ACTA UNIVERSITATIS AGRICULTURAE SUECIAE

VETERINARIA 15

The Interferon-α/β System Studied *in vitro* and *in vivo*

Maija-Leena Eloranta

SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

The interferon-α/β system studied in vitro and in vivo

Maija-Leena Eloranta

Akademisk avhandling som för vinnande av filosofie doktorsexamen kommer att offentligen försvaras i Ettan, Klinikcentrum, SLU, Uppsala, torsdagen den 12 juni 1997, kl. 09.15.

Abstract

In this thesis the interferon-α/β (IFN-α/β) system was studied, especially the IFN-α/β producing cells (IPCs) in man and mouse. The phorbol 12-myristate 13-acetate (PMA) by activating proteinkinase C efficiently inhibited expression of IFN-α/β genes induced by Sendai virus in monocytes and by Herpes simplex virus (HSV) in the natural IPC (NIPC), the two major IPCs in human blood. A physiological mechanism controlling IFN-α/β production could be involved, perhaps relevant for the tumor promoting activity of PMA. Analysis of the phenotype of human NIPC, the most versatile and perhaps important IPC, was carried out by flow cytometry and sorting. These HSV-induced IPCs did not belong to the lymphoid or myeloid developmental lineages, but had characteristic light scatter. The further characterization of IPCs was done in the mouse. Because human NIPC might be immature dendritic cells (DCs), the IFN-α/β responses of two murine DC lines were studied. One of them responded to virus and bacteria in a NIPC-like fashion, and will become a useful experimental tool. Infrequent but efficient NIPC-like cells were also detected among normal cells derived from lymphoid organs of mice. When HSV was injected intravenously in mice, highly productive IPC were almost only found in the marginal zones of splenic white pulp. The IPCs had the cell adhesion molecule sialoadhesin and may be metallophilic macrophages, but not DCs or common macrophages. Subcutanous injection of HSV in mice resulted in appearance of IPCs mainly in the regional lymph node and especially in its subcapsular parts. A concomitant early lymph node swelling due to accumulation of lymphocytes was seen, in part dependent on produced IFN-α/β. Also in mice intracerebrally infected by lymphocytic choriomeningitis virus (LCMV), IPCs appeared in splenic marginal zones and in lymph nodes. No IFN-α and little IFN-β production was seen in the brain. This differential activity of the IFN-α/β system explains in part its pivotal effects on the course of LCMV infections.

Keywords: interferon-alpha, interferon-beta, dendritic cells, spleen, lymph node, Herpes simplex virus, Sendai virus, Lymphocytic choriomeningitis virus, phorbol ester.

Distribution:
Swedish University of Agricultural Sciences
Division of Immunology
Department of Veterinary Microbiology
S-751 23 UPPSALA, Sweden

Uppsala 1997
ISSN 1401-6230
ISBN 91-576-5400-X

The Interferon-α/β System Studied *in vitro* and *in vivo*

Maija-Leena Eloranta
Department of Veterinary Microbiology
Uppsala

Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 1997

Acta Universitatis Agriculturae Sueciae
Veterinaria 15

ISSN 1401-6257
ISBN 91-576-5400-X
© 1997 Maija-Leena Eloranta, Uppsala

To *Mr Tipuli*

Abstract

Eloranta, M.-L. 1997. *The interferon-α/β system studied in vitro and in vivo*. Doctor's dissertation.
ISSN 1401-6257, ISBN 91-576-5400-X.

In this thesis the interferon-α/β (IFN-α/β) system was studied, especially the IFN-α/β producing cells (IPCs) in man and mouse. The phorbol 12-myristate 13-acetate (PMA) by activating proteinkinase C efficiently inhibited expression of IFN-α/β genes induced by Sendai virus in monocytes and by Herpes simplex virus (HSV) in the natural IPC (NIPC), the two major IPCs in human blood. A physiological mechanism controlling IFN-α/β production could be involved, perhaps relevant for the tumor promoting activity of PMA. Analysis of the phenotype of human NIPC, the most versatile and perhaps important IPC, was carried out by flow cytometry and sorting. These HSV-induced IPCs did not belong to the lymphoid or myeloid developmental lineages, but had characteristic light scatter. The further characterization of IPCs was done in the mouse. Because human NIPC might be immature dendritic cells (DCs), the IFN-α/β responses of two murine DC lines were studied. One of them responded to virus and bacteria in a NIPC-like fashion, and will become a useful experimental tool. Infrequent but efficient NIPC-like cells were also detected among normal cells derived from lymphoid organs of mice. When HSV was injected intravenously in mice, highly productive IPC were almost only found in the marginal zones of splenic white pulp. The IPCs had the cell adhesion molecule sialoadhesin and may be metallophilic macrophages, but not DCs or common macrophages. Subcutaneous injection of HSV in mice resulted in appearance of IPCs mainly in the regional lymph node and especially in its subcapsular parts. A concomitant early lymph node swelling due to accumulation of lymphocytes was seen, in part dependent on produced IFN-α/β. Also in mice intracerebrally infected by lymphocytic choriomeningitis virus (LCMV), IPCs appeared in splenic marginal zones and in lymph nodes. No IFN-α and little IFN-β production was seen in the brain. This differential activity of the IFN-α/β system explains in part its pivotal effects on the course of LCMV infections.

Keywords: interferon-alpha, interferon-beta, dendritic cells, spleen, lymph node, Herpes simplex virus, Sendai virus, Lymphocytic choriomeningitis virus, phorbol ester.

Author's address: Maija-Leena Eloranta, Division of Immunology, Department of Veterinary Microbiology, SLU, BMC, Box 588, S-751 23 UPPSALA

Contents

Appendix

Papers I-VI

This thesis is based on the following papers, which will be referred to by their Roman numerals.

I. Sandberg, K., Eloranta, M.-L., Gobl, A.E., and Alm, G.V. Phorbol ester-mediated inhibition of IFN-α/β gene transcription in blood mononuclear leukocytes. J. Immunol. 147: 3116-3121, 1991.

II. Sandberg, K., Eloranta, M.-L., Johannisson, A., and Alm, G.V. Flow cytometric analysis of natural interferon-α producing cells. Scand. J. Immunol. 34: 565-576, 1991.

III. Eloranta, M.-L., Sandberg, K., and Alm, G.V. The interferon-α/β responses of mice to Herpes simplex virus studied at the blood and tissue level in vitro and vivo. Scand. J. Immunol. 43: 355-360, 1996.

IV. Riffault, S., Eloranta, M.-L., Carrat, C., Sandberg, K., Charley, B., and Alm, G. Herpes simplex virus induces appearance of interferon-α/β producing cells and partially interferon-α/β dependent accumulation of leukocytes in murine regional lymph nodes. J. Interferon Cytokine Res. 16: 1007-1014, 1996.

V. Sandberg, K., Eloranta, M.-L., Campbell, I.L. Expression of alpha/beta interferons (IFN-α/β) and their relationship to IFN-α/β induced genes in lymphocytic choriomeningitis. J. Virol. 68: 7358-7366, 1994.

VI. Eloranta , M.-L., Sandberg, K., Ricciardi-Castagnoli, P., Lindahl, M., and Alm, G.V. Production of interferon-α/β by murine dendritic cell lines stimulated by virus and bacteria. Scand. J. Immunol., in press.

The papers are reproduced by kind permission of the publisher of the journals.

Abbreviations

APC, antigen presenting cell
CD, cluster of differentiation
CNS, central nervous system
cRNA, copy RNA
CTL, cytotoxic T lymphocyte
DC, dendritic cell
DELFIA, dissociation enhanced lanthanide fluoroimmunoassay
GM-CSF, granulocyte-macrophage colony-stimulating factor
HSV, Herpes simplex virus type 1
IFN, interferon
IFNAR, interferon-α receptor
IL, interleukin
IPC, interferon-α/β producing cell
IRE, interferon responsive element
IE, inducible element
IRF, interferon regulatory factor
i. c., intracerebral
i. v., intravenous
LCMV, lymphocytic choriomeningitis virus
mAb, monoclonal antibody
MHC, major histocompatibility complex
NDV, Newcastle disease virus
NIPC, natural IFN-α/β producing cell
NK, natural killer
NRE, negative regulatory element
OAS, 2´-5´oligoadenylate synthetase
poly I:C, polyinosinic-polycytidilic acid
s. c., subcutaneous
SV, Sendai virus
TNF, tumor necrosis factor
PALS, periarteriolar lymphoid sheath
PBMC, peripheral blood mononuclear cell
PKC, proteinkinase C
PMA, phorbol 12-myristate 13-acetate
PRD, positive regulatory domain
RPA, RNase protection assay
SAC, Staphylococcus aureus Cowan I
TGEV, transmissible gastroenteritis virus
Th, T helper cell
Tc, T cytotoxic cell
UV, ultra violet
VRE, virus responsive element

Introduction

Discovery and initial characterization of interferons

Interferon (IFN) was first described in 1957 by Isaacs and Lindenmann when they studied a phenomenon termed virus interference between inactivated and live influenza virus. They discovered a soluble factor released from fragments of chorio-allantoic membranes after incubation with inactive virus, that was able to protect the cells in the membranes against infection when challenged with live influenza virus (Isaacs & Lindenmann, 1957; Isaacs et al., 1957).

