formation in vitro by interferon gamma and correction by interferon receptor immunoadhesin. Blood **83**, 911.

**Mechti N**, Bisbal C, Leonetti JP et al (1988) Interferons and oncogenes in the control of cell growth and differentiation: Working hypothesis and experimental facts. Biochimie **70**, 869.

**Meert KL**, Ofenstein JP, Sarnaik AP (1998) Altered T cell cytokine production following mechanical trauma. Ann Clin Sci **28**, 283.

**Meffert M**, Duensing S, Atzpodien J (1997) Dose distribution of low-dose subcutaneous recombinant interleukin-2 affects the secondary release of interferon-gamma in vivo. Cancer Biother Radiopharm **12**, 149.

**Meissner K**, Weyer U, Kowalzick L, Altenhoff J (1991) Successful treatment of primary progressive follicular mucinosis with interferons. J Am Acad Dermatol **24**, 848.

**Meissner N**, Kussebi F, Jung T et al (1997) A subset of CD8+ T cells from allergic patients produce IL-4 and stimulate IgE production in vitro. Clin Exp Allergy **27**, 1402.

**Melin M**, Hartmann DJ, Magloire H et al (1989). Human recombinant gamma interferon stimulates proliferation and inhibits collagen and fibronectin production by human dental pulp fibroblasts. Cell Mol Biol **35**, 97.

**Melis M**, Spatafora M, Melodia A et al (1996) ICAM-1 expression by lung cancer cell lines: Effects of upregulation by cytokines on the interaction with LAK cells. Eur Respir J **9**, 1831.

**Melissen PM**, van Vianen W, Bidjai O et al (1993) Free versus liposome-encapsulated muramyl tripeptide phosphatidylethanolamide (MTPPE) and interferon-γ (IFN-γ) in experimental infection with Listeria monocytogenes. Biotherapy **6**, 113.

**Mellors JW**, Debs RJ, Ryan JL (1989) Incorporation of recombinant gamma interferon into liposomes enhances its ability to induce peritoneal macrophage antotoxoplasma activity. Infect Immun **57**, 132.

**Mellouk S**, Green SJ, Nacy CA, Hoffman SL (1991) IFN-γ inhibits development of *Plasmodium berghei* exoerythrocytic stages in hepatocytes by an L-arginine-dependent effector mechanism. J Immunol **146**, 3971.

**Mellouk S**, Hoffman SL, Liu ZZ et al (1994) Nitric oxide-mediated antiplasmodial activity in human and murine hepatocytes induced by gamma interferon and the parasite itself: enhancement by exogenous tetrahydrobiopterin. Infect Immun **62**, 4043.

**Melnick JL**, Adam E, Debakey ME (1993) Cytomegalovirus and atherosclerosis. Eur Heart J **14**(Suppl K), 30.

**Melnick JL**, Adam E, Debakey ME (1996) Cytomegalovirus and atherosclerosis. Arch Immunol Ther Exp Warsz **44**, 297.

**Mendelson J**, Clecner BY, Eiley S (1986) Effect of recombinant interferon alpha 2 on clinical course of first episode genital herpes infection and subsequent recurrences. Genitourin Med **62**, 97.

**Mendiratta SK**, Quezada A, Matar M et al (1999) Intratumoral delivery of IL-12 gene by polyvinyl polymeric vector system to murine renal and colon carcinoma results in potent antitumor immunity. Gene Ther **6**, 833.

**Menschikowski M**, Rosner-Schiering A, Eckey R et al (2000) Expression of secretory group IIA phospholipase A (2) in relation to the presence of microbial agents, macrophage infiltrates, and transcripts of proinflammatory cytokines in human aortic tissues. Arterioscler Thromb Vasc Biol **20**, 751.

**Merlin G**, Falcoff E, Aguet M (1985) I-Labelled human interferons- α, β and γ: Comparative receptor binding data. J Gen Virol **66**, 1149.

**Meske S**, Weber S, Wirzberger M, Peter HH (1989) Die therapie der rheumatoiden arthritis mit rekombinantem interferon gamma (rIFN-γ). Z Rheumatol **48**, 101.

**Metcalf DR**, Campbell PA (1994) How does gamma interferon mediate ressistance to Listeria monocytogenes? Trends Microbiol **2**, 206

**Meterissian S**, Angelo L, Owen-Schaub L (1994) Expression of Fas/APO-1 antigen on human melanoma cells (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **35**, A40.

**Meurs EF**, Galabru J, Barber GN et al (1993) Tumor suppressor function of the interferon-induced double-stranded RNA-activated protein kinase. Proc Natl Acad Sci USA **90**, 232.

**Meyaard L**, Otto SA, Keet IP et al (1994) Changes in cytokine secretion patterns of CD4+ T-cell clones in human immunodeficiency virus infection. Blood **84**, 4262.

**Meyaard L**, Hovenkamp E, Keet IP et al (1996) Single cell analysis of IL-4 and IFN-gamma production by T cells from HIV infected individuals: Decreased IFN-gamma in the presence of preserved IL-4 production. J Immunol **15**, 2712.

**Meyskens FL** Jr, Kopecky K, Samson M et al (1991) A phase III trial of recombinant human interferon-gamma (IFN) as adjuvant therapy of high risk malignant melanoma (MM) (meeting abstract). Proc Annu Meet Am Soc Clin Oncol **10**, A1019.

**Mezey E** (1991) Approaches to treatment of fibrogenesis in alcoholic liver disease. Alcohol Alcohol Suppl **1**, 363.

**Micallef MJ**, Yoshida K, Kawai S et al (1997) In vivo antitumor effects of murine interferon-gamma-inducing factor/interleukin-18 in mice bearing syngeneic Meth A sarcoma malignant ascites. Cancer Immunol Immunother **43**, 361.

**Michalski WP**, Shiell BJ, O`Neil TE et al (1999) Recombinant chicken IFN-gamma expressed in Escherichia coli: Analysis of C-terminal truncation and effect of biologic activity. J Interferon Cytokine Res **19**, 383.

**Michelini-Norris MB**, Blanchard DK, Barton DPJ et al (1996) Direct tumoricidal function against human ovarian carcinoma by interferon-gamma and tumor necrosis factor alpha activated human peritoneal mesothelial cells. Int J Gynecol Cancer **6**, 27.

**Michiels L**, Haelewyn J, Proost P, De Ley M (1998) The soluble extracellular portion of the human interferon-gamma receptor is a valid substitute for evaluating binding characteristics and for neutralizing the biological activity of this cytokine. Int J Biochem Cell Biol **30**, 505.

**Mikovits JA**, Young HA, Vertino P et al (1998) Infection with human immunodeficiency virus type 1 upregulates DNA methyltransferase, resulting in de novo methylation of the gamma interferon (IFN-gamma) promoter and subsequent downregulation of IFN-gamma production. Mol Cell Biol **18**, 5166.

**Miles RH**, Paxton TP, Zacheis D et al (1994) Systemic administration of interferon-gamma impairs wound healing. J Surg Res **56**, 288.

**Miller BA**, Olivieri N, Hope SM et al (1990) Interferon-γ modulates fetal hemoglobin synthesis in sickle cell anemia and thalassemia. J Interferon Res **10**, 357.

**Milligan GN**, Bernstein DI (1997) Interferon-gamma enhances resolution of herpes simplex virus type 2 infection of the murine genital tract. Virology **229**, 259.

**Minoda M**, Ohno M, Hamada K et al (1991) Interferon-gamma inhibition of interleukin 4–induced hyperproliferation of resting B cell from NZB/NZW F1 mice. J Clin Lab Immunol **35**, 57.

**Minoda M**, Ohno M, Tomioka Y et al (1992) Effects of gamma-interferon and FK506 on resting B cell proliferation of New Zealand black/white F1 mice. Microbiol Immunol **36**, 885.

**Mironova R**, Toshimitsu N, Hayashi H et al (2001) Evidence for non-enzymatic glycosylation in Escherichia colli. Mol Microbiol **39**, 1061.

**Mishra S**, Hamburger AW (1994) Interferon gamma-induced reduction in erbB-2 tyrosyl phosporylation in human ovarian carcinoma cells. Int J Cancer **58**, 538.

**Mistchenko AS**, Falcoff R (1987) Recombinant human interferon-γ inhibits adenovirus multiplication *in vitro*. J Gen Virol **68**, 941.

**Mistchenko AS**, Diez RA, Falcoff R (1987) Recombinant human interferon-γ inhibits adenovirus multiplication without modifying viral penetration. J Gen Virol **68**, 2675.

**Mistchenko AS**, Diez RA, Falcoff R (1989) Inhibitory effect of interferon-γ on adenovirus replication and late transcription. Biochem Pharmacol **38**, 1971.

**Mitragotri S**, Blankschtein D, Langer R (1995) Ultrasound-mediated transdermal protein delivery. Science **269**, 850.

**Miura H**, Arai H, Matsuoka A et al (1999) Long-term immunological study on postoperative adjuvant treatment with interferon gamma in patients with renal cell carcinoma who underwent nephrectomy. Hyniokika Kyo **45**, 79.

**Miyake Y**, Ajitsu S, Yamashita T, Sendo F (1988) Enhancement by recombinant interferon-gamma of spontaneous tumor cytostasis by human neutrophils. Mol Biother **1**, 37.

**Mizuno M**, Yoshida J, Oyama H, Sugita K (1992) Growth inhibition of glioma cells by liposome-mediated cell transfection with tumor necrosis factor-alpha gene — Its enhancement by prior gamma-interferon treatment. Neurol Med Chir Tokyo **32**, 873.

**Mizuno M**, Yoshida J, Takaoka T, Sugita K (1994) Liposomal transfection of human gamma-interferon gene into human glioma cells and adoptive immunotherapy using lymphokine-activated killer cells. J Neurosurg **80**, 510.

**Mizutani Y**, Terachi T, Kakehi Y et al (1996) Interferon gamma inhalation in patients with lung metastasis from renal cell carcinoma. Biotherapy **10**, 408.

**Mizutani Y**, Terachi T, Kakehi Y et al (1997) Low toxicity of inhaled interferon-gamma in four patients with lung metastasis from renal cell carcinoma. Oncol Rep **4**, 39.

**Mobus VJ**, Asphal W, Knapstein PG, Kreienberg R (1993) Effects of interferon gamma on the proliferation and modulation of cell-surface structures of human ovarian carcinoma cell lines. J Cancer Res Clin Oncol **120**, 27.

**Mohan K**, Sam H, Stevenson MM (1999) Therapy with a combination of low doses of interleukin 12 and chloroquine completely cures blood-stage malaria, prevents severe anemia, and induces immunity to reinfection. Infect Immun **67**, 513.

**Molinas FC**, Wietzerbin J, Falcoff E (1987) Human platelets possess receptors for a lymphokine: demonstration of high specific receptors for HuIFN-gamma. J Immunol **138**, 802.

**Momosaki S**, Yano H, Ogasawara S et al (1995) Expression of intracellular adhesion molecule 1 in human hepatocellular carcinoma. Hepatology **22**, 1708.

**Montaldo PG**, Carbone R, Corrias MV et al (1994) Synergistic differentiation-promoting activity of interferon gamma and tumor necrosis factor-alpha. Role of receptor regulation on human neuroblasts. J Natl Cancer Inst **86**, 1694.

**Montaldo PG**, Chiesa V, Bado N et al (1997) Induction of differentiation and apoptosis by interferon-gamma in human neuroblastoma cells in vitro as a dual and alternative early biological response. Cell Death Differ **4**, 150.

**Montano L**, Miescher GC, Goodall KH et al (1982) Hepatitis B virus and HLA antigen display in the liver during chronic hepatitis B virus infection. Hepatology **2**, 557.

**Moore JL**, Poiesz BJ, Zamkoff KW et al (1985) Inverse relationship between constitutive gamma-interferon production and human T-cell lymphoma/leukemia virus expression in cultured T lymphocytes. J Virol **53**, 440.

**Moore JM**. Nahlen BL, Misore A et al (1999) Immunity to placental malaria. I. Elevated production of interferon-gamma by placental blood mononuclear cells is associated with protection in an area with high transmission of malaria. J Infect Dis **179**, 1218.

**Morecki S**, Lubina-Salomon A, Slavin S, Nagler A (1998) Cytokine gene transduction into non-immunogeneic murine tumor cells. Cytokines Cell Mol Ther **4**, 87.

**Morhenn VB** (1988) Recombinant interferon gamma in psoriasis. Arch Dermatol **124**, 487.

**Morhenn VB**, Pregerson-Rodan K, Mullen RH et al (1987) Use of recombinant interferon gamma administered intramusculary for the treatment of psoriasis. Arch Dermatol **123**, 1633.

**Mori H**, Hanabayashi T, Yamada Y, Tamaya T (1990) Decrease in interferon-gamma production by peripheral blood mononuclear cells in patients with uterine cervical cancer J Clin Immunol **10**, 45.

**Morikawa K**, Fidler IJ (1989) Heterogeneous response of human colon cancer cells of the cytostatic and cytotoxic effects of recombinant human cytokines: Interferon-$\alpha$, interferon-$\gamma$, tumor necrosis factor and interleukin-1. J Biol Respir Modif **8**, 206.

**Morisaki T**, Hoon DS, Yuzuki D, Morton DL (1992) Expression of IL4 receptors (IL4R) and response to IL4 by human gastric cancer cells (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **33**, A438.

**Morita Y**, Yamamura M, Nishida K et al (1998) Expression of interleukin-12 in synovial tissue from patients with rheumatoid arthritis. Arthritis Rheum **41**, 306.

**Moritz T**, Kirchner H (1986) The effect of interferons on cellular differentiation. Blut **53**, 361.

**Morrisson CJ**, Brummer E, Stevens DA (1989) In vivo activation of peripheral blood polymorphonuclear neutrophils by gamma interferon results in enhanced fungal killing. Infection and Immunity **57**, 2953.

**Morshed SA**, Fukuma H, Kimura Y et al (1993) Interferon-gamma, interleukin (IL)-2 and IL-2 receptor expressions in hepatitis C virus–infected liver. Gastroenterol Jpn **28**, 59.

**Moss RB**, Trauger RJ, Giermakowska WK et al (1997) Effect of immunization with an inactivated gp120-depleted HIV-1 immunogen on beta-chemokine and cytokine production in subjects with HIV-1 infection. J Acquir Immune Defic Syndr Hum Retrovirol **14**, 343.

**Mossman K**, Upton C, McFadden G (1995) The myxoma virus-soluble interferon-gamma receptor homolog, M-T7, inhibits interferon-gamma in a species-specific manner. D Biol Chem **270**, 3031.

**Motoki Y**, Kikkawa Y, Kojima Y et al (1997) The correlation between interleukin-10 and interferon-gamma produced by peripheral blood mononuclear cells stimulated by house dust mite antigen in atopic dermatitis. Arerugi **46**, 376.

**Moulton TA**, Jiang H, Guarini L et al (1992) Induction of growth suppression and modification of gene expression in multi–drug-resistant human glioblastoma multiforme cells by recombinant human fibroblast and immune interferon. Int J Cancer **51**, 373.

**Moverare R**, Elfman L, Stalenheim G, Bjornsson E (2000) Study of the Th1/Th2 balance, including IL-10 production, in cultures of peripheral blood mononuclear cells from birch-pollen-allergic patients. Allergy 55, 171.

**Mrtz E**, Sareneva T, Haebel S et al (1996) Interferon gamma and glycosylation. Electrophoresis **17**, 925.

**Mueller H**, Flury N, Scheidegger S, Eppenberger U (1994) Tumor necrosis factor-alpha and interferon-gamma are selectively cytostatic for human breast cancer cells (meeting abstract). Experientia **50**, A47.

**Mueller H**, Flury N, Liu R et al (1996) Tumour necrosis factor and interferon are selectively cytostatic in vitro for hormone-dependent and hormone-independent human breast cancer cells. Eur J Cancer Part A **32**, 2312.

**Mulkerrin MG**, Wetzel R (1989) pH dependence of the reversible and irreversible thermal denaturation of gamma interferons. Biochemistry **28**, 6556**.**

**Munk ME**, Emoto M (1995) Functions of T-cell subsets and cytokines in mycobacterial infections. Eur Respir J **20**(Suppl), 668s**.**

**Munker R**, Andreeff M (1996) Induction of death (CD95/FAS), activation and adhesion (CD54) molecules on blast cells of acute myelogenous leukemias by TNF-alpha and IFN-gamma. Cytokines Mol Ther **2**, 147.

**Munoz-Bellido FJ**, Monteseirin FJ, Escribano MM et al (1998) Effect of seasonal exposure to pollen on nonspecific interleukin-4, interleukin-5, and interferon-gamma in vitro release by peripheral blood mononuclear cells from subjects with pollenosis. Allergy **53**, 420.

**Munoz-Fernandez MA**, Cano E, O'Donnell CA. et al (1994). Tumor necrosis factor-alpha (TNF-alpha), interferon-gamma and interleukin-6 but not TNF-beta induce differentiation of neuroblastoma cells: The role of nitric oxide. J Neurochem **62**, 1330.

**Murao SI**, Fukumoto Y, Katayama M et al (1985) Recombinant gamma-interferon and lipopolysaccharide enhance 1.25-dihydroxyvitamin D3–induced cell differentiation in human promyelocytic leukemia (HL-60) cells. Jpn J Cancer Res **76**, 596.

**Murohashi I**, Hoang T (1991) Interferon-gamma enhances growth factor-dependent proliferation of clonogenic cells in acute myeloblastic leukemia. Blood **78**, 1085.

**Murray HW** (1990) Gamma interferon, cytokine-induced macrophage activation, and antimicrobial host defense. In vitro, in animal models, and in humans. Diagn Microbiol Infect Dis **13**, 411.

**Murray HW** (1994) Interferon-gamma and host antimicrobial defense: current and future clinical applications. Am J Med **97**, 459.

**Murray HW (**1996a) Current and future clinical applications of interferon-gamma in host antimicrobial defense. Intensive Care Med **4**(Suppl), S456.

**Murray HW**, Juanfbhanich CW, Nathan CF, Cohn ZA (1979) Macrophage oxygen-dependent antimicrobial activity. II. The role of oxygen intermediates. J Exp Med **150**, 950.

**Murray HW**, Hillman JK, Rubin BY et al (1985) Patients at risk for AIDS-related opportunistic infections. Clinical manifestations and impaired gamma interferon production. N Engl J Med **313**, 1504.

**Murray KJ**, Grom AA, Thompson SD et al (1998) Contrasting cytokine profiles in the synovium of different forms of juvenile rheumatoid arthritis and juvenile spondyloarthropathy: prominence of interleukin 4 in restricted disease. J Rheumatol **25**, 1388.

**Murray PJ**, Young RA (1999) Increased antimycobacterial immunity in interleukin-10–deficient mice. Infect Immun **67**, 3087.

**Murray PJ**, Aldovini A, Young RA (1996b) Manipulation and potentiation of antimycobacterial immunity using recombinant bacille Calmette-Guerin strains that secrete cytokines. Proc Natl Acad Sci USA **93**, 934.

**Musch E**, Hogemann B, Gerritzen A et al (1998) Phase II clinical trial of combined natural interferon-beta plus recombinant interferon-gamma treatment of chronic hepatitis B. Hepatogastroenterology **45**, 2282.

**Muschen M**, Moers C, Warskulat U et al (2000) CD95 ligand expression as a mechanism of immune escape in breast cancer. Immunology **99**, 69.

**Musial J**, Gluszko P, Undas A et al (1998) Gamma interferon administration to patients with atopic dermatitis inhibits fibrinolysis and elevates C1 inhibitor. Thromb Res **89**, 253.

**Musiani M**, Zerbini ML, Muscari A et al (1990) Antibody patterns against cytomegalovirus and Epstein-Barr virus in human atherosclerosis. Microbiologica **13**, 35.

**Muss HB**, Caponera M, Zekan PJ et al (1986) Recombinant gamma interferon in advanced breast cancer: A phase II trial. Invest New Drugs **4**, 377.

**Muster-Bloy R**, Elsasser-Beile U, Weber W et al (1996) Immunosuppressive activity of sera from patients with colorectal and gynecological carcinomas as evaluated by impaired IFN-gamma, IL-1 alpha and TNF-alpha production of human peripheral mononuclear cells. Immunobiology **196**, 356.

**Mutch DG**, Massad LS, Kao MS, Collins JL (1990) Proliferative and antiproliferative effects of interferon-gamma and tumor necrosis factor-alpha on cell lines derived from cervical and ovarian malignancies. Am J Obstet Gynecol **163**, 1920.

## N

**Nabioullin R**, Sone S, Mizuno K et al (1994) Interleukin-10 is a potent inhibitor of tumor cytotoxicity by human monocytes and alveolar macrophages. J Leukoc Biol **55**, 437.

**Nagamura F**, Nagamura-Inoue T, Tojo A et al (1998) Establishment of novel cell lines derived from two patients with chronic myelogenous leukemia in blast crisis; IMS-BC1 and IMS BC2 which exhibit markedly different sensitivity to apoptosis. Int J Hematol **67**, 283.

**Naganuma H**, Kiessling R, Patarroyo M et al (1991) Increased susceptibility of IFN-gamma treated neuroblastoma cells to lysis lymphokine-activated killer cells: Participation of ICAM-1 induction on target cells. Int J Cancer **47**, 527.

**Naganuma H**, Halapi E, Masucci G et al (1992) Diverse effect of cytokine treatment of tumor cells on specific versus non specific cytotoxicity. Med Oncol Tumor Pharmacother **9**, 25.

**Nagao SI**, Sato K, Ogawa H, Osada Y (1986) Augmentation by bacterial lipopolysaccharide of antitumor potency of murine recombinant interferon-gamma against Lewis lung adenocarcinoma. Jpn J Cancer Res **77**, 212.

**Nagashima M**, Fukuo Y, Saito A, Terashi A (1994) Effect of tumor necrosis factor and interferon-gamma on platelat-derived growth factor (PDGF) A and B gene expression in TPH-1 monocyte cells. J Nippon Med Sch **61**, 453.

**Nagata K**, Izumi T, Kitagawa T, Yoshida N (1987) Oligomeric structure of recombinant human and murine immune interferons by means of sedimentation equilibrium. J Interferon Res **7**, 313.

**Nagoshi M**, Goedegebuure PS, Burger UL et al (1998) Successful adoptive cellular immunotherapy is dependent on induction of a host immune response triggered by cytokine (IFN-gamma and granulocyte/macrophage colony-stimulating factor) producing donor tumor-infiltrating lymphocytes. J Immunol **160**, 334.

**Nair MP**, Chadha KC, Stadler I et al (1995) Differential effects of human immunodeficiency virus type 1 envelope protein gp120 on interferon production by mononuclear cells from adults and neonates. Clin Diagn Lab Immunol **2**, 434.

**Nair S**, Ernstoff MS, Bahnson RR et al (1992) Interferon-induced reversible acute renal failure with nephrotic syndrome. Urology **39**, 169.

**Naito S**, Yasumasu T, Kumazawa J et al (1995); Treatment of advanced renal cell carcinoma with a combination of interferon alpha and gamma. Jpn J Urol **86**, 1346.

**Nakagawa M**, Wada M, Takahashi S et al (1994). Clinical study of subcutaneous administration of low-dose gamma-interferon for renal cell carcinoma. Niscinihon J Urol **56**, 542.

**Nakagawa S**, Aiba S, Tagami H (1998) Decreased frequency of interferon-gamma-producing CD4+ cells in the peripheral blood of patients with atopic dermatitis. Exp Dermatol **7**, 112.

**Nakajima H**, Hiyama Y, Tsukada W et al (1990). Effects of interferon gamma on cultured synovial cells from patients with rheumatoid arthritis: inhibition of cell growth, prostaglandin $E_2$, and collagenase release. Ann Rheum Dis **49**, 512.

**Nakajima H**, Iwamoto I, Yoshida S (1993) Aerosolized recombinant interferon-gamma prevents antigen-induced eosinophil recruitment in mouse trachea. Am Rev Respir Dis **148**, 1102.

**Nakajima R**, Brubaker RR (1993) Association between virulence of Yersinia pestis and suppression of gamma interferon and tumor necrosis factor alpha. Infect Immun **61**, 23.

**Nakajima Y**, DelliPizzi A, Mallouh C, Ferreri NR (1995) Effect of tumor necrosis factor-alpha and interferon-gamma on the growth of human prostate cancer cell lines. Urol Res **23**, 205.

**Nakamaki T**, Sakashita A, Hino KI et al(1990) Difference in effects of interferon-alpha and interferon-gamma on the induction of differentiation of retinoic acid–treated acute myeloid leukemia cells in primary culture. Leukoc Res **14**, 785.

**Nakayama J**, Yamada A, Hori Y (1989) Cutaneous lesion of adult T-cell lymphoma treated successfully with local hyperthermia in combination with intravenous administration of RINF-gamma. Nippon Hifuka Gakkai Zasshi **99**, 907.

**Nakayama J**, Guan XC, Nakashima M et al (1997) In vitro comparison between mouse B16 and human melanoma cell lines of the expression of ICAM-1 induced by cytokines and/or hyperthermia. J Dermatol **24**, 351.

**Nakazawa M**, Sugi N, Kawaguchi H et al (1997) Predominance of type 2 cytokine-producing CD4+ and CD8+ cells in patients with atopic dermatitis. J Allergy Clin Immunol **99**, 673.

**Naldini A**, Fleischmann WR Jr (1987) In vivo myelosuppression by combination interferon treatment: Antagonism of MuIFN-gamma and Mu IFN-beta myelosuppressive effects. J Biol Response Modif **6**, 546.

**Naldini A**, Carraro F, Bocci V et al (1995) Effects of hypoxia on the antiproliferative activity of human interferons. J Interferon Cytokine Res **15**, 137.

**Nambu M**, Watanabe H, Kim KM et al (1991) Regulation of Fc gamma receptor subtype expression on a human eosinophilic leukemia cell line EoL-3. Participation of cAMP and protein kinase C in the effects of interferon-gamma and phorbol ester. Cell Immunol **133**, 27.

**Nambu M**, Mayumi, Mikawa H (1992) Regulation of the expression of leukocyte function-associated antigen-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1) on a human eosinophilic leukemia cell line Eol-3. Cell Immunol **143**, 335.

**Nandi PK** (1998) Evidence of molten globule like state(s) of interferon gamma in acidic and sodium perchlorate solutions. Int J Biol Macromol **22**, 23.

**Nanes MS**, McKoy WM, Marx SJ (1989) Inhibitory effects of tumor necrosis factor-alpha and interferon gamma on deoxyribonucleic acid and collagen synthesis by rat osteosarcoma cells (ROS 17/2.8). Endocrinology **124**, 339.

**Nanni P**, De Giovanni C, Nicoletti G et al (1998) The immune response elicited by mammary adenocarcinoma cells transduced with interferon-gamma and cytosine deaminase genes cures lung metastases by parental cells. Hum Gene Ther **9**, 217.

**Nara N** (1993) Combined effect of interferon-gamma and tumor necrosis factor-alpha causing suppression of leukemic blast progenitors in acute myeloblastic leukemia. Leukoc Lymphoma **10**, 201.

**Narayanan AS**, Whithey J, Souza A, Raghu G (1992) Effect of gamma-interferon on collagen synthesis by normal and fibrotic human lung fibroblasts. Chest **101**, 1326.

**Narita M**, Ogino M, Kato K et al (1986) A case of hepatoma with a remarkable response to gamma-interferon administration. Gan To Kagaku Ryoho **13**, 2867.

**Nash KA**, Mohammed G, Nandapalan N et al (1993) T cell function in children with acute lymphoblastic leukaemia. Br J Haematol **83**, 419.

**Nassar GM**, Morrow JD, Roberts LJ et al (1994) Induction of 15-lipoxygenase by interleukin-13 in human blood monocytes. J Biol Chem **269**, 27631.

**Nastala CL**, Edington HD, McKinney TG et al (1994) Recombinant IL-12 administration induces tumor regression in association with IFN-gamma production. J Immunol **153**, 1697.

**Nathan CF** (1992) Treatment of lepromatous leprosy with recombinant interferon-gamma. In: Antiinfective application of interferon gamma (Jaffe HS, Bucalo LR, Sherwin SA, eds). Marcel Dekker, New York, p. 227.

**Nathan CF**, Silverstein SC, Bruckner LH, Cohn ZA (1979) Extracellular cytolysis by activated macrophages and granulocytes. II. Hydrogen peroxide as a mediator of cytotoxicity. J Exp Med **149**, 100.

**Nathan CF**, Murray HW, Wiebe ME, Rubin BY (1983) Identification of interferon-γ as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. J Exp Med **158**, 670.

**Nathan CF**, Prendergast TJ, Wiebe ME et al (1984) Activation of human macrophages. Comparison of other cytokines with interferon-gamma. J Exp Med **160**, 600.

**Nathan CF**, Horowitz CR, De la Harre J et al (1985) Administration of recombinant interferon-γ to cancer patients enhances monocyte secretion of hydrogen peroxide. Proc Natl Acad Sci USA **82**, 8686.

**Nathan I**, JE Groopman, SG Quan et al (1981) Immune (γ) interferon produced by a human T-lymphoblast cell line. Nature **292**, 842.

**Naujokat C**, Sezer O, Possinger K (1999) Tumor necrosis factor-alpha and interferon-gamma induce expression of functional Fas ligand on HT29 and MCF7 adenocarcinoma cells. Biochem Biophys Res Commun **264**, 813.

**Nawa A**, Kishiyama Y, Yamamoto N et al (1990) Selective suppression of human papilloma virus type 18 mRNA level in HeLa cells by interferon. Biochem Biophys Res Commun **170**, 793.

**Nayak SK**, Schiltz PM, Dillman RO (1999) Modulation of renal carcinoma cells in vitro: Comparison after transduction with retroviral vector containing a human IFN-gamma gene versus incubation with soluble IFN-gamma. J Interferon Cytokine Res **19**, 49.

**Nehme A**, Albin N, Caliaro MJ et al (1995). Mechanism of interaction between cisplatin and human recombinant interaction gamma in human ovarian cell lines. Int J Cancer **61**, 643.

**Nestle F**, Dummer R, Wiede J et al (1990) Pathophysiological implications of soluble IL-2 receptor in cutaneous T-cell lymphomas. J Invest Dermatol **95**, 482.

**Neumann-Haefelin D**, Sundmacher R, Frey H, Merk W(1985) Recombinant HuIFN-gamma prevents herpes simplex keratitis in African green monkeys: Demonstration of synergism with recombinant HuIFN-alpha 2. Med Microbiol Immunol (Berl) **174**, 81.

**Neville LF**, Mathiak G, Bagasra O (1997) The immunobiology of interferon-gamma-inducible protein 10 kD (IP-10): a novel, pleiotropic member of the C-X-C chemokine superfamily. Cytokine Growth Factor Rev **8**, 207.

**Newman HFV**, Bleehen NM, Galazka A, Scott E (1987) Small cell lung carcinoma. A Phase II evaluation of r-interferon-gamma. Cancer **60**, 2938.

**Newport JN**, Huxley CM, Huston S et al (1996) A mutation in interferon-γ–receptor gene and susceptibility to mycobacterial infection. N Engl J Med **335**, 1941.

**Newton RC** (1985) Effect of interferon on the induction of human monocyte secretion of interleukin-1 activity. Immunology **56**, 441.

**Nezu N**, Ryu K, Koide Y, Yoshida TO (1990) Regulation of HLA class II molecule expressions by IFN-γ. The signal transduction mechanism in glioblastoma cell lines. J Immunol **145**, 3126.

**Nguyen KD**, Hoang AT, Lee DA (1994) Transcriptional control of human Tenon's capsule fibroblast collagen synthesis in vitro by gamma-interferon. Invest Ophthalmol Vis Sci **35**, 3064.

**Nickoloff BJ**, Basham TY, Merigan TC, Morhenn VB (1984) Antiproliferative effects of recombinant α- and γ-interferons on cultured human keratinocytes. Lab Invest **51**, 697.

**Nickoloff BJ**, Basham TY, Merigan TC, Morhenn VB (1985) Immunomodulatory and antiproliferative effect of recombinant alpha, beta, and gamma interferons on cultured human malignant squamous cell lines, SCL-1 and SW-1271, J Invest Dermatol **84**, 487.

**Nickoloff BJ**, Basham TY, Merigan TC et al (1986) Human keratinocyte-lymphocyte reactions in vitro. J Invest Dermatol **87**, 11.

**Nickoloff BJ**, Fisher GJ, Mitra RS, Voorhees JJ (1988) Additive and synergistic antiproliferative effects of cyclosporin A and gamma interferon on cultured human keratinocytes. Am J Pathol **131**, 12.

**Nickoloff BJ**, Mitra RS, Elder JT et al (1989) Decreased growth inhibition by recombinant gamma interferon is associated with increased transforming growth factor-alpha production in keratinocytes cultured from psoriatic lesions. Br J Dermatol **121**, 161.

**Nicoletti F**, Meroni P, Di-Marco R et al (1992) In vivo treatment with a monoclonal antibody to interferon-gamma neither affects the survival nor the incidence of lupus-nephritis in the MRL/lpr-lpr mouse. Immunopharmacology **24**, 11.

**Niederle N**, Kloke O, Wandl UB et al (1993) Long term treatment of chronic myelogenous leukemia with different interferons: Results from three studies. Leukoc Lymphoma **9**, 111.

**Niell HB**, Mauer AM, Rademacher D (1994) Cytotoxic effects of alpha and gamma-interferon and tumor necrosis factor in human bladder tumor cell lines. Urol Res **22**, 247.

**Niesvizky R**, Siegel D, Michaeli J (1993) Biology and treatment of multiple myeloma. Blood Rev **7**, 24.

**Nishihara K**, Miyatake S, Sakata T et al (1988) Augmentation of tumor targeting in a line of glioma-specific mouse cytotoxic T-lymphocytes by retroviral expression of mouse gamma- interferon complementary DNA. Cancer Res **48**, 4730.

**Nishimura M**, Mitsunaga S, Akaza T et al (1994) Protection against natural killer cells by interferon-gamma treatment of K562 cells cannot be explained by augmented major histocompability complex class I expression. Immunology **83**, 75.

**Nishisaka N**, Maini A, Kinoshita Y et al (1999) Immunotherapy for lung metastases of murine renal cell carcinoma: Synergy between radiation and cytokine-producing tumor vaccines. J Immunother **22**, 308.

**Noga SJ**, Hess AD (1991) The antitumor effect of cyclosporine a (CSA)–induced autoimmune graft-vs-host-disease (AIGVHD) is potentiated by gamma interferon (IFN) (meeting abstract). Exp Hematol **19**, 580.

**Noga SJ**, Horwitz L, Kim H et al (1992) Interferon-gamma potentiates the antitumor effect of cyclosporine-induced autoimmunity. J Hematother **1**, 75.

**Noh GW**, Lee KY (1998) Blood eosinophils and serum IgE as predictors for prognosis of interferon-gamma therapy in atopic dermatitis. Allergy **53**, 1202.

**Norioka K-I**, Mitaka T, Mochizuki Y et al (1994) Interaction of interleukin-1 and interferon gamma on fibroblast growth factor–induced angiogenesis. Jpn J Cancer Res **85**, 522.

**Novellino PS**, Trejo YG, Beviacqua M et al (2000) Regulation of HLA-DR antigen in monocytes from colorectal cancer patients by in vitro treatment with human recombinant interferon-gamma. J Investig Allergol Clin Immunol **10**, 90.

**Numata A** (1992) Detection of endogenous IFN-gamma and TNF-alpha in tumor-infiltrating mononuclear cells of human colorectal cancer. Hokkaido Igaku Zasshi **67**, 40.

**Nurse B**, Haus M, Puterman AS et al (1997) Reduced interferon-gamma but normal IL-4 and IL-5 release by peripheral blood mononuclear cells from Xhosa children with atopic asthma. J Allergy Clin Immunol **100**, 662.

## O

**Obert HJ**, Brzoska J (1986) Interferon-gamma in der Therapie der chronischen Polyarthritis. Arzneimittel Forsch **36**, 1557.

**Obert HJ**, Brzoska J (1989a) Behandlung der chronischen Polyarthritis mit Interferon gamma. Ergebnisse einer therapiestudie der Phase II bei 476 patienten. Rheuma **9**, 286.

**Obert HJ**, Brzoska J (1989b) Interferon gamma in der Therapie der chronischen Polyarthritis. Die langzeitbehanlung in der Praxis. Arzneimittel Forsch **39**, 819.

**Obert HJ**, Hofschnieder PH (1985) Interferon bei chronischer Polyarthritis. Dtsch. Med Wochenstr **110**, 1766.

**Obert HJ**, Brzoska J, Hundgen MP (1989) Interferon gamma zur Behandlung rheumatoiden Arthritis. Ergebnisse von Therapiestudien bei 850 Patienten. In: Neure Ergebnisse in Osteologie (Willert HG, Heuck FHW, eds). Springer-Verlag, Berlin, p 3.

**Obiri NI**, Siegel JP, Varricchio F, Puri RK (1994) Expression of high-affinity IL-4 receptors on human melanoma, ovarian and breast carcinoma cells. Clin Exp Immunol **95**, 148.

**Ocano M**, Purtilo DT (1992) Interferons and Epstein-Barr virus infections. In: Anti-infective application of interferon gamma (Jaffe HS, Bucalo LR, Sherwin SA, eds). Marcel Dekker, New York, p 213.

**O'Connell MJ**, Ritts RA Jr, Moertel CG et al (1989) Recombinant interferon-gamma lacks activity against metastatic colorectal cancer but increases serum levels of CA 19-9. Cancer **63**, 1998.

**Offner FA**, Obrist P, Stadlmann S et al (1995) Expression of high-affinity IL-4 receptors on human melanoma, ovarian and breast carcinoma cells. Clin Exp Immunol **95**, 148.

**Ogata A**, Nishimoto N, Shima Y et al (1994) Inhibitory effect of al-trans retinoic acid on the growth of freshly isolated myeloma cells via interference with interleukin-6 signal transduction. Blood **84**, 3040.

**Ogata K**, Tamura H, Yokose N et al (1995) Effects of interleukin-12 on natural killer cell cytotoxicity and the production of interferon-gamma and tumor necrosis factor-alpha in patients with myelodysplastic syndromes. Br J Haematol **90**, 15.

**Ogawa M**, Yu WG, Umehara K et al (1998) Multiple roles of interferon-gamma in the mediation of interleukin 12–induced tumor regression. Cancer Res **58**, 2426

**Ogi S**, Oda H, Yokoyama M, Takeuchi M (1992) Complete remission of liver metastasis from renal cell carcinoma with recombinant interferon-gamma: A case report. Nishinihon J Urol **54**, 1777.

**Ogura T**, Esumi H (1996) Nitric oxide synthase expression in human neuroblastoma cell line induced by cytokines. NeuroReport **7**, 853.

**Oh SH**, Gonik B, Greenberg SB, Kohl S (1986) Enhancement of human neonatal natural killer cytotoxicity to herpes simplex virus with use of recombinant human interferons: lack of neonatal response to gamma interferon. J Infect Dis **153**, 791.

**Oh YM**, Yoo CG, Chung HS et al (1996) The mechanism of interferon-gamma induced cytoxicity on the lung cancer cell line A549. Tuberc Respir Dis **43**, 63.

**Ohashi Y**, Nakai Y, Kakinoki Y et al (1997) Immunotherapy affects the seasonal increase in specific IgE and interleukin-4 in serum of patients with seasonal allergic rhinitis. Scand J Immunol **46**, 67.

**Ohashi Y**, Nakai Y, Tanaka A et al (1998) Allergen-induced synthesis of interleukin-5, but not of IgE, is a key mechanism linked to symptomatic episodes of seasonal allergic rhinitis in sensitized individuals. Scand J Immunol **47**, 596.

**Ohno R** (1987) Interferons in the treatment of multiple myeloma. Int J Cancer **1(**Suppl), 14.

**Okada T**, Sugie I, Aisaka K (1993) Effects of gamma-interferon on collagen and histamine content in bleomycin-induced lung fibrosis in rats. Lymphokine Cytokine Res **12**, 87.

**Okadome M**, Saito T, Tsukamoto N et al (1991) Potential of human lymph node cells for antitumor activity mediated by interferon-gamma. Cancer **68**, 2378.

**Okamoto T**, Harada M, Tamada K et al (1997) Local injection of OK432 can augment the TH1-type T-cell response in tumor draining lymph node cells and increase their immunotherapeutical potential. Int J Cancer **70**, 598.

**Okamoto Y**, Tsurunaga T, Ueda M et al (1993). A study of intercellular adhesion molecule-1 (ICAM-1) in cervical cancer. Acta Obstet Gynaecol Jpn **45**, 566.

**Okamura K**, Abe Y, Yamada K et al (1986) Enhanced expression of HLA antigens on gynecologic cancer cells by interferon-gamma. Nippon Sanka Fujinka Gakkai Zasshi **38**, 945.

**Okaneya T**, Murata Y, Kinebuchi Y (1999) Complete remission of lung and hepatic metastases from renal cell carcinoma by interferon alpha-2b therapy: a case report. Hinyokika Kiyo **45**, 617.

**Olaitan A**, Johnson MA, Reid WM et al (1998) Changes to the cytokine microenvironment in the genital tract mucosa of HIV+ women. Clin Exp Immunol **112**, 100.

**Oldroyd SD**, Thomas GL, Gabbiani G, El Nahas AM (1999) Interferon-gamma inhibits experimental renal fibrosis. Kidney Int **56**, 2116.

**Oliver RTD**, Nouri AME, Crosby D et al (1989) Biological significance of beta hCG, HLA and other membrane antigen expression on bladder tumours and their relationship to tumour infiltrating lymphocytes (TIL). J Immunogenet **16**, 381.

**Onishi T**, Machida T, Masuda F, Igarashi H (1991) Immunological assessment after radical nephrectomy for renal cell carcinomas pre-treated with interferon-gamma. Acta Urol Jpn **37**, 671.

**Onishi T**, Ohishi Y, Iizuka N et al (1997) Study of the effects of treatment with interferon for the advanced renal cell carcinoma based upon the survival rate. Nippon Hinyokika Gakkai Zasshi **88**, 463.

**Onishi T**, Onishi Y, Imagawa K et al (1999) An assessment of the immunological environment based on intratumoral cytokine production in renal cell carcinoma. Br J Urol Int **83**, 488.

**Opalka B**, Wandl UB, Becher R et al (1991) Minimal residual disease in patients with chronic myelogenous leukemia undergoing long-term treatment with recombinant interferon alpha-2B alone or in combination with interferon gamma. Blood **78**, 2188.

**Orange JS**, Wang B, Terhorst C, Biron CA (1995) Requirement for natural killer cell-produced interferon gamma in defense against murine cytomegalovirus infection and enhancement of this defense pathway by interleukin 12 administration. J Exp Med **182**, 1045.

**Orlikowsky T**, Wang ZQ, Dudhane A et al (1996) The cell surface marker phenotype of macrophages from HIV-1–infected subjects reflects an IL-10–enriched and IFN-gamma–deprived donor environment. J Interferon Cytokine Res **16**, 957.

**Osaki T**, Peron JM, Cai Q et al (1998) IFN-gamma–inducing factor/IL-18 administration mediates IFN-gamma and IL-12–independent antitumor effects. J Immunol **160**, 1742.

**Osaki T**, Hashimoto W, Gambotto A et al (1999) Potent antitumor effects mediated by local expression of the mature form of the interferon-gamma inducing factor, interleukin-18 (IL-18). Gene Ther **6**, 808.

**Osanto S**, Jansen R, Naipal AM et al (1989) In vivo effects of combination treatment with recombinant interferon-gamma and alpha in metastatic melanoma. Int J Cancer **43**, 1001.

**Osanto S**, Jansen R, Vloemans M (1992) Down modulation of c-myc expression by interferon gamma and tumor necrosis factor alpha precedes growth arrest in human melanoma cells. Eur J Cancer Part A Gen Top **28**, 1622.

**Osna N**, Duk A, Sochnev A (1996) Interferon gamma production in whole peripheral blood culture in acute hepatitis B. Scand J Clin Lab Invest **56**, 145.

**Osoba D**, Zee B, Pater J, Quirt I (1993) Lessons learned from measuring health-related quality of life (QOL) in an adjuvant trial of gamma interferon (GIF) vs levamisole (LEV) in patients with resected malignant melanoma (meeting abstract). Proc Annu Meet Am Soc Clin Oncol **12**, A1497.

**Ostlund L**, Einhorn S, Robert KH et al (1986) Chronic B-lymphocytic leukemia cells proliferate and differenitate following exposure to interferon in vitro. Blood **67**, 152.

**Ottesen SS**, Ahrenkiel V, Kieler J (1990) Recombinant human interferon gamma exerts an anti-proliferative effect and modulates the expression of human leukocyte antigens A, B, C, and DR in human urothelial cell lines. Cancer Immunol Immunother **31**, 93.

**Otto F**, Mackensen A, Mertelsmann R, Engelhardt R (1995) Complete response of metastatic renal cell carcinoma to low-dose interferon-gamma treatment. Cancer Immunol Immunother **40**, 115.

**Otto T**, Goepel M, Luboldt HJ Rubben H (1995) Cytokine therapy of metastatic renal cell carcinoma. Urologie Ausgabe A **34**, 200.

**Otto U**, Schneider A, Denkhaus H, Conrad S (1988) Treatment of patients with metstatic renal cell carcinoma with recombinant alpha-2 interferon and gamma-interferon. Results of two clinical phase II and phase III studies. Oncologie **11**, 185.

**Ouchi T**, Lee SW, Ouchi M et al (2000) Collaboration of signal transducer and activator of transcription 1 (STAT1) and BRCA1 in differential regulation of IFN-gamma target genes. Proc Natl Acad Sci USA **97**, 5208.

**Overall JC**, Tze-Jou Y, Kern ER (1980) Sensitivity of herpes simplex virus 1 and 2 to three preparations of human interferon. J Infect Dis **142**, 243.

**Owens MW**, Milligan SA, Grisham MB (1996) Inhibition of pleural mesothelial cell collagen synthesis by nitric oxide. Free Radic Biol Med **21**, 601.

**Ozer H** (1988) Biotherapy of chronic myelogenous leukemia with interferon. Semin Oncol **15**(Suppl 5), 14.

**Ozer H** (1991) Interferon combination therapy in leukemias and lymphomas (meeting abstract). Cytokines in Hemopoiesis, Oncology, AIDS, June 19–22, 1996, Hannover, Germany, A98.

**Ozmen L**, Gribaudo G, Fountoulakis M et al (1993) Mouse solube IFNγ inhibitor. Distribution, antigenicity and activity after injection in mice. J Immunol **7**, 2698.

**Ozmen L**, Roman D, Fountoulakis M et al (1995) Experimental therapy of systemic lupus erythematosus: the treatment of NZB/W mice with mouse soluble interferon-gamma receptor inhibits the onset of glomerulonephritis. Eur J Immunol **25**, 6.

**Ozzello L**, Habif DV, DeRosa CM, Cantell K (1988) Treatment of human breast cancer xenografts using natural interferons-alpha and -gamma injected singly or in combination. J Interferon Res **8**, 679.

**Ozzello L**, Habif DV, DeRosa CM (1990) Antiproliferative effects of natural interferon beta alone and in combination with natural interferon gamma on human breast carcinomas in nude mice. Breast Cancer Res Treat **16**, 89.

## P

**Padrid PA**, Mathur M, Li X et al (1998) CTLA4Ig inhibits airway eosinophilia and hyperresponsiveness by regulating the development of Th1/Th2 subsets in a murine model of asthma. Am J Respir Cell Mol Biol **18**, 453.

**Paganelli R**, Scala E, Ansotegui IJ et al (1995) CD8+T lymphocytes provide helper activity for IgE synthesis in human immunodeficiency virus–infected patients with hyper-IgE. J Exp Med **181**, 423.