During the first half of the sixties many studies indicated that IFN was not a single compound but consists of different molecular species. This notion was based on observations that IFN produced by cells stimulated with viral inducers like Newcastle disease virus (NDV) or the bacterium B. abortus had different physicochemical characteristics compared to IFN induced endotoxin or PHA, such as molecular weight and stability at different pH (Hallum et al., 1965; Ke et al., 1966; Wheelock, 1965). These observations were confirmed by using different antisera which distinguished three antigenically distinct types of IFN, that is leukocyte, fibroblast and immune interferon (De Maeyer & De Maeyer-Guignard, 1988).

IFNs are defined according to their biological action as follows "to qualify as an interferon a factor must be a protein which exerts virus non-specific, antiviral activity at least in homologous cells through cellular metabolic processes involving synthesis of both RNA and protein" (Stewart, 1980).

The further characterization of IFNs was not easy due to the small amounts produced by stimulated cells and the difficulties to obtain pure IFN preparations. However at the meeting "Regulatory functions of interferons" in New York 1979, amino terminal amino acid sequences of different IFNs were presented (Knight et al., 1980; Taira et al., 1980; Zoon et al., 1980). Soon thereafter a gene coding for human leukocyte interferon was cloned, sequenced and expressed (Nagata et al., 1980).

IFN genes and proteins

Probably all vertebrates are able to produce IFNs and since 1980 IFN genes of many species have been cloned (Campos et al., 1992; Sekellick et al., 1994; Tamai et al., 1993; Weissmann & Weber, 1986). The most thoroughly studied so far are the IFN systems of man and mouse (De Maeyer & De Maeyer-Guignard, 1988). In general, the IFNs are divided into type I and type II IFNs. The type I IFN consists of five subfamilies, IFN-α -β, -ω, -τ and -δ, while type II has only one member, IFN-γ.

The type I IFN multigene family: All the type I IFN genes lack introns, an unusual feature among mammalian genes. Many of the type I IFN genes show homologies in the sequences of the 5′ flanking regions upstream of the TATA box that are involved in virus inducibility of the genes. Another common feature of IFN genes is the repeats of A and T in the 3' noncoding regions that affect the mRNA stability (De Maeyer & De Maeyer-Guignard, 1988). The IFN-α and -β genes exhibit approximately 45-50% homology at nucleotide level and it is suggested that the IFN-β gene was the first member of the type I gene family to diverge from an ancestral gene (Hughes, 1995).

The IFN-α consists of 14 closely related nonallelic genes and 4 pseudogenes in man and at least 12 nonallelic genes or pseudogenes in the mouse (De Maeyer & De Maeyer-Guignard, 1988; Diaz et al., 1996). The mature human and murine IFN-α proteins consist of 165 to 167 amino acids and most murine and several human IFN-α are glycosylated. The IFN-α genes are highly conserved within species, showing 80-100% homology in the coding region. A 50-60% homology is seen between the IFN-α of man and mouse (Weissmann & Weber, 1986).

The IFN-β is a single copy gene both in man and mouse, coding for a glycosylated protein consisting of 166 and 161 amino acids, respectively (Derynck et al., 1980; Higashi et al., 1983; Taniguchi et al., 1980). There is a 48% homology between IFN-β genes of mouse and man (Weissmann & Weber, 1986). The number of IFN-β genes varies, for example pigs have only one IFN-β gene (Artursson et al., 1992) while cows have several IFN-β genes (Leung et al., 1984).

The IFN-ω consists of 1 functional gene in man encoding a glycosylated protein of 172 amino acids, as well as 6 pseudogenes (Weissmann & Weber, 1986). The IFN-ω exhibits approximately 70% sequence homology with human IFN-ω genes. While no IFN-ω genes are found in mice or dogs, multiple IFN-ω genes are present in cows, horses and sheep (Dron & Tovey, 1992).

The IFN-τ is constitutively expressed by the trophoblast cells during the early pregnancy in ruminants and is important for the implantation and early embryonic development (Leaman et al., 1992; Roberts et al., 1991). The IFN-τ has 172 amino acids, a high degree of homology with IFN-ω and is not inducible by virus. The IFN-δ resembles the IFN-ω in that it is expressed constitutively in the trophoblast cells, but is structurally unique and has so far only been demonstrated in the pig (Lefevre & Boulay, 1993).

The type II IFN (IFN-γ): The IFN-γ is a single copy gene with introns in all studied species, that codes for a glycoprotein. The IFN-γ genes have no significant homology with type I IFN genes. The IFN-γ is mainly produced

by activated T- and NK-cells, have antiviral effects and many immunoregulatory functions, but acts on a different receptor than the type I IFNs (De Maeyer & De Maeyer-Guignard, 1988; Weissmann & Weber, 1986).

Regulation of IFN-α/β gene expression

The IFN-α and -β genes are strictly regulated and expressed transiently after stimulation with appropriate inducers (Hiscott et al., 1995; Pitha & Au, 1995). The regulatory mechanisms appear different for IFN-α and -β, although some transcription factors involved are common for both types of genes. The purine rich repetitive sequences found in 5´-flanking regions of both human and murine IFN-α/β genes contain binding sites for transcriptional regulators and confer virus inducibility and cell-type specific expression of the IFN genes. These regions in murine and human IFN-α promoter regions are termed inducible elements (IE) and virus responsible elements (VRE), respectively. Several transcription factors with binding affinities to the regulatory elements have been isolated of which some act as enhancers while others have repressive effect on the IFN-α/β expression.

The production of the transcription factor interferon regulatory factor-1 (IRF-1) is induced by virus and has been shown to increase the IFN-α gene expression. Further, a transcription regulatory complex called AF-1, probably modified upon virus stimulation, binds to a critical sequence for virus inducibility found both in human and murine IFN-α promoter regions. The AF-1 binding sequence overlaps the binding site for IRF-1 and it is suggested that effective onset of IFN-α gene transcription requires binding of both IRF-1 and AF-1 (Pitha & Au, 1995). A negatively acting factor IRF-2 has been shown to have the same binding motif as IRF-1 and thereby inhibiting the positive effect of IRF-1 in some cases. The synthesis of both IRF-1 and IRF-2 are induced both by viruses and by IFN and these transcription factors also bind to the IFN-inducible regulatory element of the MHC class I gene (Harada et al., 1989).

The IFN-β gene expression is controlled by four positive regulatory domains (PRDI to PRDIV) and at least one negative regulatory element (NRE), all within a 110-bp long sequence immediately upstream from the transcription initiation site (Hiscott et al., 1995). These regions have binding motifs for several positive transcription factors required for efficient virus induced expression of the IFN-β gene, including NF-κB, IRF-1 and ATF-1. Several negative transcription factors have also been demonstrated such as IRF-2 and PRDI-BF1.

While several positive and negative transcription regulating factors for the IFN-α/β genes have been described, their relative importance is less well documented. For instance, it was found that IRF-1 is not essential in vivo

because mice lacking the gene for IRF-1 are still able to produce IFN-α and IFN-β (Reis et al., 1994; Ruffner et al., 1993).

Inducers of IFN-α/β production and their mechanism of action

The IFN-α/β production is induced by a wide range of virus, bacteria, protozoa or synthetic compounds, but the actual mechanism of the IFN induction is still elusive (De Maeyer & De Maeyer-Guignard, 1988; Ho, 1984; Levy & Salazar, 1992). Almost all viruses are able to induce IFN-α/β production. Three different viruses were used in the present thesis, that is Herpes simplex virus type I (HSV), Sendai virus (SV) and Lymphocytic choriomeningitis virus (LCMV).