**Paganin C**, Frank I, Trinchieri G (1995) Priming for high interferon-gamma production induced by interleukin-12 in both CD4+ and CD8+ T cell clones from HIV-infected patients. J Clin Invest **96**, 1677.

**Pak CC**, Fidler IJ (1989) Activated macrophages distinguish undifferentiated-tumorigenic from differentiated-nontumorigenic murine erythroleukemia cells. Differentiation **41**, 49.

**Paludan SR**, Lovmand J, Ellermann-Eriksen S, Mogensen SC (1997) Effect of IL-4 and IL-13 on IFN-gamma–induced production of nitric oxide in mouse macrophages infected with herpes simplex virus type 2. FEBS Lett **414**, 61.

**Palumbo A**, Battaglio S, Napoli P et al (1994) Recombinant interferon-gamma inhibits the in vitro proliferation of human myeloma cells. Br J Haematol **86**, 726.

**Palumbo A**, Bruno B, Boccadoro M, Pileri A (1995a) Interferon-gamma in multiple myeloma. Leuk Lymphoma **18**, 215.

**Palumbo A**, Battaglio S, Napoli P et al (1995b). Retinoic acid inhibits the growth of human myeloma cells in vitro. Br J Haematol **89**, 555.

**Pan YC**, Stern AS, Familletti PC et al (1987) Structural characterization of human interferon γ. Heterogeneity of the carboxyl terminus. Eur J Biochem **166**, 145.

**Panayiotidis P**, Ganeshaguru K, Foroni L, Hoffbrand AV (1995) Expression and function of the FAS antigen in B chronic lymphocytic leukemia and hairy cell leukemia. Leukemia **9**, 1227.

**Pancre V**, Joseph M, Mazingue C et al (1987) Induction of platelet cytotoxic functions by lymphokines: role of interferon-γ. J Immunol **138**, 4490.

**Pancre V**, Schellekens H, Van der Meide P et al (1990) Biological effect of interferon-γ during the course of experimental infection of rat by *Schistosoma mansoni*. Cell Immunol **125**, 58.

**Pancre V**, Wolowczuk I, Guerret S et al (1994) Protective effect of rSm28GST-specific T cells in schisostosomiasis: Role of gamma interferon. Infect Immun **62**, 3723.

**Panici B**, Scambia G, Baiocchi G et al (1989) Randomized clinical trial comparing systemic interferon with diathermocoagulation in primary multiple and widespread anogenital condylomata. Obstet Gynecol **74**, 393.

**Panitch HS**, Hirsch RL, Schindler J, Johnson KP (1987) Treatment of multiple sclerosis with gamma interferon: Exacerbations associated with activation of the immune system. Neurology **37**, 1097.

**Pao CC**, Lin CY, Yao DS, Tseng CJ (1995) Differential expression of cytokine genes in cervical cancer tissues. Biochem Biophys Res Commun **214**, 1146.

**Papamichail M**, Baxevanis CN (1992) Gamma-interferon enhances the cytotoxic activity of interleukin-2-induced peripheral blood lymphocyte (LAK) cells, tumor infiltrating lymphocytes (TIL), and effusion associated lymphocytes. J Chemother **4**, 387.

**Park JG**, Kim HT, Park SH, Kim NK(1991) The combined effects of 5-fluororacil and recombinant interferon gamma on human gastric carcinoma cell lines (meeting abstract). Proc Annu Meet Am Soc Clin Oncol **10**, A418.

**Parkinson DR** (1993) Cytokines in the treatment of advanced melanoma (meeting abstract). Melanoma Res **3**(Suppl 1), 28.

**Parodi MT**, Cornaglia Ferraris P, Ponzoni M (1989) Effects of gamma-interferon on the growth morphology and membrane and cytoskeletal proteins expression of lan-1 cells. Exp Cell Res **185**, 327.

**Paroli M**, Carloni G, Franco A et al (1994) Human hepatoma cells expressing MHC antigens display accessory cell function: Dependence on LFA-1/ICAM-1 interaction. Immunology **82**, 215.

**Parr MB**, Parr EL (1999) The role of gamma interferon in immune resistance to vaginal infection by herpes simplex virus type 2 in mice. Virology **258**, 282.

**Pastore S**, Corinti S, La Placa M et al (1998) Interferon-gamma promotes exaggerated cytokine production in keratinocytes cultured from patients with atopic dermatitis. J Allergy Clin Immunol **101**, 538.

**Patterson JB**, Thomis DC, Hans SL, Samuel CE (1995) Mechanism of interferon action: Double-stranded RNA-specific adenosine deaminase from human cells is inducible by alpha and gamma interferons. Virology **210**, 508.

**Paulnock DM**, Havlin KA, Storer BM et al (1989) Induced proteins in human peripheral mononuclear cells over a range of clinically tolerable doses of interferon-gamma. J Interferon Res **9**, 457.

**Pavlidis N**, Nicolaides C, Athanassiadis A et al (1996) Phase II study of 5-fluorouracil and interferon-gamma in patients with metastatic colorectal cancer. A Hellenic Cooperative Oncology Group Study. Oncology **53**, 159.

**Pazin GJ**, Hrger JH, Armstrong JA et al (1987) Leukocyte interferon for treating first episodes of genital herpes in women. J Infect Dis **156**, 891.

**Pecherstorfer M**, Jilch R, Leitgeb C et al (1994) Combined interleukin-2/interferon-gamma treatment for chemotherapy resistant multiple myeloma (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **35**, A 1177.

**Peck R**, Bollag W (1991) Potentiation of retinoid-induced differentiation of HL-60 and U937 cell lines by cytokines. Eur J Cancer **27**, 53.

**Peeva E**, Fishman AD, Goddard G et al (2000) Rheumatoid arthritis exacerbation caused by exogenous interleukin-12. Arthritis Rheum **43**, 461.

**Pellegrini P**, Berghella AM, Del Beato T et al (1996) Disregulation in TH1 and TH2 subsets of CD4+ T cells in peripheral blood of colorectal cancer patients and involvement in cancer establishment and progression. Cancer Immunol Immunother **42**, 1.

**Pendino KJ**, Laskin JD, Shuler RL et al (1993) Enhanced production of nitric oxide by rat alveolar macrophages after inhalation of a pulmonary irritant is associated with increased expression of nitric oxide synthase. J Immunol **151**, 7196.

**Penna AB**, Del Prete G, Cavalli A et al (1997). Predominant T-helper 1 cytokine profile of hepatitis B virus nucleocapsid-specific T cells in acute self-limited hepatitis. Hepatology **25**, 1022.

**Peplinski GR**, Tsung K, Meko JB, Norton JA et al (1996) Prevention of murine breast cancer by vaccination with tumor cells modified by cytokine-producing recombinant vaccina viruses. Ann Surg Oncol **3**, 15.

**Perez L**, Vega J, Chuay C et al (1990) Production and characterization of human gamma interferon from Escherichia coli. Appl Microbiol Biotechnol **33**, 429.

**Perez R**, Lipton A, Harvey HA et al (1988); A phase I trial of recombinant human gamma interferon (IFNγ)(4A) in patients with advanced malignancy. J Biol Response Modif **7**, 309.

**Perez R**, Padavic K, Krigel R, Weiner L (1991) Antierythrocyte autoantibody formation after therapy with interleukin-2 and gamma interferon. Cancer **67**, 2512.

**Perfect JR**, Granger DL, Durack DT (1987) Effects of antifungal agents and gamma interferon on macrophage cytotoxicity for fungi and tumor cells. J Infect Dis **156**, 316.

**Perry LL**, Feilzer K, Caldwell HD (1997) Immunity to Chlamydia trachomatis is mediated by T helper 1 cells through IFN-gamma–dependent and –independent pathways. J Immunol **158**, 3344.

**Peruzzi M**, Azzari C, Rossi ME et al (2000) Inhibition of natural killer cell cytotoxicity and interferon gamma production by the envelope protein of HIV and prevention by vasoactive intestinal peptide. AIDS Res Hum Retroviruses **16**, 1067.

**Pestka S** (1992) The interferon receptors: An unfinished story. AIDS Res Hum Retroviruses **8**, 776.

**Pestka S**, Langer JA, Zoon KC, Samuel CE (1987) Interferons and their actions. Annu Rev Biochem **56**, 727.

**Peter RU**, Gottlober P, Nadeshina N et al (1999) Interferon gamma in survivors of the Chernobyl power plant accident: new therapeutic option for radiation-induced fibrosis. Int J Radiat Oncol Biol Phys **45**, 147.

**Peters PM**, Svedersky LP, Shalaby MR, Palladino MA (1985) Interferon gamma: More than a MAF. Lymphokine Res **4**, 265.

**Peterson JD**, Herzenberg LA, Vasquez K et al (1998) Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response patterns. Proc Natl Acad Sci USA **95**, 3071.

**Petricoin E 3rd**, David M, Fang H et al (1994) Human cancer cell lines express a negative transcriptional regulator of the interferon regulatory factor family of DNA binding proteins. Mol Cell Biol **14**, 1477.

**Pfuzenmaier K**, Bartsch H, Scheurich P et al (1985) Differential γ-interferon response of human colon carcinoma cells: Inhibition of proliferation and modulation of immunogenicity as independent effects of γ-interferon on tumor cell growth. Cancer Res **45**, 3503.

**Philips NC** (1989) Kupfer cells and liver metastases. Optimization and limitation of activation of tumoricidal activity. Cancer Metastasis Rev **8**, 231.

**Philips NC**, Skamene E, Chedid L (1988) Correction of defective tumoricidal activity of macrophages from A/J mice by liposomal immunomodulators. Immunopharmacology **15**, 1.

**Piasecki E** (1987) Synergistic action of interferons (IFN-α/β and IFN-γ) of different species origin. Archiv Immunol Therap Exper **35**, 473.

**Pierson GR**, Evans AE, Zier KS (1990) The development of cell mediated immunity: Very young children have strong lak activity. J Clin Lab Immunol **31**, 51.

**Pignatelli M**, Waters J, Brown D et al (1986) HLA class I antigens on the hepatocyte membrane during recovery from acute hepatitis B virus infection and during interferon therapy in chronic hepatitis B virus infection. Hepatology **6**, 349.

**Piguet V**, Carrel S, de-Tribolet-N (1986a) Modulation of HLA-DR expression and growth inhibition on glioma cells: heterogeneous responses to gamma-interferon. In: Biology of brain tumour (Walker MD, Thomas DGT, eds). Boston, Martinus Nijhoff, p 509.

**Piguet V**, Carrell S, Diserens AC et al (1986b) Heterogeneity of the induction of HLA-DR expression by human immune interferon on glioma cell lines and their clones. J Natl Cancer Inst **76**, 223.

**Pinto AJ**, Morahan PS, Brinton M et al (1990) Comparative therapeutic efficacy of recombinant interferons-alpha, -beta, and -gamma against alphatogavirus, bunyavirus, flavivirus, and herpesvirus infections. J Interferon Res **10**, 293.

**Pirker R**, Prior C, Voves R et al (1994). Cisplatin/etoposide combined with interferon-gamma in non–small cell lung cancer. Radiol Oncol **28**, 395.

**Pistoia V**, Prigione I, Facchetti P, Corrias MV (1994) The scientific foundations of novel strategies for the treatment of pediatric solid tumors: Immunotherapy and gene therapy. Pediatr Med Chir **16**, 219.

**Pittet B**, Rubbia-Brandt L, Desmouliere A et al (1994) Effect of gamma-interferon on the clinical and biologic evolution of hypertrophic scars and Dupuytren's disease: An open pilot study. Plast Reconstr Surg **93**, 1224.

**Platsoucas CD** (1986) Regulation of natural killer cytotoxicity by Escherichia coli–derived human interferon gamma. Scand J Immunol **24**, 93 [Erratum in Scand J Immunol **25**, 107, 1987].

**Pohl D**, Bockelmann C, Forster K et al (1997) Neonates at risk of atopy show impaired production of interferon-gamma after stimulation with bacterial products. Allergy **52**, 732.

**Polk HC Jr**, Cheadle WG, Sonnenfeld, G, Hershman MJ (1992) Infections associated with the surgical care of the major trauma victims. In: Antiinfective application of Interfereon gamma (Jaffe HS, Bucalo LR, Sherwin SA, eds). Marcel Dekker, New York, p 29.

**Ponzoni M**, Bocca P, Chiesa V et al (1994) Neuroblastoma as an in vitro model system to study differentiation therapy. Pediatr Med Chir **16**, 227.

**Ponzoni M**, Lanciotti M, Cornaglia, Ferraris P (1990) Gamma-interferon and retinoic acid induce human neuroblastoma cell differentiation following different biochemical pathways. Clin Chem Enzymol Commun **2**, 309.

**Ponzoni M**, Casalaro A, Cornaglia-Ferraris P (1991a) Interferon gamma synergizes with retinoic acid and tumor necrosis factor in inducing human neuroblastoma cell differentiation (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **32**, A202.

**Ponzoni M**, Lanciotti M, Montaldo PG et al (1991b) Gamma-interferon, retinoic acid and cytosine arabinoside induce neuroblastoma differentiation by different mechanisms. Cell Mol Neurobiol **11**, 397.

**Ponzoni M**, Casalaro A, Lanciotti M et al (1992a) The combination of gamma-interferon and tumor necrosis factor causes a rapid and extensive differentiation of human neuroblastoma cells. Cancer Res **52**, 931.

**Ponzoni M**, Guarnaccia F, Corrias MV, Cornaglia-Ferraris P (1992b). Induction of HLA class I and class II expression by interferon-gamma in human neuroblastoma (NB) cells (meeting abstract). European Neuroblastoma Study Group: Euro Neuro '92.

**Ponzoni M**, Montaldo PG, Carbone R et al (1992c) Reciprocal heterologous upregulation of interferon-gamma and tumor necrosis factor receptors in neuroblastoma models (meeting abstract). Chronica Dermatol **2**, 426.

**Ponzoni M**, Cornaglia-Ferraris P (1993a) Interferon-gamma–stimulated and GTP-binding protein–mediated phospholipase A2 activation in human neuroblasts. Biochem J **294**, 893.

**Ponzoni M**, Guarnaccia F, Corrias MV et al (1993b) Uncoordinate induction and differential regulation of HLA class-I and class-II expression by gamma-interferon in differentiating human neuroblastoma cells. Int J Cancer **55**, 817.

**Ponzoni M**, Lucarelli E, Corrias MV et al (1993c) Protein kinase C isoenzymes in human neuroblasts. Involvement of PKC epsilon in cell differentiation. FEBS Lett **322**, 120.

**Portier M**, Zhang XG, Caron E et al (1993) Gamma-interferon in multiple myeloma: Inhibition of interleukin-6 (IL-6)–dependent myeloma cell growth and down-regulation of IL-6 receptor expression in vitro. Blood **81**, 3076.

**Porzsolt F**, Otto AM, Trauschel B et al (1989) Rationale for combining tamoxifen and interferon in the treatment of advanced breast cancer. J Cancer Res Clin Oncol **115**, 465.

**Pope J** (1993) Treatment of systemic sclerosis. Curr Opin Rheumatol **5**, 792.

**Price G**, Brenner MK, Prentice HG et al (1987) Cytotoxic effects of tumor necrosis factor and gamma-interferon on acute myeloid leukaemia blasts. Br J Cancer **55**, 287.

**Prud'homme GL**, Kong DH, Theofilopoulos AN (1995) Quantitative polymerase chain reaction analysis reveals marked overexpression of interleukin-1 beta, interleukin-1 and interferon-gamma mRNA in the lymph nodes of lupus-prone mice. Mol Immunol **32**, 495.

**Pryor K**, Haslam PL (1992) In vivo levels and in vitro production of interferon gamma in fibrosing interstitial lung disease. Clin Exp Immunol **88**, 280.

**Pryor K**, Goddard J, Goldstein D et al (1995) Bacillus Calmette-Guerin (BCG) enhances monocyte and lymphocyte mediated bladder tumor cell killing. Br J Cancer **71**, 801.

**Puehler F**, Weining KC, Symons JA et al (1998) Vaccinia virus–encoded cytokine receptor binds and neutralizes chicken interferon-gamma. Virology **248**, 231.

**Pujade-Lauraine E**, Colombo N, Guastalla J et al (1991) Intraperitoneal R-IFN gamma for salvage therapy in ovarian carcinoma (meeting abstract). 3rd Int Congress in Neo-adjuvant Chemotherapy, Paris, p 68.

**Pujade-Lauraine E**, Guastalla JP, Colombo N et al (1996) Intraperitoneal recombinant interferon gamma in ovarian cancer patients with residual disease at second look laparotomy. J Clin Oncol **14**, 343.

**Pujol JL**, Gibney DJ, Su JQ et al (1993) Immune response induced in small-cell lung cancer by maintenance therapy with interferon gamma. J Natl Cancer Inst **85**, 1844.

**Punt CJ** (1998) The use of interferon-alpha in the treatment of cutaneous melanoma: A review. Melanoma Res **8**, 95.

**Puyana JC**, Pellegrini JD, De AK et al (1998) Both T-helper-1 and T-helper-2–type lymphokines are depressed in posttrauma anergy. J Trauma **44**, 1037.

## Q

**Quesada JR**, Kurzrock R, Sherwin SA, Gutterman JU (1987) Phase II studies of recombinant human interferon gamma in metastatic renal cell carcinoma. J Biol Response Modif **6**, 20.

**Quesada JR**, Alexanian R, Kurzrock R et al (1988) Recombinant interferon gamma in hairy cell leukemia, multiple myeloma and Waldenstrom's macroglobulinemia. Am J Hematol **29**, 1.

**Quintavalla JC**, Berg RA, Beavis AJ et al (1993) Differential induction of stromelysin mRNA by bovine articular chondrocytes treated with interferon-γ and interleukin-1α. J Cell Physiol **154**, 113.

## R

**Raad I**, Hachem R, Leeds N et al (1996) Use of adjunctive treatment with interferon gamma in an immunocompromised patient who had refractory multidrug-resistant tuberculosis of the brain. Clin Infect Dis **22**, 572.

**Rabin EM**, Mond JJ, Ohara J, Paul WE (1986) Interferon-γ inhibits the action of B cell stimulatory factor (BSF)-1 on resting B cells. J Immunol **137**, 1573.

**Raefsky EL**, Platanias LC, Zoumbos NC, Young NS (1985) Studies of interferon as a regulator of hematopoietic cell proliferation. J Immunol **4**, 2507.

**Raitano AB**, Korc M (1993) Growth inhibition of a human colorectal carcinoma cell line by interleukin 1 is associated with enhanced expression of gamma-interferon receptors. Cancer Res **53**, 636.

**Rakhit A**, Yeon MM, Ferrante J et al (1999) Down regulation of the pharmacokinetic-pharmacodynamic response to interleukin 12 during long-term administration to patients with renal cell carcinoma and evaluation of the mechanism of this "adaptive response" in mice. Clin Pharmacol Ther **65**, 615.

**Ralph SJ**, Wines BD, Payne MJ et al (1995) Resistance of melanoma cell lines to interferons correlates with reduction of IFN-induced tyrosine phosphorylation: Induction of the anti-viral state by IFN is prevented by tyrosine kinase inhibitors. J Immunol **154**, 2248.

**Ramani P**, Balkwill FR (1988) Human interferons inhibit experimental metastases of a human melanoma cell line in nude mice. Br J Cancer **58**, 350.

**Rambaldi A**, Young DC, Herrmann F et al (1987) Interferon-γ induces expression of the interleukin 2 receptor gene in human monocytes. Eur J Immunol **17**, 153.

**Ramshaw AL**, Roskell DE, Parums DV (1994) Cytokine gene expression in aortic adventitial inflammation associated with advanced atherosclerosis (chronic periaortitis). J Clin Pathol **47**, 721.

**Randal M**, Kossiakoff AA (1998) Crystallization and preliminary x-ray analysis of a 1:1 complex between a designed monomeric interferon-gamma and its soluble receptor. Protein Sci **7**, 1057.

**Rangel R**, Rocha, Ramirez JL et al (1995) Generation of memory CD4+, CD8+, CD45RO+ and CD16-lymphocytes activated with IL-2, INF-gamma and TNF-alpha with specific cytotoxicity against autologous cervical cancer cells in a mixed leukocyte-tumor cell culture. Eur Cytokine Netw **6**, 195.

**Rapoza PA**, Tahija SG, Carlin JP et al (1991) Effect of interferon on a primary conjunctival epithelial cell model of trachoma. Invest Ophthalmol Vis Sci **32**, 2919.

**Rapp F**, Wroz H (1985) Synergistic effect of human leukocyte interferon and Nonoxynol 9 against herpes simplex virus type 2. Antimicrob Agents Chemother **28**, 449.

**Rashidbaigi A**, Langer JA, Jung V et al (1986) The gene for the human immune interferon receptor is located on chromosome 6. Proc Natl Acad Sci USA **83**, 384.

**Raychaudhuri SP**, Farber EM, Raychaudhuri SK (1999**)** Immunomodulatory effects of peptide T on Th1/Th2 cytokines. Int J Immunopharmacol **21**, 609.

**Raziuddin S**, Bahabri S, Al-Dalaan A et al (1998) A mixed Th1/Th2 cell cytokine response predominates in systemic onset juvenile rheumatoid arthritis: Immunoregulatory IL-10 function. Clin Immunol Immunopathol **86**, 192.

**Reali E**, Guiliani AL, Spisani S et al (1994) Interferon-gamma enhances monoclonal antibody 17-1A–dependent neutrophil cytotoxicity toward colorectal carcinoma cell line SW11-16. Clin Immunol Immunopathol **71**, 105.

**Reano A**, Richard MH, Denoroy L et al (1993) Gamma interferon potently induces tryptophanyl-tRNA synthetase expression in human keratinocytes. J Invest Dermatol **100**, 775.

**Reddy PG**, Graham GM, Datta S et al (1991) Effect of recombinant fibroblast interferon and recombinant immune interferon on growth and the antigenic phenotype of multidrug-resistant human glioblastoma multiforme cells. J Natl Cancer Inst **83**, 1307.

**Redman BG**, Flaherty L, Chou TH et al (1990) A phase I trial of recombinant interleukin-2 combined with recombinant interferon-gamma in patients with cancer. J Clin Oncol **8**, 1269.

**Reed SG**, da Silva JS (1992) In: Antiinfective application of interferon gamma (Jaffe HS, Bucalo LR, Sherwin SA, eds). Marcel Dekker, New York, p 137.

**Reginato AM**, Sanz-Rodriguez-C, Diaz-A et al (1993) Transcriptional modulation of cartilage-specific collagen gene expression by interferon-gamma and tumor necrosis factor alpha in cultured human chondrocytes. Biochem J **294**, 761.

**Reichel RP**, Fitz R, Neumann R et al (1992) Clinical study with recombinant interferon gamma versus interferon alpha-2c in patients with condylomata acuminata. Int J STD AIDS **3**, 350.

**Reinhold U**, Kukel S, Brzoska J, Kreysel HW (1993) Systemic interferon gamma treatment in severe atopic dermatitis. J Am Acad Dermatol **29**, 58.

**Ren EC**, Wong LYM, Chan SH (1988) Effect of recombinant gamma interferon on hepatocellular carcinoma cell lines. Cancer Lett **39**, 305.

**Renzi PM**, Turgeon JP, Marcotte JE et al (1999) Reduced interferon-gamma production in infants with bronchiolitis and asthma. Am J Respir Crit Care Med **159**, 1417.

**Rep MH**, Hintzen RQ, Polman CH, van Lier RA (1996) Recombinant interferon-beta blocks proliferation but enhances interleukin-10 secretion by activated human T-cells. J Neuroimmunol **67**, 111.

**Retsas S**, Leslie M, Bottomley D (1989) Intralesional tumor necrosis factor combined with interferon gamma in metastatic melanoma. Br Med J **298**, 1290.

**Revel M**, Chebath J (1986) Interferon-activated genes. TIBS **11**, 166.

**Reynolds DS**, Boom WH, Abbas AK (1987) Inhibition of B lymphocyte activation by interferon-γ. J Immunol **139**, 767.

**Reynolds JV**, Shou J, Choi H et al (1989) The influence of natural killer cells in neuroblastoma. Arch Surg **124**, 235.

**Ribera E**, Ocana I, Martinez-Vazquez JM et al (1988) High level of interferon gamma in tuberculous pleural effusion. Chest **93**, 308.

**Ribera E**, Martinez-Vazquez JM, Ocana I et al (1990) Gamma interferon and adenosine deaminase in pleuritis. Med Clin (Barc) **94**, 364.

**Richaud-Patin Y**, Alcocer-Varela J, Llorente L (1995) High levels of TH2 cytokine gene expression in systemic lupus erythematosus. Rev Invest Clin **47**, 267.

**Richter G**, Kruger-Krasagakes S, Hein G et al (1993) Interleukin 10 transfected into Chinese hamster ovary cells prevents tumor growth and macrophage infiltration. Cancer Res **53**, 4134.

**Richtsmeier WJ** (1988) Interferon gamma induced oncolysis. Arch Otolaryngol Head Neck Surg **114**, 432.

**Richtsmeier WJ**, Styczynski P, Johns ME (1987) Selective, histamine-mediated immunosuppression in laryngeal cancer. Ann Otol Rhinol Laryngol **96**, 569.

**Ridge J**, Terle DA, Dragunsky E, Levenbook I (1996) Effects of gamma-IFN and NGF on subpopulations in a human neuroblastoma cell line: Flow cytometric and morphological analysis. In Vitro Cell Dev Biol Anim **32**, 238.

**Ridley MG**, Panayi GS, Nicholas NS, Murphy J (1986). Mechanisms of microphage activation in rheumatoid arthritis: The role of gamma-interferon. Clin Exp Immunol **63**, 587.

**Riedel D**, Lindemann A, Otto J et al (1990) Gamma-interferon interrupts growth stimulation in chronic myelogenous leukemia established by endogenous granulocyte colony-stimulating factor. Leukemia **4**, 786.

**Rigby WFC**, Ball ED, Guyre PM, Fanger MW (1985) The effects of recombinant-DNA–derived interferons on the growth of myeloid progenitor cells. Blood **65**, 858.

**Riggs DR**, Tarry WF, De Haven JI et al (1992) Immunotherapy of murine transitional cell carcinoma of the bladder using alpha and gamma interferon in combination with other forms of immunotherapy. J Urol **147**, 212.

**Rinderknecht E**, O'Connor BH, Rodriguez H (1984) Natural human interferon gamma: complete amino acid sequence and determination of sites of glycosylation. J Biol Chem **259**, 6790.

**Rinehart JJ**, Malspeis L, Young D, Neidhart JA (1986). Phase I/II trial of human recombinant interferon gamma in renal cell carcinoma. J Biol Response Modif **5**, 300.

**Rinehart JJ**, Young D, Laforge J et al (1987) Phase I/II trial of recombinant gamma-interferon in patients with renal cell carcinoma: Immunologic and biologic effects. J Biol Response Modif **6**, 302.

**Robertson LE**, Huang P, Keating MJ, Plunkett W (1994) Apoptosis in chronic lymphocytic leukemia. Cancer Bull **46**, 130.

**Robinson BW**, Pinkston P, Crystal RG (1984) Natural killer cells are present in the normal human lung but are functionally impotent. J Clin Invest **74**, 942.

**Robinson DS**, Ying S, Taylor IK et al (1994) Evidence for a Th1-like bronchoalveolar T-cell subset and predominance of interferon-gamma gene activation in pulmonary tuberculosis. Am J Respir Crit Med **149**, 989.

**Rockey DC**, Chung JJ (1994) Interferon gamma inhibits lipocyte activation and extracellular matrix mRNA expression during experimental liver injury: Implications for treatment of hepatic fibrosis. J Investig Med **42**, 660.

**Rockley PF**, Tyring SK (1995) Interferons alpha, beta and gamma therapy of anogenital human papillomavirus infections. Pharmacol Ther **65**, 265.

**Rockstroh JK**, Kreuzer KA, Sauerbruch T, Spengler U (1998) Protein levels of interleukin-12 p70 holomer, its p40 chain and interferon-gamma during advancing HIV infection. J Infect **37**, 282.

**Rodriguez N**, Yano N, Eylar E et al (1997) Mechanisms associated with defective Th1 cytokine production in HIV infection. Cell Mol Biol (Noisy-le-grand) **43**, 951.

**Rohde D**, Thiemann D, Wildberger J et al (1998) Treatment of renal carcinoma patients with gemcitabine (2,2'-difluorodeoxycytidine) and interferons: Antitumor activity and toxicity. Oncol Rep **5**, 1555.

**Rojas R**, Roman J, Torres A et al (1996) Inhibition of apoptopic cell death in B–CLL by interferon gamma correlates with clinical stage. Leukemia **10**, 1782.

**Romagnani S** (1999) Th1/Th2 cells. Inflamm Bowel Dis **5**, 285.

**Romeo G**, Fiorucci G, Rossi GB (1989) Interferons in cell growth and development. Trends Genet **5**, 19.

**Ronnelid J**, Berg L, Rogberg S et al (1998) Production of T-cell cytokines at the single-cell level in patients with inflammatory arthritides: Enhanced activity in synovial fluid compared to blood. Br J Rheumatol **37**, 7.

**Rook AH**, Gottlieb SL, Wolfe JT et al (1997) Pathogenesis of cutaneous T cell lymphoma: Implications for the use of recombinant cytokines and photopheresis. Clin Exp Immunol **107(**Suppl**)**, 16.

**Rose RM**, Wasserman AS, Weiser WY, Remold HG (1986) Deficient responses of pulmonary macrophages from healthy smokers to antiviral lymphokines in vitro. J Infect Dis **154**, 611.

**Rosenberg ES**, Billingsley JM, Caliendo AM et al (1997) Vigorous HIV-1–specific CD4+ T cell responses associated with control of viremia. Science **278**, 1447.

**Rossi GB** (1985) Interferons and cell differentiation. Interferon **6**, 31.

**Rossol S**, Marinos G, Carucci P et al (1997) Interleukin-12 induction of Th1 cytokines is important for viral clearance in chronic hepatitis B. J Clin Invest **99**, 3025.

**Rosti V**, Carlo-Stella C, Pedrazzoli P, Cazzola M (1989) In vitro and in vivo effects of recombinant interferon gamma on the growth of hematopoietic progentor cells from patients with myelodysplastic syndrome. Haematologica **74**, 435.

**Rothermel CD**, Rubin BY, Jaffe EA, Murray HW (1986) Oxygen-independent inhibition of intracellular *Chlamydia psittaci* growth by human monocytes and interferon-γ–activated macrophages. J Immunol **137**, 689.

**Rothlein R**, Czajkowski M, O'Neill MM et al (1988) Induction of intercellular adhesion molecule 1 on primary and continuous cell lines by pro-inflammatory cytokines: Regulation by pharmacologic agents and neutralizing antibodies. J Immunol **141**, 1665.

**Royer-Pekora B**, Kunkel LM, Monaco AP et al (1986) Cloning the gene for an inherited human disorder — chronic granulomatous disease — on the basis of its chromosomal location. Nature **322**, 32.

**Royuela M**, de Miguel MP, Ruiz A et al (2000) Interferon-gamma and its functional receptors overexpression in benign prostatic hyperplasia and prostatic carcinoma: Parallelism with c-myc and p53 expression. Eur Cytokine Netw **11**, 119.

**Rubin BY**, Gupta SL (1980) Differential efficacies of human type I and type II interferons as antiviral and antiproliferative agents. Proc Natl Acad Sci USA **77**, 5928.

**Ruby J**, Ramshaw I (1991) The antiviral activity of immune $CD8^+$ T cells is dependent of interferon-γ. Lymphokine Cytokine Res **10**, 353.

**Ruff MR**, Farrar WL, Pert CB (1986) Interferon γ and granulocyte/macrophage colony-stimulating factor inhibit growth and induce antigens characteristic of myeloid differentiation in small-cell lung cancer cell lines. Med Sci **83**, 6613.

**Rutenfranz I**, Bauer A, Kirchner H (1990) Interferon gamma encapsulated into liposomes enhances the activity of monocytes and natural killer cells and has antiproliferative effects on tumor cells in vitro. Blut **61**, 30.

**Ruth W**, Craig, Heather L, Buchan (1989) Differentiation-inducing and cytotoxic effects of tumor necrosis factor and interferon-gamma in myeloblastic ML-1 cells. J Cell Physiol **141**, 46.

## S

**Sacks SL**, Varner TL, Davies KS et al (1990) Randomized, double-blind, placebo-controlled, patient-initiated study of topical high- and low-dose interferon α with nonoxynol-9 in the treatment of recurrent genital herpes. J Infect Dis **161**, 692.

**Saez-Royuela F**, Porres JC, Moreno A et al (1990) High doses of recombinant α-interferon or γ-interferon for chronic hepatitis C: A randomized, controlled trial. Hepatology **13**, 327.

**Saha K**, Caruso M, Volsky DJ (1997) Human immunodeficiency virus type 1 (HIV-1) infection of herpes virus saimiri–immortalized human CD4-positive T lymphoblastoid cells: Evidence of enhanced HIV-1 replication and cytopathic effects caused by endogenous interferon-gamma. Virology **231**, 1.

**Saibara T**, Onishi S, Matsuura Y et al (1990) Enhancement of depressed lymphokine activated killer cell activity in patients with hepatocellular carcinoma. Biotherapy **2**, 1.

**Saibara T**, Maeda T, Miyazaki M et al (1993a) Assessment of lymphokine-activated killer activity and gamma-interferon production in patients with small hepatocellular carcinomas. Hepatology **17**, 781.

**Saibara T**, Maeda T, Onishi S, Yamamoto Y(1993b) Depressed immune functions in the early phase of varicella-zoster virus reactivation. J Med Virol **39**, 242.

**Saito H**, Kurose I, Ebinuma H et al (1996) Kupffer cell-mediated cytotoxicity against hepatoma cells occurs through production of nitric oxide and adhesion via ICAM-1/CD18. Int Immunol **8**, 1165.

**Saito S**, Bannerji R, Gansbacher B et al (1994) Immunotherapy of bladder cancer with cytokine gene-modified tumor vaccines. Cancer Res **54**, 3516.

**Saito T**, Berens ME, Welander CE (1986) Direct and indirect effects of human recombinant gamma-interferon on tumor cells in a clongenic assay. Cancer Res **46**, 1142.

**Saito T**, Berens ME, Welander CE (1987) Characterization of the indirect antitumor effect of γ-interferon using ascites-associated macrophages in a human tumor clonogenic assay. Cancer Res **47**, 673.

**Saito T**, Okadome M, Sugihara K et al (1994) Antiproliferative lymphokine production by human peripheral blood lymphocytes and lymph node lymphocytes detected by a modified double layer soft agarose clonogenic assay. Lymphokine Cytokine Res **13**, 55.

**Sakaguchi M**, Honda S, Ozawa M, Nishimura O (1988) Human interferon γ lacking 23 COOH-terminal amino acidis is biologiclly active. FEBS Lett **230**, 201.

**Salup RR**, Sicker DC, Finley GG, Wolmark N (1994) Estrogen receptor positive breast cancer cell lines express neu, ndf and CD 54 (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **35**, A 211.

**Salvaggio A**, Balotta C, Galli M et al (1996) CD4 count in HIV infection is positively correlated to interferon-gamma and negatively correlated to interleukin-10 in vitro production. AIDS **10**, 449.

**Samaniego F**, Markham PD, Gendelman R et al (1998) Vascular endothelial growth factor and basic fibroblast growth factor present in Kaposi's sarcoma (KS) are induced by inflammatory cytokines and synergize to promote vascular permeability and KS lesion development. Am J Pathol **152**, 1433.

**Sanceau J**, Sonderneyer P, Beranger F et al (1987) Intracellular human gamma-interferon triggers an antiviral state in transformed murine L cells. Proc Natl Acad Sci USA **84**, 2906.

**Sanchez-Bueno A**, Verkhusha V, Tanaka Y et al (1996) Interferon-gamma–dependent expression of inducible nitric oxide synthase, interleukin-12 and interferon-gamma–inducing factor in macrophages elicited by allografted tumor cells. Biochem Biophys Res Commun **224**, 555.

**Sangfelt O**, Einhorn S, Bjorklund AC et al (1996) Wild type p53-induced apoptosis in a Burkitt lymphoma cell line is inhibited by interferon-gamma. Int J Cancer **67**, 106.

**Sano E**, Kamata K, Okano K et al (1988) Structural characterization of recombinant human interferon-gamma derived from two different mammalian cells. Microbiol Immunol **32**, 499.

**Santin AD**, Rose GS, Hiserodt JC et al (1996) Effects of cytokines combined with high-dose gamma irradiation on the expression of major histocompatibility complex molecules and intercellular adhesion molecule-1 in human ovarian cancers. Int J Cancer **65**, 688.

**Saravolac EG**, Kournikakis B, Gorton L, Wong JP (1996) Effect of liposome-encapsulation on immunomodulating and antiviral activities of interferon-gamma. Antiviral Res **29**, 199.

**Sareneva T**, Cantell K, Pyhala L et al (1993) Effect of carbohydrates on the pharmacokinetics of human interferon-gamma. J Interferon Res **13**, 267.

**Sareneva T**, Pirhonen J, Cantell K et al (1994) Role of N-glycosylation in the synthesis, dimerization and secretion of human interferon-gamma. Biochem J **303**, 831.

**Sareneva T**, Pirhonen J, Cantell K, Julkunen I (1995) N-glycosylation of human interferon-gamma:glycans at Asn-25 are critical for protease resistance. Biochem J **308**, 9.

**Sareneva T**, Mortz E, Tolo H et al (1996) Biosynthesis and N-glycosylation of human interferon-gamma. Asn 25 and Asn97 differ markedly in how efficiently they are glycosylated and in their oligosaccharide composition. Eur J Biochem **242**, 191.

**Sareneva T**, Matikainen S, Kurimoto M, Julkunen I (1998) Influenza A virus–induced IFN-alpha/beta and IL-18 synergistically enhance IFN-gamma gene expression in human T cells. J Immunol **160**, 6032.

**Sargent ER**, Williams RD (1992) Immunotherapeutic alternatives in superficial bladder cancer. Interferon, interleukin-2, and keyhole-limpet hemocyanin. Urol Clin North Am **19**, 581.

**Sariban E**, Mitchell T, Griffin J, Kufe WD (1987) Effects of interferon-γ on proto-oncogene expression during induction of human monocytic differentiation. J Immunol **138**, 1954.

**Satake I**, Tari K, Nakagomi K, Ozawa K (1993) Feasibility and pharmacokinetics of continuous subcutaneous infusion of low-dose interferon-gamma: a pilot study. Jpn J Clin Oncol **23**, 356.

**Sathar MA**, Simjee AE, Coovadia YM et al (1995) Ascitic fluid gamma interferon concentrations and adenosine deaminase activity in tuberculous peritonitis. Gut **36**, 419.

**Sato K**, Akaki T, Tomioka H (1998) Differential potentiation of anti-mycobacterial activity and reactive nitrogen intermediate-producing ability of murine peritoneal macrophages activated by interferon-gamma (IFN-gamma) and tumour necrosis factor-alpha (TNF-alpha). Clin Exp Immunol **112**, 63.

**Sato N**, Beitz J, Kato J et al (1990) Inhibitory effects of gamma interferon (IFN-gamma) on angiogenesis in vitro (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **31**, A466.

**Sato T**, Selleri C, Young NS, Maciejewski JP (1997) Inhibition of interferon regulatory factor-1 expression results in predominance of cell growth stimulatory effects of interferon-gamma due to phosphorylation of Stat1 and Stat3. Blood **90**, 4749.

**Sato Y**, Tsukada K, Matsumoto Y, Abo T (1993) Interferon-γ inhibits liver regeneration by stimulating major histocompatibility complex class II antigen expression by regenerating liver. Hepatology **18**, 340.

**Saunders BM**, Zhan Y, Cheers C (1995) Endogenous interleukin-12 is involved in resistance of mice to Mycobacterium avium complex infection. Infect Immun **63**, 4011.

**Saunders NA**, Smith RJ, Jetten AM (1994) Differential responsiveness of human bronchial epithelial cells, lung carcinoma cells, and bronchial fibroblasts to interferon-gamma in vitro. Am J Respir Cell Mol Biol **11**, 147.

**Sawai N**, Kita M, Kodama T et al (1999) Role of gamma interferon in Helicobacter pylori–induced gastric inflammatory responses in a mouse model. Infect Immun **67**, 279.

**Saxne T**, Palladino MA, Heinegard D et al (1988). Detection of tumor necrosis factor a but not tumor necrosis factor b in rheumatoid arthritis synovial fluid and serum. Arthritis Rheum **31**, 1041.

**Scanga CA**, Mohan VP, Yu K et al (2000) Depletion of CD4(+) T cells causes reactivation of murine persistent tuberculosis despite continued expression of interferon-gamma and nitric oxide synthase 2. J Exp Med **192**, 347.

**Schadendorf D**, Jurgovsky K, Worm M, Czarnetzki BM (1994) In vitro sensivity of human melanoma cells to chemotherapeutic agents and interferons. Melanoma Res **4**, 243.

**Scharffetter K**, Heckmann M, Hatamochi A et al (1989) Synergistic effect of tumor necrosis factor-alpha and interferon gamma on collagen synthesis of human skin fibroblasts in vitro. Exp Cell Res **181**, 409.

**Scheibenbogen C**, Keilholz U, Maclachlan D et al (1993) Phase II study of intermittent low-dose IFN-gamma in metastatic renal cell cancer. Oncologie **16**, 461.

**Scheynius A**, Fransson J, Johansson C et al (1992) Expression of interferon-gamma receptors in normal and psoriatic skin [see comments]. J Invest Dermatol **98**, 255.

**Schiffer CA** (1991) Interferon studies in the treatment of patients with leukemia. Semin Oncol **18**, 1.

**Schiller JH**, Bittner G (1990) Anti-proliferative effects of tumor necrosis factor, gamma interferon and 5-fluorouracil on human colorectal carcinoma cell lines. Int J Cancer **46**, 61.

**Schiller JH**, Borden EC (1988) Antiproliferative and clinical antitumor effects of interferons. In: Leukolysins and cancer (Ransom JH, Ortaldo JR, eds). Humana Press, Clifton, NJ, p. 273.

**Schiller JH**, Groveman DS, Schmid SM et al (1986) Synergistic Antiproliferative effects of human recombinant α54- or $\beta_{ser}$ interferon with γ-interferon on human cell lines of various histogenesis. Cancer Res **46**, 483.

**Schiller JH**, Storer B, Willson JKV, Borden EC (1987) Phase I trial of combinations of recombinant interferons beta (ser) and gamma in patients with advanced malignancy. Cancer Treat Rep **71**, 945.

**Schiller JH**, Storer B, Bittner G et al (1988). Phase II trial of a combination of interferon-beta (ser) and interferon-gamma in patients with advanced malignant melanoma. J Interferon Res **8**, 581.

**Schiller JH**, Horisberger MA, Bittner G et al (1990a) Effects of combinations of interferon-$\beta_{ser}$ and interferon-γ on interferon-inducible proteins and on the cell cycle. J Biol Response Modif **9**, 368.

**Schiller JH**, Storer B, Paulnock DM et al (1990b) A direct comparison of biological response modulation and clinical side effects by interferon-beta$_{ser}$ Interferon-gamma or the combination of interferon beta$_{ser}$ and gamma in humans. J Clin Invest **86**, 1211.

**Schiller JH**, Storer B, Bittner G et al (1990c). Characterization of the synergistic antiproliferative effects of interferon-γ and tumor necrosis factor on human colon carcinoma cell lines. J Interferon Res **10**, 129.

**Schiller JH**, Pugh M, Kirkwood JM et al (1996) Eastern cooperative group trial of interferon gamma in metastatic melanoma: an innovative study design. Clin Cancer Res **2**, 29.

**Schirren CG**, Majewski S, Hunzelmann N et al (1992). The effect of interferon gamma on the invasiveness of HT-180 cells. Br J Dermatol **126**, 331.

**Schlaak JF**, Buslau M, Jochum W et al (1994) T cells involved in psoriasis vulgaris belong to the Th1 subset. J Invest Dermatol **102**, 145.

**Schmidt M**, Nagel S, Proba J et al (1998) Lack of interferon consensus sequence binding protein (ICSBP) transcripts in human myeloid leukemias. Blood **91**, 22.

**Schneider AW**, Otto U, Conrad S, Klosterhalphen H (1990) Long-term results of the treatment of metastatic renal cell carcinoma with recombinant alpha -2 or gamma-interferon. Helv Chir Acta **57**, 415.

**Schneider LC**, Baz Z, Zarcone C, Zurakowski D (1998) Long-term therapy with recombinant interferon-gamma (rIFN-gamma) for atopic dermatitis. Ann Allergy Asthma Immunol **80**, 263.

**Schofield L** (1992) Interferon gamma and immunity to malaria sporozoites: Implications for the development of synthetic vaccines. In: Antiinfective application of interferon gamma (Jaffe HS, Bucalo LR, Sherwin SA, eds). Marcel Dekker, New York, p. 159.

**Schofield L**, Villaquiran J, Ferreira A et al (1987). γ Interferon, $CD8^+$ T cells and antibodies required for immunity to malaria sporozoites. Nature **330**, 664.

**Schonfeld A**, Schattner A, Crespi M et al (1984) Intramuscular human interferon-βi injections in treatment of condylomata acuminata. Lancet **1**, 1038.

**Schonian U**, Maisch B (1992) Cytomegalovirus associated diseases of the heart. Herz **17**, 85.

**Schreiber RD**, Place JL, Russell SW et al (1983) Macrophage-activating factor produced by a T cell hybridoma: physicochemical and biosynthetic resemblance to gamma-interferon. J Immunol **131**, 826.

**Schuerwegh AJ**, De Clerck LS, De Schutter L et al (1999) Flow cytometric detection of type 1 (IL-2, IFN-gamma) and type 2 (IL-4, IL-5) cytokines in T-helper and T-suppressor/cytotoxic cells in rheumatoid arthritis, allergic asthma and atopic dermatitis. Cytokine **11**, 783.

**Schuger L**, Dixit VM, Carey TE, Varani J (1990) Modulation of squamous carcinoma cell growth, morphology, adhesiveness and extracellular matrix production by interferon-γ and tumor necrosis factor-α. Pathology **58**, 279.

**Schüller J**, Cassidi J, Dumont E et al (2000) Preferential activation of capecitabine in tumor following oral administration to colorectal cancer patients. Cancer Chemother Pharmacol **45**, 291**.**

**Schultz ES**, Dummer R, Becker JC et al (1994) Influence of various cytokines on the interleukin-2–dependent lysis of melanoma cells in vitro. Arch Dermatol Res **286**, 73.

**Schultz RM**, Altom, MG (1990) Protective activity of recombinant murine tumor necrosis factor-alpha and interferon-gamma against experimental murine lung carcinoma metastases. J Interferon Res **10**, 229.

**Schulze HJ**, Mahrle G (1986) Effect of interferons (rIFN-alpha 2, rIFN-gamma) on DNA-synthesis and HLA-DR expresion in psoriasis. Arch Dermatol Res **278**, 416.

**Schut RL**, Gekker G, Hu S et al (1994) Cytomegalovirus replication in murine microglial cell cultures: suppression of permissive infection by interferon-gamma. J Infect Dis **169**, 1092.

**Schwacha MG**, Somers SD (1998) Thermal injury-induced immunosuppression in mice: the role of macrophage-derived reactive nitrogen intermediates. J Leukoc Biol **63**, 51.

**Schwarz MA**, Hamilton LD, Tardelli L et al (1994) Stimulation of cytolitic activity by interleukin 10. J Immunother Emphasis Tumor Immunol **16**, 95.

**Schwarzinger I**, Bettelheim P, Lechner K (1988) Interferon treatment of malignant blood disease. Wien Klin Wochenschr 100, 497.

**Schwarzinger I**, Stain C, Bettelhocheeim P et al (1990) Gamma-interferon in myelodysplastic syndromes: A pilot study. Oncology **47**, 322.