The HSV is a large enveloped virus containing linear double stranded DNA, encoding more than 60 proteins (Nash & Cambouropoulus, 1993; Rawls, 1985). The HSV infects cells in the mucosa and epithelium and then migrates to the sensory ganglia where a productive infection can occur and also a latent persistent infection of the neurons can be established. The latent HSV can be reactivated by certain stimuli and cause mucocutaneous lesions, and in rare cases also stromal keratitis or encephalitis. Both innate immunity and later virus specific antibodies and T-cells are involved in the defence against HSV during primary infection, but the immune system is unable to affect the virus during its latency Much of the immunopathology associated with the herpes infection is due to a Th-cell mediated hypersensitivity reaction. The importance of IFN-α/β during the primary HSV infection is described below (see Biological relevance of the IFN-α/β system).

The SV is also known as parainfluenzavirus 1, and is a member of the paramyxovirus genus. The SV contains a negative stranded RNA genome encoding 5-7 proteins enclosed by an envelope containing neuraminidase and hemagglutinin. It causes often subclinical infections in many species, but severe respiratory disease in colonies of rats and mice. It is widely used as an inducer of IFN-α/β responses in experimental systems in vitro (Kingsbury, 1990).

The LCMV is an enveloped negative stranded RNA virus belonging to the arenavirus family which has rodents as natural host but also infects humans. It is common to classify LCMV in "aggressive" or "docile" strains depending on the immunopathology of the infections. When adult immunocompetent mice are infected intracerebrally (i.c) with an aggressive strain of LCMV, the animals develop an acute central nervous system (CNS) disease and usually die within 7 to 9 days. In contrast, congenital or neonatal infection, as well as infections caused by docile LCMV strains, lead to a persistent infection (Buchmeier et al., 1980). The choriomeningitis seen in the LCMV infected mice is caused by virus-specific cytotoxic T lymphocytes (CTL). Both docile and aggressive LCMV strains give rise to strong CTL

responses, but in mice infected with the docile strain no inflammation is seen in the brain (Pfau et al., 1982). Further, the docile strains of LCMV replicate to higher multiplicities compared to the aggressive strains. It has therefore been proposed that the decreased neuropathogenicity of docile strains is due to high titers of virus in multiple target organs which then causes dispersion of the pool of the cytotoxic T lymphocytes and prevents focusing of CTL to the brain (Pfau et al., 1983).

The mechanism whereby viruses induce IFN-α/β production in cells is largely unknown. Virus replication is not necessary for the IFN-α/β induction because UV-irradiation that abolishes infectivity of SV (Ito et al., 1978b), NDV (Clavell & Bratt, 1971) and HSV (unpublished results) does not destroy the IFN inducing ability of these viruses in leukocytes. However, the IFN induction in mouse L- cells by SV was destroyed by UV-irradiation, indicating a different induction mechanisms for different cell types (Ito et al., 1978a). Observations that glutaraldehyde-fixed HSV-infected cell lines are efficient inducers of IFN-α/β production in human PBMC (Gobl et al., 1988; Lebon et al., 1980; Rönnblom et al., 1988) and in murine splenocytes (own unpublished results) prove that viral replication is not necessary in some experimental systems. Results of several groups indicate that viral glycoproteins could be the actual IFN-α/β inducers. Thus, the IFN-inducing capacity is reduced by antibodies directed against viral glycoproteins (Jestin & Cherbonnel, 1991; Lebon, 1985). Further, the porcine transmissible gastroenteritis virus (TGEV), a coronavirus, with a point mutation in the M-protein has a much reduced IFN-α inducing capacity (Laude et al., 1992). Finally, there are reports of IFN-α/β production induced by purified gp120 envelope protein of HIV (Capobianchi et al., 1992).

Many bacteria, both gram negative and positive, are able to induce IFN-α/β (Niesel & Klimpel, 1992; Rönnblom et al., 1983a). The bacteria *Escherichia coli* (E. coli) and *Staphylococcus aureus* Cowan I (SAC) induce IFN-α production in human NIPC (Funa et al., 1985; Svensson et al., 1996a). Another interesting group of IFN inducers consists of protozoa, including the parasites *Trypanosoma cruzi* and *Plasmodium falciparum* (Kierszenbaum & Sonnenfeld, 1982 #381; Ojo Amaize et al., 1981; Rönnblom et al., 1983b).

Several synthetic compounds are potent IFN inducers (Levy & Salazar, 1992). They include double-stranded polyinosinic-polycytidylic acid (poly I:C) and the low molecular compounds fluorononе (Tilorone) and 10-carboxymethyl-9-acridanone (CMA). It has been shown that DNA from bacteria can induce IFN-α/β responses in murine splenocytes, and the critical inducer may be palindromic DNA sequences with CG motifs, minimally hexamer size (Sonehara et al., 1996; Yamamoto et al., 1992).

Consequently, an almost bewildering array of microrganisms and their products, as well as more simple molecules can induce production of IFN-α/β, at least in leukocytes. The question obviously arises if they use one common or several separate mechanisms in order to trigger transcription from the IFN-α/β genes. What could possibly tie the many inducers to the transcription factors regulating the IFN-α/β genes? This is not known at the present time, but it has been suggested recently that scavenger receptors such as the mannose binding lectin (MBL) on the IPCs could be involved (Milone & Fitzgerald-Bocarsly, 1996). Such molecules could conceivably interact with many different types of inducers, and through a common pathway activate the IFN-α/β genes.

The IFN-α/β producing cells (IPCs)

Many different cell types are able to produce IFN-α and -β in vitro upon stimulation with various inducers. Leukocytes isolated from human peripheral blood are the most thoroughly examined cells. In different studies, B-cells, NK-cells, monocytes, dendritic cells (DC) or null cells have been reported to be major producers of IFN-α/β. However, as reviewed (Fitzgerald-Bocarsly, 1993), the main IPCs are now considered to be the monocytes and the Natural IFN-α/β producing cells (NIPC).

In man, much work has been carried out with SV as IFN-α/β inducer in PBMC. Early studies indicated that monocytes are the main IFN-α/β producers in this case (Fitzgerald-Bocarsly, 1993). This was confirmed by two-color immunohistochemical staining of SV-stimulated PBMC for intracellular IFN-α and surface markers present on monocytes such as CD68 and HLA-DR (Akerlund et al., 1996). A small fraction of SV-stimulated IPCs has however been reported not to be monocytes, and may represent NIPC (Akerlund et al., 1996; Feldman et al., 1995).

In contrast, when human PBMC are stimulated by HSV the IFN-α/β production occurs in the NIPC which are infrequent cells, comprising only about 0.2% of all PBMC. These NIPC produce as much as 1-2 U IFN-α per cell, while the monocytes stimulated by SV produce ten times less IFN-α per cell but are at least 10 times as frequent (Cederblad & Alm, 1990; Gobl et al., 1988; Rönnblom et al., 1988; Sandberg et al., 1990). The NIPC are nonadherent, non-phagocytic and lack typical antigenic markers of B-cells, T-cells, NK-cells or monocytes (Fitzgerald-Bocarsly, 1993). In addition, many other viruses than HSV (Feldman et al., 1994; Fitzgerald-Bocarsly, 1993), several different types of bacteria (Rönnblom et al., 1983a; Svensson et al., 1996a) and parasites (Rönnblom et al., 1983b) can induce IFN-α/β production in cells resembling NIPC.

The NIPC in human peripheral blood have been shown to express MHC class II molecules, low levels of CD4 molecules, the thrombospondinreceptor

CD36 as well as the D44 antigen present on immature hematologic cells and a subpopulation of T-cells (Bandyopadhyay et al., 1986; Lebon et al., 1982; Sandberg et al., 1989; Sandberg et al., 1990). The flow cytometric analysis of HSV-induced PBMC showed that their size and granularity differed from that of monocytes (Sandberg et al., 1990). The work described in paper II in the present thesis further established that the NIPC did not obviously belong in the myeloid and lymphoid lineages, because they did not express the markers CD33, CD34, CD7, CD10, CD11b, CD13, CD14 or CD15 and CD19. In a comprehensive later study in our laboratory (Svensson et al., 1996b), another approach was used to characterize the phenotype of HSV-stimulated human NIPC. By simultaneously staining for intracellular IFN-α and different antigenic markers and analysis by flow cytometry, the NIPC were confirmed to lack leukocyte lineage specific markers, and to express CD4, CD36 and HLA-DR. Furthermore, they expressed high levels of CD44, CD45RA and CD45RB, and lower levels of CD40, CD45RO, CD72 and CD83. The NIPC expression of CD13, CD33 and FcεRI were weak but significant, while no CD5, CD11b, CD16, CD64, CD80 or CD86 were detected. It was therefore concluded that the NIPC in several respects resembled immature DC, but differed from typical mature DC which for instance express the costimulatory B7 molecules CD80 and CD86. A relationship between NIPC and DC has been suggested before (Feldman & Fitzgerald-Bocarsly, 1990; Ferbas et al., 1994; Ghanekar et al., 1996; Perussia et al., 1985). However, because of the lack of truly DC-specific markers, and the low frequency of NIPC, it cannot yet be completely excluded that the NIPC actually constitute a specialized and unique IPC population.