**Sechler JMG**, Malech HL, White CJ, Gallin JI (1988) Recombinant human interferon-γ reconstitutes defective phagocyte function in patients with chronic granulomatous disease of childhood. Proc Natl Acad Sci USA **85**, 4874.

**Seder RA**, Grabstein KH, Berzofsky JA et al (1995) Cytokine interactions in human immunodeficiency virus–infected individuals: Roles of interleukin IL-2, IL-12, and IL-15. J Exp Med **182**, 1067

**Sedmak JJ**, Siebenlist R, Grossberg SE (1985) Human IFN-gamma antiviral activity correlates with immunological reactivity in a double monoclonal antibody radiometric assay. J Interferon Res **5**, 397.

**Seelig GF**, Wijdenes J, Nagabhushan TL, Trotta PP (1988) Evidence for a polypeptide segment at the carboxyl terminus of recombinant human gamma interferon involved in expression of biological activity. Biochemistry **27**, 1981.

**Seelig GF**, Prosise-WW, Taremi-SS (1994) Synthetic mimics of juxtaposed amino- and carboxyl-terminal peptide domains of human gamma interferon block ligand binding to human gamma interferon receptor. J Biol Chem **269**, 358

**Segal DM**, Garrido MA, Perez P et al (1988) Targeted cytotoxic cells as a novel form of cancer immunotherapy. Mol Immunol **25**, 1099.

**Segal R**, Dayan M, Zinger H, Mozes E (1995) Methotrexate treatment in murine experimental systemic lupus erythematosus (SLE); clinical benefits associated with cytokine manipulation. Clin Exp Immunol **101**, 66.

**Seid JM**, Liberto M, Bonina L et al (1986) T cell-macrophage interactions in the immune response to herpes simplex virus: the significance of interferon-gamma. J Gen Virol **67**, 2799.

**Seitz M**, Napierski I, Augustin R et al (1987). Reduced production of interferon alpha and interferon gamma in leukocyte cultures from patients with active rheumatoid arthritis. Scand J Rheumatol **16**, 257.

**Seitz M**, Franke M, Kirchner H (1988a) Induction of antinuclear antibodies in patients with rheumatoid arthritis receiving treatment with human recombinant interferon gamma. Ann Rheum Dis **47**, 642.

**Seitz M**, Napierski I, Kirchner H (1988b) Depressed PPD and tetanus toxoid presentation by monocytes to T lymphocytes in patients with rheumatoid arthritis: restoration by interferon gamma. Rheumatol Int **8**, 189.

**Selby P** (1990) Clinical results with interferon and tumor necrosis factor. Br J Cancer **62**, 481.

**Seliger B**, Pfizenmaier K, Schafer R (1991) Short-term treatment with gamma interferon induces stable reversion of ras-transformed mouse fibroblasts. J Virol **65**, 6307.

**Seliger B**, Hammers S, Hohne A et al (1997) IFN-gamma–mediated coordinated transcriptional regulation of the human TAP-1 and LMP-2 genes in human renal cell carcinoma. Clin Cancer Res **3**, 573.

**Sempowski GD**, Derdak S, Phipps RP (1996) Interleukin-4 and interferon-gamma discordantly regulate collagen biosynthesis by functionally distinct lung fibroblast subsets. J Cell Physiol **167**, 290.

**Sen GC**, Lenguel P (1992) The interferon system. Bird's eye view of its biochemistry. J Biol Chem **267**, 5017.

**Serpier H**, Gillery P, Polette M et al (1992). Modulation of the organization of the extracellular matrix and the production of collagen by interferon gamma in three-dimensional cultures of normal and sclerodermic fibroblasts. Pathol Biol (Paris) **40**, 865.

**Sethi KK**, Omata Y, Brandis H (1985) Contribution of immune interferon (IFN-γ) in lymphokine-induced anti-toxoplasma activity: Studies with recombinant murine IFN-γ. Immunobiology **170**, 270.

**Sgadari C**, Angiolillo AL, Tosato G (1996) Inhibition of angiogenesis by interleukin-12 is mediated by the interferon-inducible protein 10. Blood **87**, 3877.

**Shah P**, Van der Meide PH, Borman T et al (1989) Effect of human recombinant tumor necrosis factor and rat gamma interferon on nitrosomethylurea-induced mammary tumours. Br J Cancer **59**, 206.

**Shalaby MR**, Aggarwal BB, Rinderknecht E et al (1985) Activation of human polymorphonuclear neutrophil functions by interferon-gamma and tumor necrosis factor. J Immunol **135**, 2069.

**Shapiro A**, Ratliff TL, Kelley DR, Catalona WJ (1984) Heightened interferon-gamma production by mononuclear cells from bladder cancer patients. Cancer Res **44**, 3140.

**Sharma B**, Iozzo RV (1998) Transcriptional silencing of perlecan gene expression by interferon-gamma. J Biol Chem **273**, 4642.

**Shaw EG**, Deming RL, Creagan ET et al (1995) Pilot study of human recombinant interferon gamma and accelerated hyperfractionated thoracic radiation therapy in patients with unresectable stage IIIA/B nonsmall cell lung cancer. Int J Radiat Oncol Biol Phys **31**, 827.

**Shearer M**, Taylor-Papadimitriou (1987) Regulation of cell growth by interferon. Cancer Metastasis Rev **6**, 199.

**Shiba T**, Higashi N, Nishimura Y (1998) Hyperglycaemia due to insulin resistance caused by interferon-gamma. Diabet Med **15**, 435.

**Shibata M**, Ando K, Amano S et al (1992) Effect of interferon gamma on expression of HLA and adhesion molecules of tumor cells in patients with hepacellular carcinoma (HCC), and gastric and colorectal cancers. (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **33**, A1465.

**Shibata M**, Ando K, Amano S, Kurosu Y (1993) Adoptive immunotherapy using lymphokine activated killer cells combined with interferon gamma for non-resectable hepatocellular carcinoma. Nihon Univ J Med **35**, 331.

**Shibata M**, Ando K, Takizawa H et al (1995). Effects of local administration of interferon (IFN) gamma on tumor infiltrating lymphocytes (TIL) of digestive system cancers. Biotherapy **9**, 352.

**Shih JC**, Kelemen DW (1995) Possible role of viruses in atherosclerosis. Adv Exp Med Biol **369**, 89.

**Shiiba K**, Suzuki R, Kawakami K et al (1986) Interleukin 2 activated killer cells: Generation in collaboration with interferon gamma and its supression in cancer patients. Cancer Immunol Immunother **21**, 119.

**Shiiki S**, Fuchimoto S, Orita K (1989) Synergistic antitumor effects of natural human tumor necrosis factor-alpha and natural human interferon-alpha or gamma on human cancer cell lines. Res Commun Chem Pathol Pharmacol **64**, 87.

**Shiiki S**, Fuchimoto S, Orita K (1990a). Antitumor effect of interferons with chemotherapeutic agents. Jpn J Cancer Chemother **13**, 293.

**Shiiki S**, Fuchimoto S, Orita K (1990b) Antitumor effects of natural human tumor necroisis factor beta on human cancer cell-lines: Synergistic enhancement by natural human interferon alpha and gamma. Int J Immunother **6**, 71.

**Shikata A**, Sugimoto T, Hosoi H et al (1994) Increased expression of trk proto-oncogene by gamma-interferon in human neuroblastoma cell lines. Jpn J Cancer Res **85**, 122.

**Shikata A**, Shikata T, Sotozono Y et al (2000) Neuronal differentiation in human neuroblastoma cells by nerve growth factor following TrkA up-regulation by interferon-gamma. Med Pediatr Oncol **34**, 394.

**Shima Y**, Nishimoto N, Ogata A et al (1995) Myeloma cells express Fas antigen/APO-1 (CD95) but only some are sensitive to anti-Fas antibody resulting in apoptosis. Blood **85**, 757.

**Shimada S**, Ogawa M, Schlom J, Greiner JW (1993) Comparison of the interferon-gamma–mediated regulation of tumor-associated antigens expressed by human gastric carcinoma cells. In Vivo **7**, 1.

**Shimizu H**, Fujimoto T (1990a) Susceptibility of NB-I neuroblastoma cells to tumorocidal activity of monocytes activated by gamma-interferon. Jpn J Cancer Res **81**, 1021.

**Shimizu H**, Fujimoto T (1990b) Neuroblastoma cell lysis by gamma-interferon-activated monocytes from patients receiving chemotherapy (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **31**, A 1449.

**Shimizu K**, Miyao Y, Okamoto Y et al (1986) Impaired in vitro gamma interferon production of peripheral blood from patients with malignant gliomas. Neurol Med Chir (Tokyo) **26**, 216.

**Shimizu Y**, Iwatsuki S, Herberman RB, Whiteside TL (1991) Effects of cytokines on in vitro growth of tumor-infiltrating lymphocytes obtained from human primary and metastatic liver tumors. Cancer Immunol Immunother **32**, 280.

**Shimizu Y**, Okada K, Tsukishiro T et al (1994) Modulation by cytokines on the surface expression and shedding of intracellular adhesion molecule-1 (ICAM-1) in human liver cancer cell lines. Int Hepatol Commun **2**, 56.

**Shimokata K** (1996) Analysis of cellular immunity against tuberculosis in man with special reference to tuberculous pleurisy and cytokines. Kekkaku **71**, 591.

**Shimokata K**, Saka H, Murate T et al (1991) Cytokine content in pleural effusion: Comparison between tuberculous and carcinomatous pleurisy. Chest **99**, 1103.

**Shiohara T**, Hayakawa J, Nagashima M (1989) Interferon-gamma as adjuvant therapy for resistant warts. J Am Acad Dermatol **21**, 387.

**Shirai A**, Conover J, Klinman DM (1995) Increased activation and altered ratio of interferon-gamma: interleukin-4 secreting cells in MRL-lpr/lpr mice. Autoimmunity **21**, 107.

**Shirazi Y**, Pitha PM (1998) Interferon downregulates CXCR4 (fusin) gene expression in peripheral blood mononuclear cells. J Hum Virol **1**, 69.

**Short SM**, Rubas W, Paasch BD, Mrsny RJ (1995) Transport of biological active interferon-gamma across human skin in vitro. Pharm Res **12**, 1140.

**Short SM**, Paasch BD, Turner JH et al (1996) Percutaneous absorption of biologically-active interferon-gamma in a human skin graft-nude mouse model. Pharm Res **13**, 1020.

**Shuai K**, Stark GR, Kerr IM, Darnell JE (1993) A single phosphotyrosine residue of stat 91 required for gene activation by interferon-γ. Science **261**, 1744.

**Siegel JP** (1988) Effects of interferon-γ on the activation of human T lymphocytes. Cell Immunol **111**, 461.

**Sigal E**, Sloane DL, Conrad DJ (1993) Human 15-lipoxygenase: induction by interleukin-4 and insights into positional specificity. J Lipid Mediat **6**, 75.

**Sigal RK**, Lieberman MD, Reynolds JV et al (1990) Tumor immunization: Improved results after vaccine modified with recombinant interferon gamma. Arch Surg **125**, 308.

**Sigal RK**, Lieberman MD, Reynolds JV et al (1991) Low dose interferon gamma renders neuroblastoma more susceptible to interleukin-2 immunotherapy. J Pediatr Surg **26**, 389.

**Silagi S**, Dutkowski R, Schaefer A (1988) Eradication of mouse melanoma by combined treatment with recombinant human interleukin 2 and recombinant murine interferon-gamma. Int J Cancer **41**, 315.

**Silva JS**, Morrissey PJ, Grabstein KH et al (1992). Interleukin 10 and interferon γ regulation of experimental *Trypanosoma cruzi* infection. J Exp Med **175**, 169.

**Sirianni MC**, Vincenzi L, Fiorelli V et al (1998) Gamma-interferon production in peripheral blood mononuclear cells and tumor infiltrating lymphocytes from Kaposi's sarcoma patients: correlation with the presence of human herpesvirus-8 in peripheral blood mononuclear cells and lesional macrophages. Blood **91**, 968.

**Sivinski CL**, Joshi SS, Colcher D, Tempero MA (1993) Interferon-gamma modulates the antigen recognized by MAb 17-1A on HT29 colorectal carcinoma cells in vitro (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **34**, A2715.

**Sivinski CL**, Lindner DJ, Borden EC Tempero MA (1995) Modulation of tumor-associated antigen expression on human pancreatic and prostate carcinoma cells in vitro by alpha- and gamma-interferons. J Immunother **18**, 156.

**Siziopikou KP**, Harris JE, Casey L et al (1991) Impaired tumoricidal function of alveolar macrophages from patients with non–small cell lung cancer. Cancer **68**, 1035.

**Skoskiewicz MJ**, Colvin RB, Schneeberger EE et al (1985) Widespread and selective induction of major histocompatibility complex-determined antigens *in vivo* by γ interferon. J Exp Med **162**, 1645.

**Slodowski O**, Bohm J, Schone B, Otto B (1991) Carboxy-terminal truncated rhuIFN-gamma with a substitution of Gln133 or Ser132 to leucine leads to higher biological activity than in the wild type. Eur J Biochem **202**, 1133.

**Small EJ**, Weiss GR, Malik UK et al (1998) The treatment of metastatic renal cell carcinoma patients with recombinant human gamma interferon. Cancer J Sci Am **4**, 162.

**Smeets TJ**, Dolhain RJ, Breedveld FC, Tak PP (1998) Analysis of the cellular infiltrates and expression of cytokines in synovial tissue from patients with rheumatoid arthritis and reactive arthritis. J Pathol **186**, 75.

**Smith DD**, Gowen M, Mundy GR (1987) Effects of interferon gamma and other cytokines on collagen synthesis in fetal rat bone cultures. Endocrinology **120**, 2494.

**Smith DM**, Mayhew E, Reszka R et al (1990) Antiviral and antiproliferative properties of liposome-associated human interferon-gamma. J Interferon Res **10**, 153.

**Smith JW II**, Urba WJ, Clark JW et al (1991). Phase I evaluation of recombinant tumor necrosis factor given in combination with recombinant interferon-gamma. J Immunother **10**, 355.

**Smith MR**, Muegge K, Keller Jr et al (1990) Direct evidence for an intracellular role for IFN-gamma. Microinjection of human IFN-gamma induces Ia expression on murine macrophages. J Immunol **144**, 1777.

**Smith PM**, Wolcott RM, Chervenak R, Jennings SR (1994) Control of acute cutaneous herpes simplex virus infection: T cell–mediated viral clearance is dependent upon interferon-gamma (IFN-gamma). Virology **202**, 76.

**Smith TJ**, Higgins PJ (1993) Interferon gamma regulation of de novo protein synthesis in human dermal fibroblasts in culture is anatomic site dependent. J Invest Dermatol **100**, 288.

**Smythies LE**, Waites KB, Lindsey JR et al (2000) Helicobacter pylori–induced mucosal inflammation is Th1 mediated and exacerbated in IL-4, but not IFN-gamma, gene-deficient mice. J Immunol **165**, 1022.

**Snapper CM**, Paul WE (1987) Interferon-γ and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. Science **236**, 944.

**Soderblom T**, Nyberg P, Teppo AM et al (1996) Pleural fluid Interferon-gamma and tumor necrosis factor-alpha in tuberculous and rheumatoid pleurisy. Eur Respir J **9**, 1652.

**Soderlund A**, Gabrielsson S, Paulie S et al (1997) Allergen induced cytokine profiles in type I allergic individuals before and after immunotherapy. Immunol Lett **57**, 177.

**Sodhi A**, Singh RK, Pai K (1990) Activation of murine macrophages by tumor necrosis factor, interleukin-1, interferon-gamma and cisplan. Immunol Lett **26**, 45.

**Sodhi A**, Suresh A, Singh SM (1993) Effect of interferon-gamma, tumor necrosis factor, interleukin-1 and interleukin-6 on the modulation of anti-tumor responses of bone marrow derived macrophages. Int J Immunopharmacol **15**, 327.

**Soh J**, Donnelli RO, Kotenko S et al (1994) Identification and sequence of an accessory factor required for activation of the human interferon γ receptor. Cell **76**, 793.

**Sohn M**, Markos-Pusztai S, Kempeni J et al (1992). Tumor necrosis factor alpha and interferon gamma or alpha: Multicenter trials in metastatic renal cell carcinoma (meeting abstract). Proc Annu Meet Am Soc Clin Oncol **11**, A639.

**Sokoloff MH**, Tso CL, Kaboo R et al (1996) In vitro modulation of tumor progression-associated properties of hormone refractory prostate carcinoma cell lines by cytokines. Cancer **77**, 862.

**Sokolova TM**, Lopatina OA, Nosik NN, Cheshik SG (1993b) Clinical trials of recombinant gamma-interferon. Circulating interferon and the 2,5-oligoadenylate synthetase activity after intramuscular and inhalation administrations. Biull Eksp Biol Med **115**, 633.

**Solary E**, Prud'Homme JF, Gauville C et al (1991) Modulation of proliferation, estradiol receptors and estrogen regulated protein Ps2/BCEI in human breast cancer cell lines by gamma interferon. J Biol Regul Homeostatic Agents **5**, 98.

**Soliman AA**, el-Aggan HA, el-Hefnawy AM et al (1994) The value of ascites adenosine deaminase activity and interferon gamma level in discriminating tuberculous from non-tuberculous ascites. J Egypt Soc Parasitol **24**, 93**.**

**Somoskovi A**, Zissel G, Zipfel PF et al (1999) Different cytokine patterns correlate with the extension of disease in pulmonary tuberculosis. Eur Cytokine Netw **10**, 135.

**Sone S**, Lopez-Berestein G, Fidler IJ (1986a) Potentiation of direct antitumor cytotoxicity and production of tumor cytolytic factors in human blood monocytes by human recombinant interferon-gamma and muramyl dipeptide derivatives. Cancer Immunol Immunother **21**, 93.

**Sone S**, Tandon P, Utsugi T et al (1986b) Synergism of recombinant human interferon gamma with liposome-encapsulated muramyl tripeptide in activation of the tumoricidal properties of human monocytes. Int J Cancer **38**, 495.

**Sone S**, Okubo A, Inamura N et al (1988) Synergism of synthetic acyltripeptide and its analogs with recombinant interferon γ for activation of antitumor properties of human blood monocytes. Cancer Immunol Immunother **27**, 33.

**Song YL**, Ford JW, Gordon D, Shanley CJ (2000) Regulation of lysyl oxidase by interferon-gamma in rat aortic smooth muscle cells. Arterioscler Thromb Vasc Biol **20**, 982.

**Sotomayor EM**, Cheng XF, Fu YX et al (1993) Impaired activation of tumoricidal function in macrophages from mammary tumor bearers: The role of IFN-gamma. Int J Oncol **3**, 719.

**Spath-Schwalbe E**, Porzsolt F, Digel W et al (1989) Elevated plasma cortisol levels during interferon-γ treatment. Immunopharmacology **17**, 141.

**Spellman JE**, Gollnick SO, Zhang PJ, Tomasi TB (1996) Cytokine production by human soft tissue sarcomas: implications for immunosuppression within the tumour bed. Surg Oncol **5**, 237.

**Spranzi E**, Djeu JY, Hoffman SL et al (1993) Lysis of human monocytic leukemia cells by extracellular adenosine triphosphate: Mechanism and characterization of the adenosine triphosphate receptor. Blood **82**, 1578.

**Sprekeler R** (1988) Die Behandlung der rheumatoiden Arthritis (RA) mit rekombinantem Interferon gamma. Aktuelle Immunologie **4**, 35.

**Sprekeler R**, Lemmel EM, Obert HJ (1990). Correlation of clinical and serological findings in patients with rheumatoid arthritis treated for one year with interferon-gamma Z. Rheumatol **49**, 1.

**Sprietsma JE** (1997) Zinc-controlled Th1/Th2 switch significantly determines development of diseases. Med Hypotheses **49**, 1.

**Squires KE**, Rosenkaimer F, Sherwood JA et al (1993) Immunotherapy for visceral leischmaniasis: A controlled pilot trial of antimony versus antimony plus interferon-gamma. Am J Trop Med Hyg **48**, 666.

**Sredni B**, Tichler T, Shani A et al (1996) Predominance of TH1 response in tumor–bearing mice and cancer patients treated with AS101. J Natl Cancer Inst **88**, 1276.

**Stadler R**, Mayer-da-Silva A, Bratzke B et al (1989) Interferons in dermatology. J Am Acad Dermatol **20**, 650.

**Stanciu LA**, Shute J, Promwong C et al (1997) Increased levels of IL-4 in CD8+ T cells in atopic asthma. J Allergy Clin Immunol **100**, 373.

**Stanton GJ**, Jordan C, Hart A et al (1987) Nondetectable levels of interferon gamma is a critical host defense during the first day of herpes simplex virus infection. Microb Pathog **3**, 179.

**Statistical Bull DHSS (**1988**)** New cases at NHS genito-urinary medicine centers in England 1976–1986, **2**.

**Stavropoulos NE**, Ioachim E, Pappa L et al. (1999) Antiproliferative activity of interferon gamma in superficial bladder cancer. Anticancer Res **19** (5C) 4529.

**Stefanos S**, Ahn YH, Pestka S (1989) Characterization of human interferon-γ receptor purified from placenta. J Interferon Res **9**, 719.

**Stein MD**, Gamble DN, Klimpel KD et al (1984) Natural killer cell defects resulting from thermal injury. Cell Immunol **86**, 551.

**Stein U**, Walther W, Shoemaker RH **(**1996) Modulation of mdr1 expression by cytikines in human colon carcinoma cells. Br J Cancer **74**, 1384.

**Steinbeck MJ**, Roth JA, Kaeberle ML (1986) Activation of bovine neutrophils by recombinant interferon-γ. Cell Immunol **98**, 137.

**Steinbeck MJ**, Webb DSA, Roth JA (1989) Role for arachidonic acid metabolism and protein synthesis in recombinant bovine interferon-γ–induced activation of bovine neutrophils. J Leukoc Biol **46**, 450.

**Steiner A**, Wolf Ch, Pehamberger H (1987) Comparison of the effects of three different treatment regimes of recombinant interferons (r-IFN gamma and r-IFN alpha + cimetidine) in disseminated malignant melanoma. J Cancer Res Clin Oncol **113**, 459.

**Stella CC**, Cazzola M, Ganser A et al (1988) Recombinant gamma-interferon induces in vitro monocytic differentiation of blast cells from patients with acute nonlymphocytic leukemia and myelodysplastic syndromes. Leukemia **2**, 55.

**Stemme S**, Faber B, Holm J et al (1995) T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. Proc Natl Acad Sci USA **92**, 3893.

**Stenquist**, **B**. Wennberg, A.-M., Gisslen, H., Larko. O. (1992) Treatment of aggressive basal cell carcinoma with intralesional interferon: evaluation of efficacy by Mohs surgery. J Am Acad Dermatol **27**, 65.

**Steplewski Z**, Blaszczyk M, Herlyn D et al (1985) Effector cells in ADCC with anti-breast cancer monoclonal antibodies. Dev Oncol **35**, 134.

**Stevens DA** (1992) Interferon-gamma and fungal infections. In: Antiinfective application of interferon gamma (Jaffe HS, Bucalo LR, Sherwin SA, eds). Marcel Dekker, New York, p 279.

**Stevens SR**, Hanifin JM, Hamilton T et al (1998) Long-term effectiveness and safety of recombinant human interferon gamma therapy for atopic dermatitis despite unchanged serum IgE levels. Arch Dermatol **134**, 799.

**Stewart WE**, Blaloc JL, Burke DC et al (1980) Interferon nomenclature. Eur J Immunology **10**, 660.

**Stoeckle MY**, Falck-Pederson E, Rubin BY et al (1996) Delivery of human interferon-gamma via gene transfer in vitro: prolonged expression and induction of macrophage antimicrobial activity. J Interferon Cytokine Res **16**, 1015**.**

**Stolzenburg T**, Binz H, Fontana A et al (1988). Impaired mitogen-induced interferon-gamma production in rheumatoid arthritis and related diseases. Scand J Immunol **27**, 73.