Studies of IFN-α/β producing cells in pigs have revealed a cell type resembling human NIPC in being nonphagocytic, nonadherent, expressing MHC II and CD4, but lacking markers of B or T-cells (Charley & Lavenant, 1990; Nowacki & Charley, 1993). The frequencies of porcine NIPC induced by Aujeszky's disease virus (ADV) or TGEV were in the order 5 per 104 PBMC (Artursson et al., 1992; Nowacki & Charley, 1993), which is lower than in man, but comparable to what was observed in the mouse (Paper III).

The IFN-α/β system is relatively well studied in mice (De Maeyer & De Maeyer-Guignard, 1988). However, there are very few studies available describing the characteristics of the IPC in vitro or in vivo. Early experiments suggested that the HSV-induced IFN-α/β production by spleen cells was due to nonphagocytic cells that were not T-cells and did not adhere to plastic (Kirchner et al., 1980). Because the cells adhered to nylon wool columns, it was suggested that the IPC could be B-cells although other cell types such as immature macrophages could not be ruled out. The IPCs among peritoneal exudate cells from mice injected i.p. with HSV or stimulated in vitro by HSV were claimed to be of macrophage lineage (Brucher et al., 1984; Kirchner et al., 1983). Finally, the phenotype of IPC among influenza virus stimulated

murine splenocytes was suggested to be a nonadherent cell expressing Ia, LFA-1 and Mac-1, because monoclonal antibodies (mAbs) to these markers reduced the IFN-α production when added in vitro (Shoshkes Reiss et al., 1984). It can therefore be concluded that although the phenotype(s) of murine IPCs remains to be determined, they may well turn out to be similar to the IPCs described in man and the pig.

Dendritic cells (DCs)

The DCs are bone marrow derived cells that efficiently take up, process and present antigen in association with MHC class I or II to naive T-cells, as reviewed (Cella et al., 1997; Peters et al., 1996; Steinman, 1991). Immature DCs with active macropinocytosis can capture antigen in peripheral tissues, undergo a maturation process and migrate to the lymphoid organs where they become the essential professional antigen presenting cells. The efficiency of the antigen presentation is attributed to the expression of high levels of costimulatory molecules such as CD80 and CD86 on the DC. Several cytokines such as GM-CSF, TNF-α and IL-4 affect the maturation and function of DCs (Cella et al., 1997). When activated by CD40 crosslinking or by some viruses or bacteria or bacterial products, the DCs can also produce cytokines such as IL-12 (Cella et al., 1997). The IL-12 together with IFN-γ are key molecules in directing the immune response to Th1-type immunity (Heufler et al., 1996; Romagnani, 1992; Romagnani, 1996). It has also been shown that IFN-γ in synergy with IFN-α can increase the IL-12 production in DC (Wenner et al., 1996).

The human DCs have also been reported to produce IFN-α when stimulated by viruses such as HSV-1, SV and HIV-1 (Feldman & Fitzgerald-Bocarsly, 1990; Ferbas et al., 1994; Ghanekar et al., 1996), and as outlined above it cannot be excluded that the NIPC are DC. It is therefore desirable to further investigate whether DC produce IFN-α/β in a NIPC-like fashion. This could preferably be done with DC lines, as further discussed in the introduction of Paper VI.

Effects of cytokines on the IFN-α/β production

It is well known that cells preincubated with low concentrations of IFN-α/β before addition of an IFN inducer increases their IFN-α/β production and this phenomenon is known as "priming"(Stewart, 1979). The molecular mechanisms of priming are not known, but it has been suggested that because priming with IFN-α/β enhances the production also of other cytokines such as IL-6 and TNF-α, the priming effect should be on an early step in the signal transduction common for the viral induction of several cytokines (Rosztoczy & Pitha, 1993). Also, treatment with other cytokines than IFN-α/β has been shown to stimulate the IFN-α/β production induced by virus (Cederblad & Alm, 1991). It was for instance demonstrated that the HSV-induced IFN-α response by human NIPC was elevated about twofold by priming with IFN-α,

IFN-γ, IL-3, or GM-CSF. Further, the kinetics of the IFN-α response varied for the IFN- and CSF-stimulated cells, suggesting different mechanisms for the stimulatory effects seen for these two groups of cytokines. The cytokine IL-4, in contrast, decreased the IFN-α and -β response by human monocytes stimulated with SV, both at the mRNA and protein levels (Gobl & Alm, 1992). It has also been reported that IL-4 reduces constitutive IFN-β mRNA expression by cultured murine bone marrow macrophages and makes the cells susceptible for virus infection (Nickolaus & Zawatsky, 1994). However, no IL-4 mediated inhibition was observed on IFN-α/β production by NIPC (Cederblad & Alm, 1991), but in these cells the cytokine IL-10 causes a marked reduction of the IFN-α/β response (Payvandi & Fitzgerald-Bocarsly, 1995).

Cytokines are therefore able to either enhance or suppress the IFN-α/β response. A stimulatory effect of certain cytokines (priming) may actually be required for normal IFN-α/β responses. This possibility is supported by the finding that antibodies to cytokines inhibit the HSV-induced IFN-α/β responses in NIPC (Cederblad & Alm, 1991).

Studies of the IFN-α/β response in vivo

Most previous studies of the activities of the IFN-α/β system in vivo have focused on determining the significance of the IFN-α/β in resistance to experimental viral infections (see below). Many studies have however shown that injection of various IFN inducers cause appearance of IFN-α/β in the blood.

Probably the first studies where IFN-α/β production was studied at the cellular level in vivo were performed in the pig (Artursson, 1993; Artursson et al., 1995). It was shown that intradermal injection of glutaraldehyde-fixed ADV-infected cells resulted in appearance of IFN-α and-β mRNA containing cells (IPCs) in the regional lymph nodes within 6 h, but not in other tissues and organs. The IPCs were localized adjacent to ADV-infected cells trapped in the cortical areas of the lymph node and only occasionally in the skin near the injected cells. Low levels of IFN-α were measured in blood at 8h and 24h post injection, but not later (Artursson et al., 1995). In pigs, a significant local IFN-α response was recorded in subcutaneous tissue chambers injected with poly I:C or ADV-infected cells (Wattrang et al., 1997). Recently, i.v. injections of the inactivated coronavirus TGEV in pigs resulted in appearance of IPC in the spleens (Riffault, 1997). Mice injected with NDV had significant quantities of IFN-α and -β mRNA in the spleens, but not in other organs except for low levels in the lungs (Babu et al., 1992). This could again indicate that lymphoid organs are a major site of IFN-α/β production. When HSV was injected i.p. in mice, significant levels of predominantly IFN-α were detected in serum, while peritoneal cells produced mostly IFN-β (Bhuiya et al., 1994). When the IFN-α/β producing ability of cells derived from

lymphoid organs were examined in vitro, cells were found to produce IFN-β, except for bone marrow cells which produced also IFN-α. These results differ from the results given in Paper III (see Results and Discussion).

The IFN-α/β producing capacity of various mouse strains differ, and their IFN-α/β responses correlate with their resistance to infections (De Maeyer & De Maeyer-Guignard, 1988). The magnitude of the IFN-α/β response is affected by autosomal If loci, which have high and low producer alleles. The C57BL/6 mice are high responders to Newcastle Disease Virus (NDV), HSV-1 and SV, while BALB/c mice are low responders. The If-1 locus which is the best characterized of the several If loci in mice regulates the NDV induced IFN-α and -β production both in vitro and vivo (Babu et al., 1992). Interestingly, priming the macrophages from If-1^h and If-1^l mice with IFN-α/β prior to addition of the virus equalized the IFN production between the low- and high responder cells (Babu et al., 1992). The higher levels of IFN-α/β in the high responder mice are due to a higher IFN production per cell, rather than elevated frequency of IPC (De Maeyer Guignard et al., 1988). The If-1 locus also influences the NDV-induced expression of the cytokines TNF-α and IL-6, and it has therefore been proposed that the If-1 locus affects the early stages of a signal transduction pathway which is common to the virus-mediated induction of both IFN-α/β and these cytokines (Babu et al., 1992).

Mechanisms for the antiviral action of type I IFN

All type I IFNs seem to bind to the same type of receptor, designated IFNAR (Cleary et al., 1994; Uzé et al., 1995), which consists of at least two subunits (IFNAR1 and 2) in man (Novick et al., 1994). The binding affinity of the different type I IFNs to the IFNAR varies, at least partially explaining the differences in their biological activities. By binding to IFNAR, IFN activates the Janus kinases (Jak) Tyk2 and Jak1 (Levy, 1995). These kinases phosphorylate the Stat proteins Stat1 and Stat2 which form the ISGF3α, that translocates to the nucleus and associates with the ISGF3γ p40 polypeptide. This complex, ISGF3, binds to the IFN-α stimulated response element (ISRE) (Levy et al., 1989; Levy et al., 1988) located in the promoter of IFN-α/β inducible genes (ISG), activating their transcription. The Jak-Stat pathway appears essential for the biologic effect of IFN, because Stat1 knock-out mice do not respond to IFN-α or IFN-γ stimulation and are highly vulnerable to viral and bacterial infections (Durbin et al., 1996; Meraz et al., 1996). The deficient response also to IFN-γ is explained by the fact that the receptor for this cytokine also uses the Stat1 protein (Levy, 1995).

The type I IFNs are very potent antiviral proteins, active at levels as low as 10^{-12} to 10^{-13} M. The antiviral effect is due to proteins encoded by IFN-α/β inducible genes (ISG). A large number of such genes have been identified, but the actual function of the corresponding proteins have been clarified in

only a few cases (Sen & Ransohoff, 1993; Staeheli, 1990). The IFN-α/β induced proteins can affect various steps of the viral multiplication, for example viral penetration, uncoating of the virions, transcription, translation and assembly of progeny virus. Examples of such ISG products are given below.

One example is the 2'-5'A system which show specificity for at least some picornaviruses such as encephalomyocarditis virus and mengo virus. It consists of at least three enzymes, 2´-5´oligoadenylate synthetase (OAS), RNAse L and 2´-5´phosphodiesterase that are induced by type I and type II IFN and activated by dsRNA. The 2´-5´oligoadenylate synthetase catalyzes the formation of oligoadenylates which activate dormant RNAse L which in turn inhibits viral replication by degradation of viral and cellular RNA (Sen & Ransohoff, 1993).

The double-stranded RNA-dependent protein kinase (PKR) is another product of an ISG. The synthesis of PKR is enhanced 5-10 fold by both type I and type II IFNs. The PKR becomes autophosphorylated upon activation by dsRNA and inhibits the initiation of protein synthesis by phosphorylating the translation initiation factor (eIF-2) and thereby inhibits multiplication of many types of virus (Williams, 1995).

Another antiviral system consists of the specifically IFN-α/β induced Mx proteins that inhibit replication of a variety of negative-strand RNA viruses, including influenza virus, measles and vesicular stomatitis virus (Pavlovic et al., 1993; Pavlovic & Staeheli, 1991) The precise mechanism of action of the Mx proteins, which are large GTPases, has not been determined (Pavlovic et al., 1993). The Mx protein have been used as a sensitive indicator of IFN-α/β action in man, but unfortunately (for the mice and me) all common inbred mouse strains lack functional Mx genes.

With regard to the action of type I IFNs on the viruses used in the present thesis, the targets of IFN-α/β action on HSV are probably the uncoating of virions and transcription of immediate early viral genes, as well as expression of viral glykoproteins gpD and gpB. The identity of the IFN-induced proteins responsible for these effects is not known (Staeheli, 1990). Also the replication of LCMV in cells is sensitive to IFN-α/β, although again the mechanism of inhibition by IFN is unknown. It is however interesting that there was a correlation between the relative resistance of different LCMV strains to IFN-α/β and their capacity to establish persistent infections in adult immunocompetent mice (Moskophidis et al., 1994).

Immunomodulatory effects of IFN-α/β

The IFN-α/β are pleiotropic cytokines which have many documented immunomodulatory effects, mostly described in mice and man (Belardelli,

1995; Belardelli & Gresser, 1996). Lately, the interest has focused on the regulatory role of IFN-α in development of different subsets of T-cells (Belardelli & Gresser, 1996; Gray, 1996). It has been shown that IFN-α promotes, at least in synergy with IFN-γ and IL-12, the development of the Th1 type of immunity characterized by a Th cell cytokine production profile consisting of IFN-γ, IL-2 and TNF-β and effector mechanisms such as killing of intracellular parasites by activated macrophages, delayed-type hypersensitivity reactions and formation of IgG2a antibodies (Romagnani, 1996; Wenner et al., 1996).

It is also known that immunoglobulin production of the IgG2a isotype dominates and that there is little or no production of IgE in mice injected with poly I:C which elicits an IFN-α response (Finkelman et al., 1991). That effect was additionally enhanced by in vivo administration of IFN-α and abolished by neutralizing antibodies against IFN-α/β. Other observations that indicate a favorable effect of IFN-α on development of Th1 immune responses are that IFN-α can inhibit IL-5 production by human CD4-positive T-cells, as well as the IL-4 induced IgE production and splenic IL-4 mRNA levels in mice (Finkelman et al., 1991; Nakajima et al., 1994). Recently, it was also shown that IFN-α/β inhibits the IL-12 and IFN-γ production induced by SAC in splenocytes in vitro and by the viruses LCMV and MCMV in vivo (Biron, 1997; Cousens et al., 1997).
These inhibitory effects of IFN-α/β on IL-12 and IFN-γ production were however not complete. They may in addition be less marked for other viral IFN-α/β inducers, such as HSV. In fact, HSV rapidly induced IFN-γ and IL-12 production, besides IFN-α, in regional lymph nodes in the same experimental model as described in paper IV, and here neutralization of IFN-α/β actually decreased the IL-12/IFN-γ response (Riffault, 1997).

Two recent and relevant findings have been made regarding the regulation of the receptor for IL-12 (IL-12R) by type I and II IFNs. In mice, the IL-12R was maintained in an upregulated and functional state on activated Th cells by IFN-γ (Szabo et al., 1997), while in man actually IFN-α/β but not IFN-γ was active (Rogge et al., 1997). This indicates major species differences in the control of IL-12R expression, which is assumed to be decisive for promoting development of T cells along the Th1/Th2 pathways.

Another interesting aspect is that IFN-α/β produced during viral infections may lead to proliferation of bystander Tc-cells and maintenance of Tc cell memory (Gray, 1996; Tough et al., 1996).

Adding further to the complexity of the IFN-α/β system, it has been shown that IFN-α actually can induce synthesis in cells such as macrophages of a new cytokine, ISG15, which then triggers production of IFN-γ in T-cells (D'Cunha et al., 1996a; D'Cunha et al., 1996b; Recht et al., 1991). Whether

this new cytokine is active in vivo and of biologic relevance remains to be determined.

Finally, there are many other effects of type I IFN on the immune system (Belardelli & Gresser, 1996; Biron, 1997; De Maeyer & De Maeyer-Guignard, 1988; Fitzgerald-Bocarsly, 1993). These include activation of NK-cells, cytotoxic for virusinfected cells. Further, IFN-α/β can enhance transcription of MHC class I genes, because they possess an enhancer element with ISRE homologies. Finally, the type I IFN can modulate expression of cell adhesion molecules (CAM), which regulate for instance the traffic of cells within the immune system. This issue is dealt with below.

Effects of IFN-α/β on cell migration

Injections of IFN-α/β in mice caused accumulation of mononuclear cells in axillary and inguinal lymph nodes (Gresser et al., 1981). It was postulated that this was due to reduced exit of cells from the lymph nodes, because cell numbers in peripheral blood and in thoracic duct lymph was decreased. Also, IFN-α injections in sheep resulted in markedly reduced numbers of cells leaving lymph nodes (Hein & Supersaxo, 1988; Kalaaji et al., 1988). Furthermore, when mice were infected by Vesicular Stomatitis Virus (VSV) by i.p. injection, a massive leukocyte redistribution in spleen and lymph nodes and reduced blood lymphocyte numbers were seen (Schattner et al., 1983). The changes in the spleen, but not in the lymph nodes, were inhibited by anti-IFN-α/β antibody injections and thus appeared IFN-mediated. Similar IFN-β dependent cellular changes have been seen in the spleens of poly I:C injected mice, associated with a pronounced accumulation of cells in the white pulp (Ishikawa & Biron, 1993). NK-like cells appear important for the migration of cells to the marginal zones of the spleen, and for the IFN-γ production in these structures in mice treated with polyI:C, LCMV or MCMV (Salazar-Mather et al., 1996).