**Stone RM**, Spriggs DR, Arthur KA et al (1993) Recombinant human gamma interferon administered by continuous intravenous infusion in acute myelogenous leukemia and myelodysplastic syndromes. Am J Clin Oncol Cancer Clin Trials **16**, 159.

**Storey A**, Thomas M, Kalita A et al (1998) Role of a p53 polymorphism in the development of human papilloma-virus–associated cancer. Nature, **393**, 229.

**Storkus WL**, Nowell DN, Salter RD et al (1987) NK susceptibility varies inversely with target cell class I. HLA antigen expression. J Immunol **138**, 1657.

**Strander H** (1989) The action of interferons on virus-associated human neoplasms. Cancer Surv **8**, 755.

**Straus SE**, Tahiff HE, Seidlin M et al (1984) Suppression of frequently. 4, recurrent genital herpes. A placebo-controlled double-blind trial of oral acyclovir. N Engl J Med **310**, 1545.

**Straus SE**, Seidlin M, Tahiff HE et al (1986) Double-blind comparison of weekend and daily regimens of oral acyclovir for suppression of recurrent genital herpes. Antiviral Res **6**, 151.

**Strickland RW**, Wahl LM, Finbloom DS (1986) Corticosteroids enhance the binding of recombinant interferon gamma to cultured human monocytes. J Immunol **137**, 1577.

**Strieter RM**, Kunkel SL, Arenberg DA et al (1995) Interferon gamma–inducible protein 10 (IP-10) a member of the C-X-C chemokine family, is an inhibitor of angiogenesis. Biochem Biophys Res Commun **210**, 51.

**Stuber D**, Friedlein A, Fountoulakis M et al (1993). Alignment of disulfide bonds of the extracellular domain of the interferon γ receptor and investigation of their role in biological activity. Biochemistry **32**, 2423.

**Stukart MJ**, Rijnsent A, Roos E, Suzuki et al (1987) Induction of tumoricidal activity in isolated rat liver macrophages by liposomes containing recombinant rat gamma-interferon supplemented with lipopolysaccharide or muramyldipeptide. Cancer Res **47**, 3880.

**Su N**, Ojeifo JO, MacPherson A, Zweibel JA (1994) Breast cancer gene therapy: Transgenic immunotherapy. Breast Cancer Res Treat **31**, 349.

**Suarez-Pestana E**, Bjorklund G, Larsson R et al (1996) Effects of interferons and tumour necrosis factor-alpha on human lung cancer cell lines and the development of an interferon-resistant lung cancer cell line. Acta Oncol **35**, 473.

**Subauste CS**, Remington JS (1992) The role of interferon-gamma in resistance to Trypanosoms gondii. In: Antiinfective application of interferon gamma (Jaffe HS, Bucalo LR, Sherwin SA, eds). Marcel Dekker, New York, p 87.

**Subratty AH**, Hooloman NK (1998) Role of circulating inflammatory cytokines in patients during an acute attack of bronchial asthma. Indian J Chest Dis Allied Sci **40**, 17.

**Sugimoto T**, Horii Y, Hino T et al (1989) Differential susceptibility of HLA class II antigens induced by gamma-interferon in humanneuroblastoma cell lines. Cancer Res **49**, 1824.

**Summersgill JT**, Powell LA, Buster BL et al (1992) Killing of Legionella pneumophila by nitric oxide in gamma interferon–activated macrophages. J Leukoc Biol **52**, 625.

**Sun H**, Gutierrez P, Jackson MJ et al (2000) Essential role of nitric oxide and interferon-gamma for tumor immunotherapy with interleukin-10. J Immunother **23**, 208.

**Sundmacher R**, Mattes A, Neumann-Haefelin D et al (1987) The potency of interferon-alpha 2 and interferon-gamma in a combination therapy of dendritic keratitis. A controlled clinical study. Curr Eye Res **6**, 273.

**Sur P**, Matsuo Y, Otani T, Minowada J (1991) Modulation of methotrexate cytotoxicity with natural interferon upon human leukemia cell line HL-60. Oncology **48**, 469.

**Sutas Y**, Hurme M, Isolauri E (1997) Oral cow milk challenge abolishes antigen-specific interferon-gamma production in the peripheral blood of children with atopic dermatitis and cow milk allergy. Clin Exp Allergy **27**, 277.

**Suzuki**, **H**, Shibano, K., Okane, M. et al (1989) Interferon-γ modulates messenger RNA levels of c-sis (PDGF-B chain), PDGF-A chain, and IL-1β genes in human vascular endothelial cells. Am J Pathol **134**, 35.

**Suzuki Y**, Orellana MA, Schreiber RD, Remington JS (1988) Interferon-γ: The major mediator of resistance against *Toxoplasma gondii.* Science **240**, 516.

**Svane IM**, Engel AM, Nielsen M, Werdelin O (1997) Interferon-gamma induced MHC class I expression and defects in Jak/Stat signalling in methylcholanthrene-induced sarcomas. Scand J Immunol **46**, 379.

**Sveinbjornsson B**, Olsen R, Seternes OM, Seljelid R (1996) Macrophage cytotoxicity against murine meth A sarcoma involves nitric oxide–mediated apoptosis. Biochem Biophys Res Commun **223**, 643.

**Swaak AJ**, van den Brink HG, Aarden LA (1997) Cytokine production in whole blood cell cultures of patients with rheumatoid arthritis. Ann Rheum Dis **56**, 693.

**Swaminathan S**, Gong J, Zhang M et al (1999) Cytokine production in children with tuberculous infection and disease. Clin Infect Dis **28**, 1290.

**Sweet MJ**, Stacey KJ, Kakuda DK et al (1998) IFN-gamma primes macrophage responses to bacterial DNA. J Interferon Cytokine Res **263**.

**Sylvester DM**, Liu SY, Meadows GG (1990) Augmentation of antimetastatic activity of interferon and tumor necrosois factor by heparin. Immunopharmacol Immunotoxicol **12**, 161.

**Symington FW** (1989) Lymphotoxin, tumor necrosis factor, and gamma interferon are cytostatic for normal human keratinocytes. J Invest Dermatol **92**, 798.

**Szabo G** (1998) Monocytes, alcohol use, and altered immunity. Alcohol Clin Exp Res **5**(Suppl), 216S.

**Szabo SK**, Hammerberg C, Yoshida Y et al (1998) Identification and quantitation of interferon-gamma producing T cells in psoriatic lesions: localization to both CD4+ and CD8+ subsets. J Invest Dermatol **111**, 1072.

**Szente BE**, Johnson HM (1994) Binding of IFNγ and its C-terminal peptide to a cytoplasmic domain of its receptor that is essential for function. Biochem Biophys Res Commun **1**, 215.

**Szente BE**, Soos JM, Johnson HW (1994) The C-terminus of IFN gamma is sufficient for intracellular function. Biochem Biophys Res Commun **30**, 1645.

## T

**Tabibzadeh SS**, Satyaswaroop GP, Rao NP (1987) Antiproliferative effect of interferon-gamma in human endometrial epithelial cells *in vitro*: Potential local growth modulatory role in endometrium. J Clin Endocrinol Metabol **67**, 131.

**Tagliaferri P**, Barile C, Caraglia M et al (1998) Daily low-dose subcutaneous recombinant interleukin-2 by alternate weekly administration: antitumor activity and immunomodulatory effects. Am J Clin Oncol **21**, 48.

**Tajima F**, Kawatani T, Endo A, Kawasaki H (1996) Natural killer cell activity and cytokine production as prognostic factors in adult acute leukemia. Leukemia **10**, 478.

**Takagi K**, Suzuki F, Barrow RE et al (1998) Recombinant human growth hormone modulates Th1 and Th2 cytokine response in burned mice. Ann Surg **228**, 106.

**Takahashi T**, Mitsuhashi N, Sakurai H, Niibe H (1995). Modifications of tumor-associated antigen expression on human lung cancer cells by hyperthermia and cytokine. Anticancer Res **15**, 2601.

**Takaku F** (1987) Treatment of various malignancies with recombinant IFN gamma (S 6810) The Interferon Gamma Study Group. Gan To Kagaku Ryoho **14**, 645.

**Takamuku K**, Baba K, Arinaga S et al (1996) Apoptosis in antibody-dependent monocyte-mediated cytotoxicity with monoclonal antibody 17-1A against human colorectal carcinoma cells: enhancement with interferon gamma. Cancer Immunol Immunother **43**, 220.

**Takayama H**, Takagi H, Saito S et al (1990) A basic study for cytotoxic effect of tumor necrosis factor-alpha on the hepatoma cell line PLC/PRF/5. Jpn J Gastroenterol **87**, 195.

**Takebayashi Y**, Yamada K, Ohmoto Y et al (1995) The correlation of thymidine phosphorylase activity with the expression of interleukin 1 alpha, interferon alpha and interferon gamma in human colorectal carcinoma. Cancer Lett **95**, 57.

**Takebayashi Y**, Akiyama SI, Haraguchi M et al (1996) Angiogenesis and thymidine phosphorylase. Biotherapy (Japan) **10**, 993.

**Takeda T** (1991) Mechanism of growth inhibition of a hepatocellular carcinoma cell line by interferon. J Osaka City Med Ctr **40**, 427.

**Takeda T**, Nishiguchi S, Kuroki T (1989) Effects of interferon on the cell cycle of human hepatoma HLF cells analyzed by flow cytometry. Jpn J Gastroenterol **86**, 876.

**Takei M**, Takeda K, Konno K (1984) The role of interferon-gamma in induction of differentiation of human myeloid leukemia cell lines ML-1 and HL-60. Biochem Biophys Res Commun **124**, 100.

**Taki H**, Sugiyama E, Mino T et al (1998) Differential inhibitory effects of indomethacin, dexamethasone, and interferon-gamma (IFN-gamma) on IL-11 production by rheumatoid synovial cells. Clin Exp Immunol **112**, 133.

**Takiguchi M**, Ting JPY, Buessow SC et al (1985) Response of glioma cells to interferon-gamma: Increase in class II RNA, protein and mixed lymphocyte reaction-stimulating ability. Eur J Immunol **15**, 809.

**Takuma T**, Takeda K, Konno K (1987) Synergism of tumor necrosis factor and interferon-gamma in induction of differentiation of human myeloblastic leukemic ML-1 cells. Biochem Biophys Res Commun **145**, 514.

**Talal N**, Flescher N (1988) Rheumatoid arthritis: an editorial perspective based on cytokine imbalance J Autoimmun **1**, 309.

**Talpaz M**, Kurzrock R, Kantarjian H et al (1991) A phase II study alternating alpha-2a-interferon and gamma-interferon therapy in patients with chronic myelogenous leukemia. Cancer **68**, 2125.

**Talpaz M**, Kantarjian H, Kurzrock R, Gutterman JU (1992) Bonne marrow hypoplasia and aplasia complicating interferon therapy for chronic myelogenous leukemia. Cancer **69**, 410.

**Tamura S**, Miyata K, Matsuo K et al (1996) Acceleration of influenza virus clearance by Th1 cells in the nasal site of mice immunized intranasally with adjuvant-combined recombinant nucleoprotein. J Immunol **15**, 3892.

**Tanabe K**, Campbell SC, Alexander JP et al (1997) Molecular regulation of intercellular adhesion molecule 1 (ICAM-1) expression in renal cell carcinoma. Urol Res **25**, 231.

**Tanaka M**, Kimura K, Yoshida S (1987) Inhibition of mammalian DNA polymerases by recombinant α-interferon and γ-interferon Cancer Res **47**, 5971.

**Tang Q**, Hendricks RL (1996) Interferon gamma regulates platelet endothelial cell adhesion molecule 1 expression and neutrophil infiltration into herpes simplex virus-infected mouse corneas. J Exp Med **1**, 1435.

**Tang Q**, Chen W, Hendricks RL (1997) Proinflammatory functions of IL-2 in herpes simplex virus corneal infection. J Immunol **158**, 1275.

**Tank B**, Habets JM, Naafs B et al (1989) Intralesional treatment of basal cell carcinoma with low-dose recombinant interferon gamma. J Am Acad Dermatol **21**, 734.

**Tannenberger S**, Hrelia P (1996) Interferons in precancer and cancer prevention: Where are we? J Interferon Cytokine Res **16**, 339.

**Tascon RE**, Stavropoulos E, Lukacs KV, Colston MJ (1998) Protection against Mycobacterium tuberculosis infection by CD8+ T cells requires the production of gamma interferon. Infect Immun **66**, 830.

**Tashiro M**, Yokoyama K, Nakayama M et al (1996) A case of nephrotic syndrome developing during postoperative gamma interferon therapy for renal cell carcinoma. Nephron **73**, 685.

**Taylor CW**, Chase EM, Hersh EM et al (1990) TI: A phase I study of the sequential combination of recombinant gamma-interferon (IFN gamma) and recombinant interleukin-2 (IL2) in patients with disseminated malignant disease (meeting abstract). Proc Annu Meet Am Soc Clin Oncol **9**, A742.

**Taylor CW**, Chase EM, Whitehead RP et al (1992). A Southwest Oncology Group Phase I study of the sequential combination of recombinant interferon-gamma and recombinant interleukin-2 in patients with cancer. J Immunother **11**, 176.

**Taylor JL**, Little SD, O'Brien WJ (1998) The comparative anti-herpes simplex virus effects of human interferons. J Interferon Cytokine Res **18**, 159.

**Taylor MW**, Feng G (1991) Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism. FASEB J **5**, 2516.

**Taylor RS**, Griffiths CE, Brown MD et al (1990) Constitutive absence and interferon-gamma–induced expression of adhesion molecules in basal cell carcinoma. J Am Acad Dermatol **22**, 721.

**Taylor-Robinson AW**, Phillips RS (1998) Infective dose modulates the balance between Th1- and Th2-regulated immune responses during blood-stage malaria infection. Scand J Immunol **48**, 527.

**Tellides G**, Tereb DA, Kirkles-Smith NC et al (2000) Interferon-γ elicits arteriosclerosis in the absence of leukocytes. Nature **403**, 207.

**Ten Hacken NH**, Oosterhoff Y, Kauffman HF (1998) Elevated serum interferon-gamma in atopic asthma correlates with increased airways responsiveness and circadian peak expiratory flow variation. Eur Respir J **11**, 312.

**Ten Hagen TL**, Vossen AC, van Vianen W et al (1995) Enhancement of nonspecific resistance by liposome-encapsulated immunomodulators does not affect skin graft rejection in mice. Transplantation **60**, 1211.

**Teramoto T**, Fukao T, Tashita H et al (1998) Serum IgE level is negatively correlated with the ability of peripheral mononuclear cells to produce interferon gamma (IFNgamma): Evidence of reduced expression of IFNgamma mRNA in atopic patients. Clin Exp Allergy **28**, 74.

**Teramura Y**, Watanabe Y, Kan N et al (1993) Interferon-gamma–producing tumor induces host tumor specific T cell responses. Jpn J Cancer Res **84**, 689.

**Testa U**, Ferbus D, Gabbianelli M et al (1988) Effect of endogenous and exogenous interferons on the differentiation of human monocyte cell line U937. Cancer Res **48**, 82.

**Thaler J** (1993) Interferon-alpha for the treatment of chronic myelogenous leukemia. Acta Med Austriaca **20**, 65.

**Than S**, Hu R, Oyaizu N, Romano J (1997) Cytokine pattern in relation to disease progression in human immunodeficiency virus–infected children. J Infect Dis **175**, 47.

**Thiele K**, Kirchner H (1988) Effects of different interferons on the replication of herpes simplex virus in human T lymphocytes. J Interferon Res **8**, 507.

**Thom AK**, Alexander HR, Andrich MP et al (1995) Cytokine levels and systemic toxicity in patients undergoing isolated limb perfusion with high-dose tumor necrosis factor, interferon gamma, and melphalan. J Clin Oncol **13**, 264.

**Thoma-Greber E**, Froschl M, Stolz W, et al (1993) Interferon-gamma. Therapy of recurrent furunculosis in HIV infections. Hautarzt **44**, 587.

**Thomassen MJ**, Barna BP, Wiedemann HP et al (1990). Immunologic studies of alveolar macrophages from patients with metastatic renal cell carcinoma. Am Rev Respir Dis **141**, 1256.

**Thompson DC**, Porter SE, Bauer AK et al (1998) Cytokine-induced nitric oxide formation in normal but not in neoplastic murine lung epithelial cell lines. Am J Physiol **274**, 922.

**Thompson JA**, Cox WW, Lindgren CG et al (1987) Subcutaneous recombinant gamma interferon in cancer patients: Toxicity, pharmacokinetics and immunomodulatory effects. Cancer Immunol Immunother **25**, 47.

**Tiemessen CT**, Kidd AH (1993) Sensitivity of subgroup F adenoviruses to interferon. Arch Virol **128**, 1.

**Tiggelman AM**, Boers W, Linthorst C et al (1995) Collagen synthesis by human liver (myo) fibroblasts in culture: evidence for a regulatory role of IL-4, TGF beta and IFN gamma. J Hepatol **23**, 307.

**Till S**, Durham S, Dickason R et al (1997) IL-13 production by allergen-stimulated T cells is increased in allergic disease and associated with IL-5 but not IFN-gamma expression. Immunology **91**, 53.

**Ting LM**, Kim AC, Cattamanchi A, Ernst JD (1999) Mycobacterium tuberculosis inhibits IFN-gamma transcriptional responses without inhibiting activation of STAT1. J Immunol **163**, 3898**.**

**Toda S**, Ishii N, Okada E et al (1997) HIV-1 specific cell-mediated immune responses induced by DNA vaccination were enhanced by mannan-coated liposomes and inhibited by anti–interferon-gamma antibody. Immunology **92**, 111.

**Tohda Y**, Iwanaga T, Uejima H et al (1996) Effects of cytokines on oxygen radical production by peripheral blood monocytes and alveolar macrophages in patients with lung cancer. Tumori **82**, 382.

**Tohda Y**, Nakahara H, Kubo H et al (1998) Theophylline suppresses the release of interleukin-4 by peripheral blood mononuclear cells. Int Arch Allergy Immunol **115**, 42.

**Tohyama K**, Ohmori S, Ueda T et al (1989) Cooperative effects of gamma-interferon and 1 alpha, 25-dihydroxyvitamin D-3 on in vitro differentiation of the blast cells of RAEB and RAEB T. Blut **58**, 181.

**Toledano M**, Mathiot C, Michon J et al (1989) Interferon-γ (IFN-γ) and interleukin-2 in the generation of lymphokine-activated killer cell cytotoxicity-IFN-γ–induced suppressive activity. Cancer Immunol Immunother **30**, 57.

**Toretsky JA**, Shahidi NT, Finlay JL (1986) Effects of recombinant human interferon gamma on hematopoietic progenitor cell growth. Exp Hematol **14**, 182.

**Torres MA**, Furst DE (1990) Treatment of generalized systemic sclerosis. Rheum Dis Clin North Am **16**, 217.

**Torseth JW**, Merigan TC (1986) Significance of local gamma interferon in recurrent herpes simplex infection. J Infect Dis **153**, 979.

**Toshimitsu** M, Takahashi R, Mihara K et al (1985) Cooperative regulation of c-*myc* expression in differentiation of human promyelocytic leukemia induced by recombinant γ-interferon and 1,25-dihydroxyvitamin D3. Cancer Res **45**, 4366.

**Toth CA**, Thomas P (1990) The effect of interferon treatment on 14 human colorectal cancer cell lines: growth and carcinoembryonic antigen secretion in vitro. J Interferon Res **10**, 579.

**Toth M**, Endresz V, Toth S, Beladi I (1985) Human interferons alpha and beta have more potent priming activities than interferon gamma. J Gen Virol **66**, 893.

**Totterman TH**, Danersund A, Carlsson M, Nilsson K (1987) Effects of recombinant interferon-alpha and -gamma on B-CLL cells in serum-free medium: Expression of activation, differentiation and CALLA antigens. Leukemia **1**, 667.

**Tovey** MG, Streuli M, Gresser I et al (1987) Interferon messenger RNA is produced constitutively in the organs of normal individuals. Proc Natl Acad Sci USA **84**, 5038.

**Towata T**, Hayashi N, Katayama K et al (1991) Signal transduction pathways in the induction of HLA class I antigen expression on HUH 6 cells by interferon-gamma. Biochem Biophys Res Commun **177**, 610.

**Trachtman H**, Futterweit S, Singhal P (1995) Nitric oxide modulates the synthesis of extracellular matrix proteins in cultured rat mesangial cells. Biochem Biophys Res Commun **207**, 120.

**Traversari C**, Meazza R, Coppolecchia M et al (1997) IFN-gamma gene transfer restores HLA-class I expression and MAGE-3 antigen presentation to CTL in HLA-deficient small cell lung cancer. Gene Ther **4**, 1029.

**Tredget EE**, Wang R, Shen Q et al (2000) Transforming growth factor-beta mRNA and protein in hypertrophic scar tissues and fibroblasts: antagonism by IFN-alpha and IFN-gamma in vitro and in vivo. J Interferon Cytokine Res **20**, 143.

**Tremisi PJ**, Bich Thuy LT (1991) Restoration of high immunoglobulin gene expression in chronic lymphoid leukemia. A possible application for gene therapy. Cell Immunol **135**, 326.

**Trent JM**, Olson S, Lawn RM (1982) Chromosomal localization of human leukocyte, fibroblast and immune interferon genes by means of *in situ* hybridization. Proc Natl Acad Sci USA **79**, 7809.

**Treon SP**, Broitman SA (1992) Beneficial effects of post-transfusional hepatitis in acute myelogenous leukemia may be mediated by lipopolysaccharides, tumor necrosis factor alpha and interferon-gamma. Leukemia **6**, 1036.

**Treon SP**, Anand B, Chou IN, Broitman SA (1991) Differentiation of HL60 promyelocytic leukemia cells by tumor necrosis factor-alpha (TNF-alpha), interferon-gamma (IFN-gamma) and lipopolysaccharide (LPS) is accompanied by reorganization of the microtubular network (meeting abstract). FASEB J **5**, A1619.

**Trinchieri G** (1997) Function and clinical use of interleukin-12. Curr Opin Hematol **4**, 59.

**Trinchieri G**, Rosen M, Perussia B (1987) Retinoic acid cooperates with tumor necrosis factor and immune interferon in inducing differentiation and growth inhibition of the human promyelocytic leukemic cell line HL-60. Blood **69**, 1218.

**Trizna Z**, Clayman G, Goepfert H, Lotan R (1994) Modulation of growth and human papilloma virus E6/E7 expression in human squamous carcinoma cells by interferon-alpha, interferon-gamma and retinoic acid alone or in combination (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **35**, A3506.

**Trizna Z**, Evans T, Bruce S et al (1998) A randomized phase II study comparing four different interferon therapies in patients with recalcitrant condylomata acuminata. Sex Transm Dis **25**, 361.

**Trotta PP**, Harrison SD Jr (1987) Evaluation of the antitumor activity of recombinant human gamma-interferon employing human melanoma xenografts in athymic nude mice. Cancer Res **47**, 5347.

**Trubiani O**, Bosco D, Di-Primio R (1994) Interferon gamma (IFN-gamma) induces programmed cell death in differentiated human leukemic B cell lines. Exp Cell Res **215**, 23.

**Trumphfheller C**, Tenner-Racz K, Racz P et al (1998) Expression of macrophage inflammatory protein (MIP)-1 alpha, MIP-1 beta, and RANTES genes in lymph nodes from HIV+ individuals: Correlation with a Th1-type cytokine response. Clin Exp Immunol **112**, 92.

**Tsokos GC** (1996) Lymphocytes, cytokines, inflammation, and immune trafficking. Curr Opin Rheumatol **8**, 395.

**Tsokos GC**, Koyacs B, Sfikakis PP et al (1996) Defective antigen-presenting cell function in patients with systemic lupus erythematosus. Arthritis Rheum **39**, 600.

**Tsuruoka N**, Sugiyama M, Tawaragi Y et al (1988) Inhibition of in vitro angiogenesis by lymphotoxin and interferon gamma. Biochem Biophys Res Commun **155**, 429.