The mechanisms whereby IFN-α/β could regulate leukocyte migrations in lymphoid organs are not yet clear, but probably various cell adhesion molecules are involved. It has thus been shown that expression of L-selectin on human B-cells is upregulated by IFN-α (Evans et al., 1993). The L-selectin expressed on lymphocytes is involved in the initial binding of the cells to high endothelial venules (HEV) and homing to the lymph nodes (Rosen & Bertozzi, 1994). The spleen has no HEV but the marginal zone surrounding the white pulp may be an HEV analogue (Lyons & Parish, 1995). The blood from the central arterioles of the white pulp actually directly empties into the marginal zones, which consist of sinuses and loose lymphoid tissue harboring many macrophages, DCs as well as B and T lymphocytes. In this way these cells come into contact with antigen and other agents present in the blood. The DCs are often located in parts of the marginal zone near the periarteriolar lymphoid sheath (PALS) (Metlay et al.,

1990). At least two types of macrophages are present in the marginal zones, the marginal zone macrophages and the marginal metallophilic macrophages (Kraal, 1992). The marginal zone macrophages are highly phagocytic MHC class II negative cells which seem to be in close proximity to B-lymphocytes (Humphrey & Grennan, 1981; Kraal, 1992). The metallophilic macrophages have low phagocytic activity and express sialoadhesin molecules which mediate binding of lymphocytes (Crocker & Gordon, 1989; Van den Berg et al., 1992; Van den Berg et al., 1996). Similar metallophilic macrophages are also present in the subcapsular sinuses of lymph nodes. It has been reported that IFN-β and certain other cytokines enhance the expression of sialoadhesin (Van den Berg et al., 1996). Therefore, IFN-α/β might well influence cell traffic in the spleen and lymph nodes via sialoadhesin. Many other cell adhesion molecules influenced by type I IFNs and involved in cell migration through lymphoid organs may however be relevant, such as members of the integrin family (Springer, 1995).

In Papers III-V in this thesis further evidence is presented demonstrating that much of the IFN-α/β production occurs in the lymphoid organs and that it can influence the cell traffic at least in the lymph node (Paper IV). The relevance of the IFN-α/β system in the recruitment of cells to lymphoid organs and the furtherance of immune responses therefore deserve more attention.

The biological relevance of the type I IFN in resistance against viruses

Two principal approaches have been used to demonstrate the relevance of the type I IFN system in resistance to viral infections, that is injections of heterologous antibodies to IFN-α/β in virus infected mice and deletion in mice of the receptor for IFN-α/β (IFNAR) by homologous recombination (IFNAR knock-outs). It has not so far been possible to delete all type I IFN genes.

In a large number of early studies it was shown that injections of anti-IFN-α/β antibodies in mice dramatically decreased resistance to various viruses, such as HSV, encephalomyocarditis virus (EMCV), VSV and LCMV (Gresser, 1984). In the case of LCMV, anti-IFN-α/β has a paradoxical effect in that LCMV replication dramatically increases, but the mice are protected against the fatal meningitis (Pfau et al., 1983; Sandberg et al., 1994). This may be due to a dispersion of the cytotoxic T cells due to the increased virus load in peripheral tissues. Injection of anti-IFN-α/β antibodies also impaired resistance to several oncogenic viruses such as Moloney sarcoma virus, Friend leukemia virus and Polyoma virus, with increased incidence of tumors (Gresser, 1984). The latter findings are interesting in view of the results presented in paper I, that is that the commonly used murine tumor promoter phorbol 12-myristate 13-acetate (PMA) inhibited production of IFN-α/β.

Also IFNAR knock-out mice showed a highly increased susceptibility to infections by VSV, Semliki Forest virus (SFV), LCMV, Theiler's virus and vaccinia virus (Fiette et al., 1995; Müller et al., 1994; Van den Broek et al., 1995). By comparison with mice lacking functional receptors for IFN-γ, that is IFNGR knock-outs, it was found that resistance to VSV, Theiler's virus and SFV was essentially IFN-α/β dependent, while resistance to the vaccinia virus and LCMV depended on both IFN-α/β and IFN-γ pathways.

The fact that LCMV-infected IFNAR knock-out mice had a deficient antiviral CTL-response might indicate that it is IFN-α/β dependent. Other abnormalities in the immune responses in the mice have so far not been found. However, more extensive studies are required to define the mechanisms whereby the IFN-α/β exerts its antiviral effects in vivo. Such studies must take into account the problems with redundancies in cytokine actions, because several IFN-α/β actions may be compensated for by other cytokine systems. Furthermore, important species differences may exists, such as the differential regulation of the IL-12R by IFN-α/β and IFN-γ in mice and man (Rogge et al., 1997; Szabo et al., 1997).

Aims of the present study

The general aim of this thesis has been to study the IFN-a/b system in vitro and in vivo, especially the IFN-α/β producing leukocytes (IPCs). The work started with a characterization of the IPCs in human blood and continued with an analysis of the IFN-α/β response in mice in vivo, which was supplemented with characterization of the IFN-α/β production in murine leukocytes in vitro, especially in two dendritic cell lines. The specific aims were:

1. To follow up an initial finding that the phorbolester phorbol 12-myristate 13-acetate (PMA) inhibited the IFN-α response induced by HSV in human PBMC. This was done because PMA is an activator of PKC which is involved in intracellular signalling, and it was thought that insight might be gained regarding the control of the IFN-α/β production. Furthermore PMA represents a class of tumor promoters, extensively studied in mice. We asked whether PMA could inhibit in vitro responses to SV in monocytes and HSV in the NIPC, whether both IFN-α and IFN-β production was affected, and whether the PMA effect was actually due to activation of PKC. We finally attempted to define the cellular level of PMA action.

2. To further define the phenotype of the NIPC. This was done because at the time of this part of the study many questions regarding the identity of these cells had no answer. We asked whether the NIPC, defined as very efficient IPCs induced by HSV, belonged to the lymphoid or myeloid developmental

lineages. An experimental approach was developed, consisting of flow cytometry and sorting of human PBMC according to their light scattering properties and expression of different antigenic markers, and then testing for HSV-inducible NIPC.

3. To characterize the IFN-α/β response induced by HSV in mice in vitro and in vivo. This involved development of required reagents and methods (for immunoassays, immunohistochemistry and in situ hybridization), determination of the capacity of cells from different organs to produce IFN-α and -β in vitro, and finally characterization of the IFN-α/β response induced in mice by HSV injected intravenously. In particular the tissue localization of the IPCs in vivo was determined, and to some extent also their phenotype.

4. To study the IFN-α/β response in the skin and in the lymph nodes. An experimental model was adopted for this purpose, which involved injection of HSV in the ears of mice and determination of IPCs at injection sites, in regional lymph nodes and in more distant organs. We asked whether the IFN-α/β response was confined to the regional lymph nodes or not. We further investigated whether the noted rapid enlargement of regional lymph nodes was caused by produced IFN-α/β, and if it was due to accumulation of any special lymphocyte subset.

5. To study the activity of the IFN-α/β system during the course of lymphocytic choriomeningitis virus (LCMV) infection in mice, in particular to determine if there was a restricted production of IFN-α/β in the central nervous system, and to what extent and where IFN-α/β was produced in lymphoid organs and other virus-infected tissues.

6. To determine whether two murine dendritic cell lines, D2SC/1 and FSDC, could produce IFN-α/β upon stimulation with the viruses SV and HSV as well as the bacteria SAC or E. coli. Further, we asked if the HSV-induced IFN-α/β production by these DC lines could be modulated by exposing the cells to the cytokines IL-4, IL-10, TNF-α, IFN-β, IFN-γ and GM-CSF.

Results and Discussion

Phorbol ester-mediated inhibition of IFN-α/β gene transcription (Paper I)

Monocytes are the main IFN-α/β producers when human PBMC are stimulated with SV, whereas the NIPC produce IFN-α/β upon stimulation with HSV (see Introduction). The two cell types appear to use different pathways for induction of the transcription of their IFN-α/β genes as indicated by the fact that transcription of the IFN-α/β genes in NIPC and the IFN-α gene in monocytes require de novo protein synthesis, while the transcription of the

IFN-β gene in monocytes does not (Cederblad et al., 1991; Gobl et al., 1992). The phorbol ester PMA, which is a tumor promoter (Boutwell, 1974; Boutwell, 1985), activates protein kinase C (PKC) irreversibly by substituting for the physiological and reversible PKC activator diacylglycerol (DAG) (Nishizuka, 1984). We wanted to study the possible involvement of PKC in the regulation of IFN-α/β gene expression because PKC is participating in intracellular signalling of many cellular responses (Kikkawa et al., 1989).