**Turano A**, Caruso A (1993) The role of human autoantibodies against gamma-interferon. J Antimicrob Chemother **32**(Suppl A), 99.

**Tuttle TM**, McCrady CW, Inge TH et al (1993) gamma-interferon plays a key role in T-cell–induced tumor regression. Cancer Res **53**. 833.

**Tuyt LML**, Bregman K, Lummen C et al (1998) Differential binding activity of the transcription factor LIL-STAT in immature and differentiated normal and leukemic myeloid cells. Blood **92**, 1364.

**Twigg HL III**, Spain BA, Soliman DM et al (1999) Production of interferon-gamma by lung lymphocytes in HIV-infected individuals. Am J Physiol **276**, 256.

## U

**Ucar K**, Seeger RC, Challita PM et al (1995) Sustained cytokine production and immunophenotypic changes in human neuroblastoma cell lines transduced with a human gamma interferon vector. Cancer Gene Ther **2**, 171.

**Ucer U**, Bartsch H, Scheurich P et al (1986) Quantitation and characterization of γ-interferon receptors on human tumor cells. Cancer Res **46**, 5339.

**Uchio E**, Ono SY, Ikezawa Z, Ohno S (2000) Tear levels of interferon-gamma, interleukin (IL)-2, IL-4 and IL-5 in patients with vernal keratoconjunctivitis, atopic keratoconjunctivitis and allergic conjunctivitis. Clin Exp Allergy **30**, 103.

**Ulchaker J**, Panuto J, Rayman P et al (1999) Interferon-gamma production by T lymphocytes from renal cell carcinoma patients: evidence of impared secretion in response to interleukin-12. J Immunother **22**, 71.

**Ullum H**, Cozzi Lepri A, Bendtzen K et al (1997) Low production of interferon gamma is related to disease progression in HIV infection; evidence from a cohort of 347 HIV-infected individuals. AIDS Res Hum Retroviruses **13**, 1039.

**Unemori EN**, Bair MJ, Bauer EA, Amento EP (1991) Stromelysin expression regulates collagenase activation in human fibroblasts. J Biol Chem **266**, 23477.

**Uotila P (**1996) The role of cyclic AMP and oxygen intermediates in the inhibition of cellular immunity in cancer. Cancer Immunol Immunother **43**, 1.

**Upton C**, Mossman K, McFadden G (1992) Encoding of a homolog of the IFN-gamma receptor by myxoma virus. Science **258**, 1369.

**Urabe T**, Mizuno Y, Terada M et al (1989) A case with hepatocellular carcinoma with complete remission due to gamma-IFN therapy. J Jpn Soc Cancer Ther **24**, 1616.

**Urbani F**, Maleci A, La Sala A et al (1995) Defective expression of interferon-gamma, granulocyte-macrophage colony-stimulating factor, tumor necrosis factor alpha, and interleukin-6 in activated peripheral blood lymphocytes from glioma patients. J Interferon Cytokine Res **15**, 421.

**US Department of Health and Human Services** (1980) 0-311-201/3052. US Government Printing Office, Washington, DC.

**Usuki K**, Kitamura K, Urabe A, Takaku F (1988) Herpes zoster, postherpetic neuralgia, and interferon-gamma. Ann Intern Med **108**, 643.

**Utsugi T**, Sone S, Tandon P et al (1986) Induction of tumorocidal properties in human monocytes by synergism between interferon-gamma and liposome-entrapped muramyl tripeptide. Gan To Kagaku Ryoho **13**, 3161.

## V

**Vadhan-Raj S**, Al Katib A, Bhalla R et al (1986a) Phase I trial of recombinant interferon gamma in cancer patients. J Clin Oncol **4**, 137.

**Vadhan-Raj S**, Nathan CF, Sherwin SA et al (1986b) Phase I trial of recombinant interferon gamma by 1-hour iv infusion. Cancer Treat Rep **70**, 609.

**Vaglini M**, Belli F, Ammatuna M et al (1994a) Treatment of primary or relapsing limb cancer by isolation perfusion with high-dose alpha-tumor necrosis factor, gamma-interferon, and melphalan. Cancer **73**, 483.

**Vaglini M**, Santinami M, Manzi R et al (1994b) Treatment of in-transit metastases from cutaneous melanoma by isolation perfusion with tumor necrosis factor-alpha (TNF-alpha), melphalan and interferon-gamma (IFN-gamma). Dose-finding experience at the National Cancer Institute of Milan. Melanoma Res **4** (Suppl 1), 35.

**Vairo G**, Vadiveloo PK, Royston AK et al (1995) Deregulated c-myc expression overrides IFNγ-induced macrophage growth arrest. Oncogene **10**, 1969.

**Valdes L**, San Jose E, Alvarez D et al (1993) Diagnosis of tuberculous pleurisy using the biologic parameters adenosine deaminase, lysozyme and interferon gamma. Chest **103**, 458.

**Valdez H**, Lederman MM (1997) Cytokines and cytokine therapies in HIV infection. AIDS Clin Rev 187.

**Vambutas A**, Bonagura VR, Steinberg BM (2000) Altered expression of TAP-1 and major histocompatibility complex class I in laryngeal papillomatosis: Correlation of TAP-1 with disease. Clin Diagn Lab Immunol **7**, 79.

**Vance JC**, Davis D (1990) Interferon alpha-2b injections used as an adjuvant therapy to carbon dioxide laser vaporization of recalcitrant ano-genital condylomata acuminata. J Invest Dermatol **95**, 146S.

**Van Crevel R**, Karyadi E, Preyers F et al (2000) Increased production of interleukin 4 by CD4+ and CD8+ T cells from patients with tuberculosis is related to the presence of pulmonary cavities. J Infect Dis **181**, 1194.

**Van de Loosdrecht AA**, Ossenkoppele GJ, Beelen RHJ et al (1992) Role of interferon gamma and tumor necrosis factor alpha in monocyte-mediated cytostasis and cytotoxicity against a human histocytic lymphoma cell line. Cancer Immunol Immunother **34**, 393.

**Van de Loosdrecht AA**, Beelen RHJ, Ossenkoppele GJ et al (1993a) Cellular and cytokine dependent monocyte-mediated leukemic cell death: Modulation by interferon-gamma and tumor necrosis factor-alpha. Exp Hematol **21**, 461.

**Van de Loosdrecht AA**, Ossenkoppele GJ, Beelen RHJ et al (1993b) Cell cycle specific effects of tumor necrosis factor alpha in monocyte mediated leukemic cell death and the role of beta-2-integrins. Cancer Res **53**, 4399.

**Van den Broek MF**, Muller U, Huang S et al (1995) Antiviral defense in mice lacking both alpha/beta and gamma interferon receptors. J Virol **69**, 4792.

**van de Stolpe A**, van der Saag PT (1996) Intercellular adhesion molecule-1. J Mol Med **74**, 13.

**Van Furth R** (1990) Mycobacteria and macrophage activation**.** Res Microbiol **141**, 256.

**van Moorselaar RJA**, Beniers AJMC, Hendriks BTh et al (1990) In vivo antiproliferative effects of gamma-interferon and tumor necrosis factor alpha in a rat renal cell carcinoma model system. J Urol **143**, 1247.

**van Moorselaar RJA**, Hendriks BT, van Stratum P et al (1991a) Synergistic antitumor effects of rat gamma-interferon and human tumor necrosis factor alpha against androgen-dependent and -independent rat prostatic tumors. Cancer Res **51**, 2329.

**van Moorselaar RJ**, Van Stratum P, Borm G et al (1991b) Differential antiproliferative activities of alpha- and gamma-interferon and tumor necrosis factor alone or in combinations against two prostate cancer xenografts transplanted in nude mice. Prostate **18**, 331.

**Van Roon JA**, Verhoef CM, van Roy JL et al (1997) Decrease in peripheral type 1 over type 2 T cell cytokine production in patients with rheumatoid arthritis correlates with an increase in severity of disease. Ann Rheum Dis **56**, 656.

**Van der Graaff WL**, Prins AP, Niers TM et al (1999) Quantitation of interferon gamma- and interleukin-4–producing T cells in synovial fluid and peripheral blood of arthritis patients. Rheumatology (Oxf) **38**, 214.

**Van der Pouw-Kraan TC**, Boeije LC, de Groot ER et al (1997) Reduced production of IL-12 and IL-12–dependent IFN-gamma release in patients with allergic asthma. J Immunol **158**, 5560.

**van Zandwijk N**, Groen HJ, Postmus PE et al (1997) Role of recombinant interferon-gamma maintenance in responding patients with small cell lung cancer. A randomised phase III study of the EORTC Lung Cancer Cooperative Group. Eur J Cancer **33**, 1759.

**Varani J**, Mitra RS, McClenic BJ et al (1989) Modulation of fibronectin production in normal human melanocytes and malignant melanoma cells by interferon-γ and tumor necrosis factor-α. Am J Pathol **134**, 827.

**Varesio L**, Blasi E, Thurman GB et al (1984) Potent activation of mouse macrophages by recombinant interferon-gamma. Cancer Res **44**, 4465.

**Vasaturo F**, Modesti A, Scarpa S (1998) Interferon gamma modifies fibronectin and laminin synthesis in human neuroblastoma cell lines. Int J Oncol **12**, 895.

**Vassileva-Atanassova A**, Mironova R, Nacheva G, Ivanov I (1999) N-terminal methionine in recombinant proteins expressed in two different Escherichia coli strains. J Biotechnol **69**, 63.

**Vassiliadis S**, Athanassakis I (1992) Type II interferon may be a potential hazardous therapeutic agent during pregnancy. Br J Haematol **82**, 782.

**Vassiliadis S**, Guilbert LJ (1991) Immune interferon induces early and late events in differentiation of HL-60 cells that are part of the same maturation program. Exp Hematol **19**, 250.

**Vedrenne J**, Assier E, Pereno R et al (1997) Inhibitor (IK) of IFN-γ induced HLA class II antigens expression also inhibits HLA class II constitutive expression in the human Raji B cell line. Oncogene **14**, 1453.

**Venkataprasad N**, Coombes AG, Singh M et al (1999) Induction of cellular immunity to a mycobacterial antigen adsorbed on lamellar particles of lactide polymers. Vaccine **17**, 1814.

**Vergara U**, Ferreira A, Schellekens H, Nussenzweig V (1987) Mechanism of escape of exoerythrocytic forms (EEF) of malaria parasites from the inhibitory effects of interferon-γ. J Immunol **138**, 4447.

**Verhaar MJ**, Damen CA, Zonnenberg BA, Blijham GH (1999) In vitro upregulation of carcinoembryonic antigen expression by combinations of cytokines. Cancer Lett **139**, 67.

**Verhoef CM**, van Roon JA Vianen ME et al (1999) The immune suppressive effect of dexamethasone in rheumatoid arthritis is accompanied by upregulation of interleukin 10 and by differential changes in interferon gamma and interleukin 4 production. Ann Rheum Dis **58**, 49.

**Verma VN**, Shenoy CN, Nadkarni JJ (1996) Augmentation of cisplatin cytotoxicity using cytokines on cervical carcinoma cell lines. Cancer Biother Radiopharmacol **11**, 349.

**Veys EM**, Mielants HY, Verbruggen G et al (1988). Interferon gamma in rheumatoid arthritis — a double blind study comparing human recombinant interferon gamma with placebo. J Rheumatol **15**, 570.

**Viens P**, Blaise D, Stoppa AM et al (1992) Interleukin-2 in association with increasing doses of interferon-gamma in patients with advanced cancer. J Immunother **11**, 218.

**Vigano A**, Balotta C, Trabattoni D et al (1997) Long-term resistance to HIV infection in vertical HIV infection; cytokine production, HIV isolation, and HIV phenotype define long-term resistant hosts. Pathobiology **65**, 169.

**Vilcek J**, Henriksen-Destefsno D, Siegel D et al (1985a) Regulation of IFN-γ induction in human peripheral blood cells by exogenous and endogenously produced interleukin-2. J Immunol **135**, 1851.

**Vilcek J**, Kelker HC, Le J, Yip YK (1985b) Structure and function of human interferon-gamma. In: Mediators in cell growth and differentiation (Ford RJ, Maizel AL, eds). Raven Press, New York, p 299.

**Vile RG**, Castleden S, Marshall J et al (1997) Generation of an anti-tumor immune response in a non-immunogenic tumour: HSVtk killing in vivo stimulates a mononuclear cell infiltrate and a Th1-like profile of intratumoral cytokine expression. Int J Cancer **71**, 267.

**Villacres MC**, Bergmann CC (1999) Enhanced cytotoxic T cell activity in IL-4–deficient mice. J Immunol **162**, 2663.

**Vincent F**, De la Salle H, Bohbot A et al (1993) Synthesis and regulation of complement components by human monocytes/macrophages and by acute monocytic leukemia. DNA Cell Biol **12**, 415.

**Vingerhoets J**, Bisalinkumi E, Penne G et al (1998) Altered receptor expression and decreased sensitivity of T-cells to the stimulatory cytokines IL-2, IL-7 and IL-12 in HIV infection. Immunol Lett **61**, 53.

**Visani G**, Russo D, Damiani D et al (1988) Sensitivity of Ph 1+CFU-GM to human recombinant interferon α and γ alone and in combination. Blut **57**, 41.

**Vita JR**, Edwalds GM, Gorey T et al (1988) Enhanced in vitro growth suppression of human glioblastoma cultures treated with the combination of recombinant fibroblast and immune interferons. Anticancer Res **8**, 297.

**Vitolo D**, Zerbe T, Kanbour A et al (1992) Expression of mRNA for cytokines in tumor-infiltrting mononuclear cells in ovarian adenocarcinoma and invasive breast cancer. Int J Cancer **51**, 573.

**Vlachoyiannopoulos PG**, Tsifetaki N, Dimitriou I et al (1996) Safety and efficacy of recombinant gamma interferon in the treatment of systemic sclerosis. Ann Rheum Dis **55**, 761.

**Vodovotz Y**, Kwon NS, Pospischil M et al (1994) Inactivation of nitric oxide synthase after prolonged incubation of mouse macrophages with IFN-gamma and bacterial lipopolysaccharide. J Immunol **152**, 4110.

**Volc-Platzer B**, Steiner A, Radaszkiewicz Th, Wolff K (1988) Recombinant gamma-interferon and in vivo induction of HLA-DR antigens. Br J Dermatol **119**, 155.

**Volk HD**, Diamantstein T (1986) IL-2 normalizes defective suppressor T cell function of patients with systemic lupus erythematosus in vitro. Clin Exp Immunol **66**, 525.

**Volpes R**, Van-den-Oord JJ, Desmet VJ, Yap SH (1992) Induction of intercellular adhesion molecule-1 (CD54) on human hepatoma cell line Hep G2; Influence of cytokines and hepatitis B virus-DNA transfection. Clin Exp Immunol **87**, 71.

**Vowels BR**, Lessin SR, Cassin M et al (1994) Th2 cytokine mRNA expression in skin in cutaneous T-cell lymphoma. J Invest Dermatol **103**, 669.

**Vrouenraets BC**, Klaase JM, Van Geel AN et al (1993) Regional isolated limb perfusion in patients with malignant melanoma. Oncologie **16**, 163.

**Vrouenraets BC**, Keus RB, Nieweg OE, Kroon BB (1997) Complications of combined radiotherapy and isolated limb perfusion with tumor necrosis factor alpha +/– interferon gamma and melphalan in patients with irresectable soft tissue tumors. J Surg Oncol **65**, 88.

## W

**Wada M**, Okamura H, Nagata K et al (1985) Cellular mechanisms *in vivo* production of gamma interferon induced by lipopolysaccharide in mice infected with *Mycobacterium bovis* BCG. J Interferon Res **5**, 431.

**Wada RK**, Pai DS, Huang J et al (1997) Interferon-gamma and retinoic acid down-regulate N-myc in neuroblastoma through complementary mechanisms of action. Cancer Lett **121**, 181.

**Wadee AA** (1990) A 25kDa fraction from Mycobacterium tuberculosis that inhibits leukocyte bactericidal activity: Reversal by gamma interferon and clofazimine. Res Microbiol **141**, 249.

**Wadee AA**, Paterson A, Coplan KA, Reddy SG (1994) HLA expression in hepatocellular carcinoma cell lines. Clin Exp Immunol **97**, 328.

**Wadler S**, Wersto R, Weinberg V et al (1990) Interaction of fluorouracil and interferon in human colon cancer cell lines: Cytotoxic and cytokinetic effects. Cancer Res **50**, 5735.

**Wakeham J**, Wang J, Magram J et al (1998) Lack of both types 1 and 2 cytokines, tissue inflammatory responses, and immune protection during pulmonary infection by Mycobacterium bovis bacille Calmette-Guerin in IL-12–deficient mice. J Immunol **160**, 6101.

**Wakiguchi H**, Kubota H, Hisakawa H et al (1994) Defective natural killer cell activity and deficient production of interferon-gamma in children with acute lymphoblastic leukemia. Acta Paediatr Jpn Overs Ed **36**, 361.

**Wallace WA**, Ramage EA, Lamb D, Howie SE (1995) A type 2 (Th2-like) pattern of immune response predominates in the pulmonary interstitium of patients with cryptogenic fibrosing alveolitis (CFA). Clin Exp Immunol **101**, 436.

**Wallace WA**, Howie SE (1999) Immunoreactive interleukin 4 and interferon-gamma expression by type II alveolar epithelial cells in interstitial lung disease. J Pathol **187**, 475.

**Wallach D** (1982) Regulation of susceptibility to natural killer cells cytotoxicity and regulation of HLA synthesis: Differing efficacies of alpha, beta and gamma interferons. J Interferon Res **2**, 329.

**Wallach D** (1983) Interferon-induced resistance to the killing by NK cells. A preferential effect of IFN-γ. Cell Immunol **75**, 390.

**Wallach D**, Fellous M, Revel M (1982) Preferential effect of γ interferon on the synthesis of HLA antigens and their m RNAs in human cells. Nature **299**, 833.

**Wallis RS**, Ellner JJ, Shiratsuchi H (1992) Macrophages, mycobacteria and HIV: the role of cytokines in determining mycobacterial virulence and regulating viral replication. Res Microbiol **143**, 398.

**Waltenbaugh C**, Vasquez K, Peterson JD (1998) Alcohol consumption alters antigen-specific Th1 responses: mechanisms of deficit and repair. Alcohol Clin Exp Res **22**(5 Suppl), 220S.

**Walther W**, Stein U **(**1994**)** Influence of cytokines on mdr1 expression in human colon carcinoma cell lines. J Cancer Res Clin Oncol **120**, 471.

**Wandl UB**, Nagel-Hiemke M, May-D et al (1992a) Lupus-like autoimmune disease induced by interferon therapy for myeloproliferative disorders. Clin Immunol Immunopathol **65**,70.

**Wandl UB**, Opalka B, Kloke O et al (1992b) Treatment of chronic myelogenous leukemia with different cytokines. Semin Oncol **19**, 88.

**Wang S**, Fan Y, Brunham RC, Yang X (1999) IFN-gamma knockout mice show Th2-associated delayed-type hypersensitivity and the inflammatory cells fail to localize and control chlamydial infection. Eur J Immunol **29**, 3782.

**Wang X**, Yue TL, Ohlstein EH et al (1996) Interferon-inducible protein-10 involves vascular smooth muscle cell migration, proliferation, and inflammatory response. J Biol Chem **271**, 24286.

**Waranowska-Stewart M**, Lin LS, Beaude IA, Stewart WE (1980) Production, partial purification and characterization of human and mouse interferon type II. Mol Immunol **17**, 625.

**Waschutza G**, Li V, Schafer T et al (1996) Engineered disulfide bonds in recombinant human interferon-gamma: the impact of the N-terminal helix A and the AB-loop on protein stability. Protein Eng **9**, 905.

**Wasserman D**, Ioannovich JD, Hinzmann RD et al (1998) Interferon-gamma in the prevention of severe burn-related infections: A European phase III multicenter trial. The Severe Burns Study Group. Crit Care Med **26**, 434**.**

**Watanabe H**, Chisaka T, Higuchi T, Tanaka A et al (1989) Effect of human interferons on morphological differentiation and suppression of N-myc gene expression in human neuroblastoma cells. Jpn J Cancer Res Gann **80**, 1072.

**Watanabe I**, Horiuchi T, Fujita S (1995) Role of protein kinase C activation in synthesis of complement components C2 and factor B in interferon-gamma–stimulated human fibroblasts, glioblastoma cell line A172 and monocytes. Biochem J **305**, 425.

**Watanabe M**, Shimamoto Y, Yoshida SI et al (1993) Intracellular multiplication of Legionella pneumophila in HL-60 cells differentiated by 1,25-dihydroxyvitamin D-3 and the effct of interferon gamma. J Leukoc Biol **54**, 40

**Watanabe Y**, Kuribayashi K, Miyatake S (1989) Exogenous expression of mouse interferon gamma cDNA in mouse neuroblastoma C 1300 cells results in reduced tumorigenicity by augmented anti-tumor immunity. Proc Natl Acad Sci USA **86**, 9456.

**Watson RB**, Galvani DW, Nash JR, Cawley JC (1990) Gamma IFN receptor expression in haemic malignancies. Leukoc Res **14**, 657.

**Wautier JL** (1989) Monocyte-endothelium relations. J Mal Vasc **14**(Suppl A), 13.

**Weck PhK**, Buddin DA, Whisnant JK (1988) Interferons in the treatment of genital human papillomavirus infections. Am J Med **85**(Suppl 2A), 159.

**Webb DSA**, Gerrard TL (1990) IFN-alpha, and IFN-gamma can affect both monocytes and tumor cells to modulate monocyte-mediated cytoxicity. J Immunol **144**, 3643.

**Weber JR**, Wright TL (1992) The clinical significance of hepatitis C viral infection. Infect Med **9**, 17-24+49.

**Weber P**, Wiedmann KH, Klein R, et al (1994) Induction of autoimmune phenomena in patients with chronic hepatitis B treated with gamma-interferon. J Hepatol **20**, 321.

**Weigent DA**, Huff TL, Peterson JW et al (1986) Role of interferon in streptococcal infection in the mouse. Microbial Pathog **1**, 399.

**Weinberg JB**, Larric JW (1987) Receptor-mediated monocytoid differentiation of human promyelocytic cells by tumor necrosis factor. Synergistic actions with interferon-gamma and 1,25-dihydroxyvitamin D-3. Blood **70**, 994.

**Weinberg JB**, Misukonis MA, Hobbs MM, Borowitz MJ (1986) Cooperative effects of gamma interferon and 1-alpha, 25-dihydroxyvitamin D-3 in inducing differentiation of human promyelocytic leukemia (HL-60) cells. Exp Hematol **14**, 138.

**Weiner LM**, Padavic Shaller K, Kitson J et al (1991) Phase I evaluation of combination therapy with interleukin 2 and gamma-interferon. Cancer Res **51**, 3910.

**Weiner LM**, Li W, Holmes M et al (1994) Phase I trial of recombinant macrophage colony-stimulating factor and recombinant gamma-interferon: Toxicity, monocytosis and clinical effects. Cancer Res **54**, 4084.

**Weitkamp B**, Cullen P, Plenz G et al (1999) Human macrophages synthesize type VIII collagen in vitro and in the atherosclerotic plaque. FASEB J **13**, 1445.

**Welander CE** (1988) Interferon in the treatment of ovarian cancer. Semin Oncol **15**, 26.

**Welander ChE**, Homesley HD, Smiles KA, Peets EA (1990) Intralesional interferon alpha-2B for the treatment of genital warts. Am J Obstet Gynecol, **162**, 348.

**Wen Q**, Zhang G, Kong X (1994) Modulation effect of cimetidine on the production of IL-2 and interferon-gamma in patients with gastric cancer. Chin J Oncol **16**, 299.

**Werner ER**, Bitterlich G, Fuchs D et al (1987a) Human macrophages degrade tryptophan upon induction by interferon-gamma. Life Sci **41**, 273.

**Werner ER**, Hirsch-Kauffman M, Fuchs D et al (1987b) Interferon-γ–induced degradation of tryptophan by human cells in vitro. Biol Chem Hoppe Seyler **368**, 1407.