We showed that PMA efficiently inhibited the secretion of IFN-α and -β by human PBMC stimulated in vitro by SV or glutaraldehyde-fixed HSV-infected WISH cells in a concentration dependent manner. The HSV-induced IFN-α/β response in NIPC was more sensitive than the SV-induced response in monocytes, 90% inhibition occurring at approximately 0.4 ng/ml versus 2 ng/ml. In contrast, the PMA analogue 4–α-phorbol, which does not activate PKC, had no effect on the IFN-α/β response. Northern blot analysis of IFN-α and -β mRNA revealed reduced mRNA levels in PMA treated cells that paralleled the results at the IFN protein level. Similar effects were seen with a synthetic diacylglycerol, 1-oleoyl-2-acetylglycerol (OAG) which also is a potent activator of PKC (Kaibuchi et al., 1983). The PKC inhibitor staurosporin (35nM) (Davis et al., 1989) was able to block the PMA-mediated inhibition of the IFN-α/β response. These observations together suggest that the inhibition of IFN-α/β response may act through activation of PKC.

To clarify if PMA affected the transcription of the IFN-α/β genes, nuclear run-on transcription assays were performed on nuclei isolated from SV-stimulated monocytes. The results showed that the SV-induced transcription of the IFN-α/β as well as IL-6 and IRF-1 genes were reduced in the presence of PMA, although the suppression of the IFN-α/β genes was more pronounced. In contrast, PMA enhanced the SV-induced TNF-α gene transcription and the PMA treatment alone of the cells induced TNF-α gene transcription. Nuclear run-on transcription assays could not be performed with HSV-induced NIPC because of the low frequency of these cells.

Interestingly, the PMA mediated inhibition of SV-induced IFN-β mRNA production in PBMC was abolished by cycloheximide (CHX), demonstrating that de novo protein synthesis is required for the suppression of the IFN-β response by PMA. It was not possible to elucidate if protein synthesis is necessary for inhibition of IFN-α mRNA production since the IFN-α mRNA response itself requires de novo protein synthesis. The mechanism by which PMA exerts the inhibitory effect on the IFN-α and IFN-β mRNA synthesis is not clear. It is possible that some of the virus-induced DNA binding transcription regulatory factors that bind to the regulatory domains of the IFN-α and -β genes are involved. The PMA could thus inactivate positive transcription factors, or alternatively activate or cause synthesis of negative

transcription factors. With regard to the former mechanism, IRF-1 could possibly be down-regulated, and we actually noted that the transcription of the IRF-1 gene was markedly inhibited by PMA. Furthermore, the phorbolester TPA causes complete disappearance NF-κB in human monocytes exposed to phorbol ester (Mufson et al., 1992). The NF-κB binds to the positive regulatory element II (PRDII) on the IFN-β gene promoter, but may however not be involved in IFN-α gene regulation (Pitha & Au, 1995). Another potential target for PMA is the negatively acting transcription factor IRF-2, which binds to motifs in the promoters of both IFN-α and -β genes and inhibits transcription. Other factors described to be involved in the regulation of the IFN-α/β genes (Hiscott et al., 1995; Pitha & Au, 1995) might however also be involved.

We have thus found that phorbol esters via activation of PKC are potent inhibitors of the IFN-α/β response. It may indicate a physiologically relevant pathway for repression of IFN-α/β genes. In a subsequent study the inhibitory effect of PMA on the IFN-α response of PBMC induced by HSV was confirmed, whereas PMA in combination with a calcium ionophore increased the IFN-α production (Li et al., 1996).

Furthermore, the ability of phorbol esters to inhibit IFN-α/β responses might be one mechanism whereby they promote development of tumors, because the direct antiviral effects of IFNs could limit replication of tumor viruses and the many immunoregulatory effects of IFNs could stimulate immune responses to tumors (see Introduction).

Flow cytometric analysis of natural IFN-α producing cells (Paper II)

At the time the present study started, the NIPC triggered by HSV were known to be infrequent cells lacking antigenic markers associated with the phenotype of T, B, NK-cells or monocytes. The human NIPC were also known to express MHC II class antigens, low levels of CD4 molecules (Sandberg et al., 1990) and the thrombospondin receptor CD36 (Sandberg et al., 1989). Further, NIPC were characterized as relatively large non-phagocytic null cells with forward- and right-angle light scatter distinct from monocytes (Sandberg et al., 1990). In the present study we wanted to further explore the expression of phenotypic cell surface markers on the NIPC by means of flow cytometric analysis and sorting of cells followed by stimulation with HSV in culture and subsequent in situ hybridization using 35S-labelled cRNA probes for IFN-α mRNA to detect and to determine the frequency of NIPC in the sorted populations. In particular we examined the possibility that the NIPC could represent early stages in the lymphoid and myeloid lineages.

A major problem with the flow cytometry and sorting of NIPC and the further analysis of these cells in vitro was their low frequency and decline in activity when cultured at low cell concentrations in vitro (Cederblad & Alm, 1990). These problems were at least partially solved in the present study by initially enriching for the NIPC by T cell depletion and density gradients (see below). Further, use of feeder cells, conditioned medium and addition of GM-CSF to the culture medium allowed IFN-α/β responses at low cell concentrations.

Three different populations of human PBMC were isolated from buffy coats by Ficoll-paque density gradient centrifugation (population I), further depleted of T-cells (population II) and finally a population consisting of the upper light band from Percoll-gradients of T-cell depleted PBMC (population III). The frequency of NIPC increased from 0.03% in population I to 0.3% in population III defined by means of in situ hybridization with IFN-α cRNA probe. The cell population III was analyzed by flow cytometry and five electronic gates were defined according to orthogonal- and forward light scattering properties which were used for sorting. The highest frequency of NIPC was detected among cells with low or intermediate orthogonal light scatter and relatively high forward light scatter, similar to that of early hematopoietic progenitors. We therefore examined the expression of the stem cell marker CD34 on the NIPC. When cells were sorted according to CD34 expression, 80% of the totally 84% NIPC that were recovered after sorting were found in the CD34 negative population.

To reveal the possible relationship of NIPC to cells of the myeloid lineage, the NIPC-enriched cell population was stained for CD11b, CD13, CD14, CD15 and CD33 antigens. These antigenic markers cover the range of cells from the myeloid progenitors expressing CD33, myeloblast and monoblast expressing CD13, mature granulocytes expressing CD11b and CD15 and monocytes expressing CD11b, CD14 and CD15. However it was found that the NIPC expressed none of these antigens.

To test the possibility that NIPC belong to the lymphoid lineage, the NIPC-enriched population was stained for expression of CD7, CD10, CD19 and CD33 antigens. The results showed that NIPC did not express CD10, a marker of early B-cells or CD19, expressed by B cells from pre-B cell stage until maturation into plasma cells. Further, NIPC did not express the early T-cell marker (CD7).

The results of the present study, together with earlier reports (see Introduction), supported the contention that the NIPC represent a unique population of cells in peripheral blood, not obviously representing progenitor cells or later differentiation stages of the lymphoid or myeloid lineages, and distinct from NK cells and DCs in peripheral blood. With regard

to the latter cells, it has been claimed that the NIPC stimulated by HSV are related to dendritic cells (Feldman & Fitzgerald-Bocarsly, 1990; Perussia et al., 1985) but these results were based on experiments with depletion of T-cells, B-cells, monocytes and NK-cells and enrichment for HLA-DR+ cells, and therefore not conclusive. Another study actually provided evidence that the NIPC were not identical with dendritic cells (Chehimi et al., 1989). In a subsequent study using direct flow cytometric analysis of HSV-induced PBMC using simultaneous staining for IFN-α and surface antigens, the phenotype of NIPC indicated that they could be immature but definitely not mature DCs or constitute a unique population of cells (Svensson et al., 1996b).

The IFN-α/β responses of mice to HSV vitro and vivo (Paper III)

A great deal is known regarding the antiviral and immunomodulatory effects of IFN-α/β in different experimental systems (see Introduction), although the physiological relevance of many of the proposed actions of the IFN-α/β must be further clarified. Much is also known about the IPCs, the IFN-α/β genes and how they are activated by different inducers (see Introduction). Such work has almost completely been carried out in vitro. With regard to leukocytes, in vitro work with especially peripheral blood cells in man and pigs have defined the NIPC and monocytes as the major IFN-α/β producers (see Introduction and Papers I and II). However quite little is known regarding the IFN-α/β response in vivo, for instance the identity of the IPCs involved, as well as their organ and tissue localization.