**Werner FG**, Werner ER, Fuchs D et al (1989) Characteristics of interferon tryptophan metabolism in human cells in vitro. Biochim Biophys Acta Ser Mol Cell Res **1012**, 140.

**Westby M**, Marriott JB, Guckian M et al (1998) Abnormal intracellular IL-2 and interferon-gamma (IFN-gamma) production as HIV-1–associated markers of immune dysfunction. Clin Exp Immunol **111**, 257.

**Wetzel R**, Perry LJ, Veilleux C, Chang G (1990) Mutational analysis of the C-terminus of human interferon-gamma. Protein Eng **3**, 611.

**Wexler L**, Thiele C, McClure L et al (1992) Adoptive immunotherapy (AI) of refractory neuroblastoma (NB) with tumor-infiltrating lymphocytes (TIL), interferon-gamma (IFN-gamma) and interleukin-2 (IL-2) (meeting abstract). Proc Annu Meet Am Soc Clin Oncol **11**, A1269.

**Weyand CM**, Goronzy JJ (1999) T-cell responses in rheumatoid arthritis: systemic abnormalities–local disease. Curr Opin Rheumatol **11**, 210**.**

**Wheelock EF** (1965) Interferon-like virus inhibitor induced in human leukocytes by phytohemagglutinin. Science **149**, 310.

**Whittington WI**, Cates WJ Jr (1984) Acyclovir therapy for genital herpes: enthusiasm and caution in equal doses. JAMA **251**, 2116.

**Widschwendter M**, Daxenbichler G, Marth C, Dapunt O (1994) Interaction of interferon with retinoic acid. Gynakol Geburtshilfliche Rundsch **34**, 168.

**Widschwendter M**, Daxenbichler G, Dapunt O, Marth C et al (1995a) Synergism of retinoic acid and interferon-gamma in breast cancer cells: a mechanism model (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **36**, A 3062.

**Widschwendter M**, Daxenbichler G, Dapunt O, Marth C et al (1995b) Effects of retinoic acid and gamma-interferon on expression of retinoic acid receptor and cellular retinoic acid-binding protein in breast cancer cells. Cancer Res **55**, 2135.

**Widschwendter M**, Daxenbichler G, Bachmair F et al (1996) Interaction of retinoic acid and interferon-alpha in breast cancer cell lines. Anticancer Res **16**, 369.

**Wiebke EA**, Custer MC, Rosenberg SA, Lotze MT (1990) Cytokines alter target cell susceptibility to lysis: I. Evaluation of non-major histocompability complex-restricted effectors reveals differential effects on natural and lymphokine-activated killing. J Biol Response Modif **9**, 113.

**Willheim M**, Silacci P, Gessl A et al (1995). Tumor necrosis factor-alpha induction of major histocompatibility complex class II antigen expression is inhibited by interferon-gamma in a monocytic cell line. Eur J Immunol **25**, 3202.

**Willheim M**, Ebner C, Baier K et al (1997) Cell surface characterization of T lymphocytes and allergen-specific T cell clones: Correlation of CD26 expression with T(H1) subsets. J Allergy Clin Immunol **100**, 348.

**Williams BRG** (1991) Transcriptional regulation of interferon-stimulated genes. Eur J Biochem **200**, 1.

**Wills RJ** (1990) Clinical pharmacokinetics of interferons. Clin Pharmacokinet **19**, 390.

**Wilsher ML**, Hagan C, Prestidge R et al (1999) Human in vitro immune responses to Mycobacterium tuberculosis. Tuberc Lung Dis **79**, 371.

**Windbichler GH**, Hensler E, Widschwendter M et al (1996) Increased radiosensitivity by a combination of 9-cis-retinoic acid and interferon-gamma in breast cancer cells. Gynecol Oncol **61**, 387.

**Windbichler GH**, Hausmaninger H, Stummvoll W et al (2000) Interferon-gamma in the first-line therapy of ovarian cancer: a randomized phase III trial. Br J Cancer **82**, 1138.

**Winters AL**, Seftor EA, Scuderi P, Hendrix MJ (1990). Interferon-gamma lowers the invasive ability and induces HLA-DR expression in highly metastatic melanoma cells (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **31**, A379.

**Wirth JJ**, Kierszenbaum F, Sonnenfeld G, Zlotnik A (1985) Enhancing effects of gamma interferon on phagocytic cell association with and killing of *Trypanosoma cruzi*. Infect Immun **49**, 61.

**Witkin SS**, Chaudhry A (1989) Circulating interferon-$\gamma$ in women sensitized to sperm: New mechanisms of infertility. Fertil Steril **52**, 867.

**Witt PL**, Goldstein D, Storer BE et al (1992) Absence of biological effects of orally administered interferon-$\beta_{ser}$. J Interferon Res **12**, 411.

**Wong LH**, Hatzinisiriou I, Devenish RJ, Ralph SJ (1998) IFN-gamma priming up-regulates IFN-stimulated gene factor 3 (ISGF3) components, augmenting responsiveness of IFN-resistant melanoma cells to type I IFNs. J Immunol **160**, 5475.

**Wong VL**, Rieman DJ, Aronson L et al (1989) Growth-inhibitory activity of interferon-beta against human colorectal carcinoma cell lines. Int J Cancer **43**, 526.

**Wongtim S**, Silachamroon U, Ruxrungtham K et al (1999) Interferon gamma for diagnosing tuberculous pleural effusions. Thorax **54**, 921.

**Wood JM**, Ehrke C, Schallreuter KU (1991) Tryptophan protects human melanoma cells against gamma-interferon and tumor necrosis factor-alpha: A unifying mechanism of action. Melanoma Res **1**, 177.

**Woodworth CD**, Evans CH, Di Paolo JA (1990) Modulation of human papillomavirus type 16 (HPV16) early gene expression by TGF beta1, gamma-interferon (gamma-IFN), and leukoregulin (LR) (meeting abstract). Proc Annu Meet Am Assoc Cancer Res **31**, A1933.

**Worm M**, Makki A, Dippel E (1995) Interferon-gamma down regulates epidermal growth factor receptors on human melanoma cells. Exp Dermatol **4**, 30.

**Wrone-Smith T**, Johnson T, Nelson B et al (1995) Discordant expression of Bcl-x and Bcl-2 by keratinocytes in vitro and psoriatic keratinocytes in vivo. Am J Pathol **146**, 1079.

**Wu J**, Danielsson A (1994) Inhibition of hepatic fibrogenesis: A review of pharmacologic candidates. Scand J Gastroenterol **29**, 385.

**Wuarin L**, Verity MA, Sidell N (1991) Effects of interferon-gamma and its interaction with retinoic acid on human neuroblastoma differentiation. Int J Cancer **48**, 136.

## X

**Xie K**, Huang S, Wang Y et al (1996) Bcl-2 protects cells from cytokine-induced nitric-oxide-dependent apoptosis. Cancer Immunol Immunother **43**, 109.

**Xie K**, Wang Y, Huang S et al (1997) Nitric oxide–mediated apoptosis of K-1735 melanoma cells is associated with downregulation of Bcl-2. Oncogene **15**, 771.

**Xie QW**, Kashiwabara Y, Nathan C (1994) Role of transcription factor NF-kappa B/Rel in induction of nitric oxide synthase. J Biol Chem **269**, 4705.

**Xu R**, Ying B, Zhao S et al (1997) Construction and identification of a recombinant adenovirus which expresses human interferon-gamma. Chin J Biotechnol **13**, 1.

**Xu S**, Vancheeswaran R, Bou-Gharios G et al (1995). Scleroderma-derived human fibroblasts retain abnormal phenotypic and functional characteristics following retroviral transduction with the SV 40 tsT antigen. Exp Cell Res **220**, 407.

**Xue H**, McCauley RL, Zhang W, Martini DK (2000) Altered interleukin-6 expression in fibroblasts from hypertrophic burn scars. J Burn Care Rehabil **21**, 142.

## Y

**Yagi H**, Tokura Y, Furukawa F, Takigawa M (1996) Th2 cytokine mRNA expression in primary cutaneous CD30-positive lymphoproliferative disorders: Successful treatment with recombinant interferon gamma. J Invest Dermatol **107**, 827.

**Yakubovskaya EL**, Makedonov GP, Zasukhina GD (1988) Natural and recombinant interferons protect the human cells with disorder in the repair system (Marfan syndrome) against the action of mutagens. Genetika **24**, 2050.

**Yamabe H**, Osawa H, Kaizuka M et al (2000) Platelet-derived growth factor, basic fibroblast growth factor, and interferon gamma increase type IV collagen production in human fetal mesangial cells via a transforming growth factor-beta–dependent mechanism. Nephrol Dial Transplant **15**, 872.

**Yamaga KM**, Bolen H, Kimura L, Lance EM (1993) Enhanced chrondrocyte destruction by lymphokine-activated killer cells. Possible role in rheumatoid arthritis. Arthritis Rheum **36**, 500.

**Yamamoto T**, Osaki T, Yoneda K, Ueta E (1993) Immunological investigation of adult patients with primary herpes simplex virus-1 infection. J Oral Pathol Med **22**, 263.

**Yamamoto T**, Sasaki G, Sato T et al (1995) Cytokine profile of tumor cells in mycosis fungoides: Successful treatment with intra-lesional interferon-gamma combined with chemotherapy. J Dermatol **22**, 650.

**Yamasaki T**, Kagawa T, Takamura M, Moritake K (1995) Pharmacokinetics of intratumoral interferon-gamma activity following subcutaneous administration of recombinant interferon-gamma in a patient with metastatic brain tumor derived from renal cancer. Neurol Surg **23**, 169.

**Yanagawa H**, Sone S, Okubo A et al (1990) Decrease of lymphokine (interleukin-2)-activated killer activity in peripheral blood after administration of natural interferon-gamma. Jpn J Clin Oncol **20**, 356.

**Yanagawa H**, Yano S, Hako T et al (1996) Interleukin-1 receptor antagonist in pleural effusion due to inflammatory and malignant lung disease. Eur Respir J **9**, 1211.

**Yanagawa H**, Haku T, Hiramatsu K et al (1997) Intrapleural instillation of interferon gamma in patients with malignant pleurisy. Cancer Immunol Immunother **45**, 93.

**Yanagisawa K**, Yamauchi H, Kaneko M et al (1998) Suppression of cell proliferation and the expression of a bcr-abl fusion gene and apoptotic cell death in a new human chronic myelogenous leukemia cell line, KT-1, by interferon-alpha. Blood **91**, 641.

**Yanagiyo H**, Kudoh S, Takahashi S, Suzuki T (1996) Characterization of tumor infiltrating lymphocytes from patients with renal cell carcinoma — Influence of combination therapy with interferon alpha and gamma. Nippon Hinyokika Gakkai Zasshi **87**, 928.

**Yang J**, Cleland JL (1997) Factors affecting the in vitro release of recombinant human interferon-gamma (rhIFN-gamma) from PLGA microspheres. J Pharm Sci **86**, 908.

**Yang R**, Liu Q, Rescorla FJ et al (1995) Experimental liver cancer: Improved response after hepatic artery ligation and infusion of tumor necrosis factor-alpha and interferon-gamma. Surgery **118**, 768.

**Yang X**, Gartner J, Zhu L et al (1999) IL-10 gene knockout mice show enhanced Th1-like protective immunity and absent granuloma formation following Chlamydia trachomatis lung infection. J Immunol **162**, 1010.

**Yang Y**, Xiang Z, Ertl HC, Wilson JM (1995) Upregulation of class I major histocompatibility complex antigens by interferon gamma is necessary for T-cell–mediated elimination of recombinant adenovirus-infected hepatocytes in vivo. Proc Natl Acad Sci USA **92**, 7257.

**Yano H**, Fukuda K, Haramaki M et al (1996) Expression of Fas and anti-Fas–mediated apoptosis in human hepatocellular carcinoma cell lines. J Hepatol **25**, 454.

**Yano T**, Yokoyama H, Ueda T et al (1994) Inhalation therapy of interferon-gamma against bronchoalveolar carcinoma of the lung; a case report. J Jpn Soc Cancer Ther **29**, 1778.

**Yarden I**, Kimchi A (1986) Tumor necrosis factor reduces c-*myc* expression and cooperates with interferon-γ in HeLa cells. Science **234**, 1419.

**Yasumoto K** (1995) Basic aspects of BRM therapy against lung cancer. Biotherapy **9**, 269.

**Yasumoto K**, Okamoto SI, Mukaida N et al (1992) Tumor necrosis factor alpha and interferon gamma synergistically induce interleukin 8 production in a human gastric cancer cell line through acting concurrently on AP-1 and NF-κB–line binding sites of the interleukin 8 gene. J Biol Chem **267**, 22506.

**Yasunaga Y**, Nishimura K, Miura H et al (1995). Immunological study of the postoperative adjuvant treatment with interferon gamma in patients with renal cell cancer. Measurement of peripheral blood lymphocyte subsets with three color flow cytometry. Acta Urol Jpn **41**, 85.

**Yawalkar N**, Karlen S, Hunger R et al (1998) Expression of interleukin-12 is increased in psoriatic skin. J Invest Dermatol **111**, 1053.

**Ybarrondo B**, Chong A, Scuderi P, Hersh E (1990) Regulation of tumor necrosis factor (TNF) and interferon-gamma (IFN-GAMMA) secretion in human interleukin-2 (IL-2) activated peripheral blood leukocytes (PBL) (meeting abstract). FASEB J **4**, A 1742.

**Ye J**, Ortaldo JR, Conlon K et al (1995) Cellular and molecular mechanisms of IFN-gamma production induced by IL-2 and IL-12 in a human NK cell line. J Leukoc Biol **58**, 225.

**Ye J**, Cippitelli M, Dorman L et al (1996) The nuclear factor YY1 suppresses the human gamma interferon promoter through two mechanisms: Inhibition of AP1 binding and activation of a silencer element. Mol Cell Biol **16**, 4744.

**Yim JH**, Wu SJ, Casey MJ et al (1997) IFN regulatory factor-1 gene transfer into an aggressive, nonimmunogenic sarcoma suppresses the malignant phenotype and enhances immunogenicity in syngeneic mice. J Immunol **158**, 1284.

**Yin D**, Kondo S, Takeuchi J et al (1994) Interferon-gamma induces a decrease in the susceptibility of human glioma cells to lysis by lymphokine-activated killer cells. Neurosurgery **35**,113.

**Yin Z**, Siegert S, Neure L et al (1999) The elevated ratio of interferon gamma-/interleukin-4–positive T cells found in synovial fluid and synovial membrane of rheumatoid arthritis patients can be changed by interleukin-4 but not by interleukin-10 or transforming growth factor beta. Rheumatology (Oxf) **38**, 1058.

**Yip YK**, Barrowclough BB, Urban C, Vilcek J (1982) Purification of two subspecies of human gamma (immune) interferon. Proc Natl Acad Sci USA **79**, 1820.

**Yocum DE**, Esparza L, Dubry S et al (1989). Characteristics of tumor necrosis factor production in rheumatoid arthritis. Cell Immunol **122**, 131.

**Yokota T**, Shimokado K, Kosaka C et al (1992) Mitogenic activity of interferon gamma on growth-arrested human vascular smooth muscle cells. Arterioscler Thromb **12**, 1393.

**Yokozeki M**, Baba Y, Shimokawa H et al (1999) Interferon-gamma inhibits the myofibroblastic phenotype of rat palatal fibroblasts induced by transforming growth factor-beta1 in vitro. FEBS Lett **442**, 61.

**Yonemitsu Y**, Kaneda Y, Komori K et al (1997) The immediate early gene of human cytomegalovirus stimulates vascular smooth muscle cell proliferation in vitro and in vivo. Biochem Biophys Res Commun **231**, 447.

**Yoneto T**, Yoshimoto T, Wang CR et al (1999) Gamma interferon production is critical for protective immunity to infection with blood-stage Plasmodium berghei XAT but neither NO production nor NK cell activation is critical. Infect Immun **67**, 2349.

**Yoshida Y**, Okuma M, Uchino H (1991) Current state of differentiation therapy in myelodysplasias. Serono Symp Publ, Raven Press **82**, 441.

**Yoshimatsu T**, Hanagiri T, Sugio K et al (1996). Activation of alveolar macrophages from patients with primary lung cancer by interferon-gamma. Biotherapy **10**, 403.

**Yoshimura A**, Sone S (1990) Role of phosphatidylserine in membrane actions of tumor necrosis factor and interferons alpha and gamma. Biochem Int **20**, 697.

**Yoshino S**, Kato M, Okada K (2000) Clinical significance of angiogenesis, proliferation and apoptosis in renal cell carcinoma. Anticancer Res **20**, 591.

**Yoshioka K**, Kakumu S, Tahara H et al (1989) Effect of interferon $\alpha$, $\gamma$ and tumor necrosis factor $\alpha$ on the HLA-A, B, C expression of cell lines derived from human liver. Liver **9**, 14.

**Young HA**, Ortaldo JR (1987) One-signal requirement for interferon-$\gamma$ production by human large granular lymphocytes. J Immunol **139**, 724.

**Young HA**, Hardy KJ (1990) Interferon-$\gamma$: Producer cells, activation stimuli, and molecular genetic regulation. Pharmacol Ther **45**, 137.

**Young HA**, Dray JF, Parrar WL (1986) Expression of transfected human interferon $\gamma$ DNA: Evidence for cell-specific regulation. J Immunol **136**, 4700.

**Young MR**, Wright MA (1992) Myelopoiesis-associated immune suppressor cells in mice bearing metastatic Lewis lung carcinoma tumors: gamma interferon plus tumor necrosis factor alpha synergistically reduces immune suppressor and tumor growth-promoting activities of bone marrow cells and diminishes tumor recurrence and metastasis. Cancer Res **52**, 6335.

**Yphantis DA**, Arakawa T (1987) Sedimentation equilibrium measurements of recombinant DNA derived human interferon γ. Biochemistry **26**, 5422.

**Yu WG**, Ogawa M, Mu J et al (1997) IL-12–induced tumor regression correlates with in situ activity of IFN-gamma produced by tumor-infiltrating cells and its secondary induction of anti-tumor pathways. J Leukoc Biol **62**, 450.

**Yu WG**, Yamamoto N, Takenaka H et al (1996a) Molecular mechanisms underlying IFN-gamma–mediated tumor growth inhibition induced during tumor immunotherapy with rIL-12. Int Immunol **8**, 855.

**Yu Z**, Manickan E, Rouse BT (1996b) Role of interferon-gamma in immunity to herpes simplex virus. J Leukoc Biol **60**, 528.

**Yuan J**, Wegenka UM, Lutticken C et al (1994) The signalling pathways of interleukin-6 and gamma interferon converge by the activation of different transcription factors which bind to common responsive DNA elements. Mol Cell Biol **14**, 1657.

**Yuan W**, Yufit T, Li L et al (1999) Negative modulation of alpha1 (I) procollagen gene expression in human skin fibroblasts: transcriptional inhibition by interferon-gamma. J Cell Physiol **179**, 97.

**Yuan YC**, Shen SY (1994) Inhibition of proto-oncogene c-myc expression with recombinant human tumor necrosis factor-alpha and interferon-gamma in HL-60 cell lines and acute myelocytic leukemia fresh cells. Chung Hua Nei Ko Tsa Chih **33**, 302.

**Yudoh K**, Matsuno H, Nakazawa F et al (2000) Reduced expression of the regulatory CD4+ T cell subset is related to Th1/Th2 balance and disease severity in rheumatoid arthritis. Arthritis Rheum **43**, 617.

**Yufit T**, Vining V, Wang L et al (1995) Inhibition of type I collagen mRNA expression independent of tryptophan depletion in interferon-gamma treated human dermal fibroblasts. J Invest Dermatol **105**, 388.

## Z

**Zabel P**, Kreiker C, Schlaak M (1990) Initial results of a controlled study of small cell and squamous cell cancers of the lung. Pneumologie Sonderh **44**, 586.

**Zangerle PF**, De Groote D, Lopez M et al (1992). Direct stimulation of cytokines (IL-1β, TNF-α, IL-6, IL-2, IFN-γ and GM-CSF) in whole blood: II. Application to rheumatoid arthritis and osteoarthritis. Cytokine **4**, 568.

**Zanussi S**, Simonelli C, Andrea M et al (1996) CD8+ lymphocyte phenotype and cytokine production in long-term non-progressor and in progressor patients with HIV-1 infection. Clin Exp Immunol **105**, 220.

**Zasukhina GD**, Sinelschikova T, Vasiljeva II, Kirkova S (1981) An approach to modification of the repair activity in eucariotic cells by biological factors. Mutat Res **81**, 123.

**Zawatzky R**, Gresser I, DeMaeyer E, Kirchner H (1982) The role of interferon in the resistance of C57BL/6 mice to various doses of herpes simplex virus type I. J Infect Dis **146**, 405.

**Zedler S**, Bone RC, Baue AE et al (1999) T-cell reactivity and its predictive role in immunosuppression after burns. Crit Care Med **27**, 66.

**Zella D**, Barabitskaja O, Burns JM et al (1998) Interferon-gamma increases expression of chemokine receptors CCR1, CCR3, and CCR5, but not CXCR4 in monocytoid U937 cells. Blood **91**, 4444.

**Zhan Y**, Cheers C (1993) Endogenous gamma interferon mediates resistance to Brucella abortus infection. Infect Immun **61**, 4899.

**Zhang JJ**, Vinkemeier U, Gu W et al (1996). Two contact regions between Stat1 and CBP/p300 in interferon gamma signaling. Proc Natl Acad Sci USA **93**, 15092.

**Zhang Y**, Broser M, Cohen H et al (1995) Enhanced interleukine-8 release and gene expression in macrophages after exposure to Mycobacterium tuberculosis and its components. J Clin Invest **95**, 586.

**Zhang Z**, Tong KT, Belew M et al (1992) Production, purification and characterization of recombinant human interferon gamma. J Chromatogr **604**, 143.

**Zhou YF**, Guetta E, Yu ZX et al (1996) Human cytomegalovirus increases modified low density lipoprotein uptake and scavenger receptor mRNA expression in vascular smooth muscle cells. J Clin Invest **98**, 2129.

**Zhu J**, Xu J, Wang G (1995) Correlative study on cell immunoregulatory factor levels and the prognosis and treatment of patients with late gastric cancer. Chin J Microbiol Immunol **15**, 49.

**Ziesche R**, Hofbauer E, Wittmann K et al. (1999) A preliminary study of long-term treatment with interferon gamma-1b and low-dose prednisolone in patients with idiopathic pulmonary fibrosis. N Engl J Med **341**, 1264.

**Zimiring JC**, Goodbourn S, Offermann MK (1998) Human herpesvirus 8 encodes an interferon regulatory factor (IRF) homolog that represses IRF-1–mediated transcription. J Virol **72**, 701.

**Zlateva T**, Boteva R, Salvato B, Tsanev R (1999) Factors affecting the dissociation and aggregation of human interferon-gamma. Intern J Biolog Macromol **26**, 357.

**Zoller M**, Strubel A, Hammerling G et al (1988) Interferon-gamma treatment of B16 melanoma cells: Opposing effects for non-adaptive and adaptive immune defense and its reflection by metastatic spread. Int J Cancer **41**, 256.

**Zou JP**, Yamamoto N, Fujii T et al (1995) Systemic administration of rIL-12 induces complete tumor regression and protective immunity: Response is correlated with a striking reversal of supressed IFN-gamma production by anti-tumor T cells. Int Immunol **7**, 1135.

**Zouboulis CC**, Buttner P, Orfanos CE (1992) Systemic interferon gamma as adjuvant therapy for refractory anogenital warts: A randomized clinical trial and meta-analysis of the available data. Arch Dermatol **128**, 1413.

**Zwaveling JH**, Maring JK, Clarke FL et al (1996) High plasma tumor necrosis factor (TNF)-alpha concentrations and a sepsis-like syndrome in patients undergoing hyperthermic isolated limb perfusion with recombinant TNF-alpha, interferon-gamma, and melphalan. Crit Care Med **24**, 765.