We therefore studied the IFN-α/β response of C57BL/6 mice to UV-irradiated HSV. First, mononuclear leukocytes isolated from lymphoid organs and blood were stimulated by HSV in vitro. The frequency of IFN-α and IFN-β mRNA expressing cells was determined by in situ hybridization using 35S-labelled cRNA probes for IFN-α and -β mRNA. Furthermore, dissociation-enhanced lanthanide fluoroimmunoassays (DELFIA) specific for IFN-α and IFN-β were developed and used to measure secreted IFNs. The highest production of IFN-α and -β was detected in bone marrow cell cultures (about 400 U/ml) while the IFN-α and -β levels in cell cultures with splenocytes, lymph node cells and blood leukocytes were in the 50-100 U per ml range. Thymocytes produced about 40 U per ml of IFN-α but less than 3 U of IFN-β, in contrast to the cells from the other sources which produced about equal amounts of IFN-α and -β. This is in contrast to the results of others, showing that predominantly IFN-β was produced by HSV-stimulated leukocytes in vitro (Bhuiya et al., 1994).

The highest frequency of IFN-α mRNA expressing cells was found among bone marrow cells ($15/10^4$), followed by spleen ($4/10^4$), lymph node cells ($2/10^4$), blood ($1/10^4$) and finally thymus ($0.2/10^4$). IFN-α production per cell was estimated to be about 0.2 U based on the IPC frequencies and

immunoassay results. The frequency of IFN-β mRNA expressing cells among bone marrow cells and splenocytes was determined and was about the same compared to the IFN-α mRNA expressing IPC (results not shown). Thus, infrequent but relatively efficient IPCs cells were found in the mice.

To localize the IFN-α and -β producing cells in vivo, HSV was injected i.v in mice. The IFN-α and IFN-β concentrations were measured in blood by DELFIA and IFN-α and IFN-β mRNA expressing cells were localized in organs by in situ hybridization at various time points post injection of HSV. Already 3 h after virus injection high levels of IFN-α and -β were detected in blood, maximum levels were seen at 6 h and only very low levels of IFN-α/β were detected after 24 h. The IFN-α levels in blood were generally somewhat higher than the IFN-β levels. The IFN-α mRNA expressing IPCs were mainly detected in the spleen and only occasionally in lymph nodes, whereas no IPCs were found in bone marrow, liver or kidneys. Furthermore, no IPCs were seen among blood leukocytes and in the lungs (results not shown). The absence of IPCs in the bone marrow is surprising, since such cells showed the highest IFN-α/β responses in vitro. Possible explanations to this discrepancy are that HSV in vivo never reaches the bone marrow, or that the putative IPC leave bone marrow upon stimulation by HSV, or that the cells are unable to respond in vivo. No IFN-β mRNA expressing cells could be detected in any of the organs, despite the fact that high levels of IFN-β were detected in blood by DELFIA. The production of this IFN may therefore occur in other locations or at a low levels in many cells in e.g. the spleen, below the limit of detection.

In the spleens, the IFN-α mRNA expressing IPCs were almost exclusively restricted to the marginal zones bordering the follicular area of the white pulp, and were heavily labelled in the autoradiographs. The IPCs were absent from the periarteriolar lymphoid sheath (PALS) and its border against the red pulp. A similar distribution of IPCs in the spleens was obtained when they were stained for intracellular IFN-α/β using polyclonal anti-IFN-α/β antibodies. The identity of the IPC is not clear, but many different cell types are present in the marginal zones, including DCs, T- and B cells as well as subpopulations of macrophages consisting of marginal zone macrophages and metallophilic macrophages (Kraal, 1992). The first results of our efforts to characterize the IPCs will be presented later as an addendum at the end of the Results and Discussion.

We estimated the number of IPC to be about $40x10^3$ per spleen at 6h after HSV injection, each producing perhaps 0.2 U IFN-α, suggesting that the spleen is a major source of the IFN-α detected in the blood. Obviously, the marginal zone localization of IPCs should expose both splenocytes and cells in blood to high concentrations of at least IFN-α. The role of this IFN-α might be to limit the viral replication and thus protect lymphoid tissue.

Immunoregulatory functions are also very likely, as further described in the Introduction, including regulation of cell migration and of the function of cells involved in immune responses.

The HSV-induced IFN-α/β production in lymph nodes and its effect on accumulation of leukocytes (Paper IV)

In the previous paper (III), methods were developed for the study of the murine IFN-α/β system, the in vitro responses of leukocytes from different sources to HSV were studied, and finally HSV was injected to study the systemic in vivo IFN-α/β response. The latter appeared to primarily occur in the spleens. It was of interest to assess more directly the local IFN-α/β response, because most virus infections begin with the local replication of virus in tissues at bodily surfaces and the draining lymph nodes will first be exposed to viral particles. We used an experimental model previously described (Nash et al., 1980) where subcutaneous (s.c.) injections of HSV in the ears of mice cause a local inflammation and an antiviral immune reaction with appearance of antigen-specific Tc and Th lymphocytes in the draining lymph nodes. In the present study, UV-irradiated HSV was injected s.c. in the right ear of mice and the IFN-α/β response in lymph nodes and spleen and at the blood level was followed. Because a marked HSV-induced reaction of the draining lymph nodes was seen, its relation to the produced IFN-α/β was studied by means of injection of neutralizing antibodies to IFN-α/β before the HSV injection.

When UV-irradiated HSV was injected into one ear of C57BL/6 mice, an IFN-α/β response was induced with relatively high levels of IFN-α/β measurable in blood, the peak occurring at approximately 6 h. Using in situ hybridization and 35S-labelled cRNA probes for IFN-α, IFN-α mRNA containing cells were detected in the lymph node draining the site of the HSV injection, primarily localized in the outer cortex and between follicles. A few IFN-α mRNA containing cells were occasionally detected in the marginal zones of the spleens, but no such cells were found in contralateral lymph nodes. Immunohistochemical staining for IFN-α/β containing cells confirmed these results. No IFN-α/β positive cells were found 24 h after HSV injections.

In the HSV injected (right) ears, a red edema and infiltration of mononuclear cells were observed at the site of HSV-injection at 6 h and 24 h whereas no infiltration and a slight or no edema were seen in the control ears injected with PBS. However, except for one experiment, no IFN-α mRNA positive cells were found in the ears.

Consequently, we have a model where much if not all of the IFN-α/β response occurs in the local draining lymph node, and where the IPCs are localized in the lymph node periphery. Locally produced IFN should therefore drain through the lymph node and expose cells to very high IFN concentrations.

Possibly, much of the IFN-α/β circulating at the blood level is lymph node derived. These results agree with previous data obtained in pigs after injections of ADV-infected cells in the skin (Artursson et al., 1995).

An almost threefold increase in the total number of cells in the lymph node draining the HSV-injected ears was noted 6 h after HSV injections, but no further increase was seen at 24 h. A marked infiltration of neutrophilic granulocytes at 6 h and 24 h in lymph nodes draining the side of HSV-injection was also noted. Analysis by flow cytometry did not reveal any preferential increase of either T (Thy1.2+), Tc (CD8+), Th (CD4+) or B (sIg+) lymphocytes in the lymph nodes draining the HSV injected ear, compared to the control lymph node draining the PBS-injected ear. This early and marked enlargement of the lymph node must be due to profound alterations in the migration of leukocytes through the organ, and thus appears to involve all major populations of lymphocytes. It might be caused by the locally produced IFN-α/β, because similar effects have been seen after administration of IFN-α/β or IFN-α/β inducers in mice and sheep (see Introduction).

We directly examined if the accumulation of cells in lymph nodes caused by injected HSV was related to production of IFN-α/β, by injecting antibodies neutralizing IFN-α/β in the base of both ears 1 h before HSV and PBS injections. These anti-IFN-α/β antibodies neutralized the IFN appearing in blood, although it was not possible to determine whether they actually completely neutralized all IFN activity in the local lymph node. We observed an approximately 50% reduction in the increase in size (weight and cell number) of the lymph node caused by HSV 6 h after injection, but no effects were seen at 24 h. These results suggest that IFN-α/β at least partially is responsible for the observed local lymph node response. This partial inhibition could be due to an incomplete neutralization of IFN-α/β, but is perhaps more likely due to production of other cytokines involved in regulation of cell traffic in the lymph node. For instance, IL-12 and IFN-γ could be involved. In fact, both IL-12 and IFN-γ has been shown to be produced at the same time as IFN-α/β in the HSV-draining lymph nodes examined (Riffault, 1997).

The results presented in Papers III and IV, together with other studies in especially pigs (Artursson et al., 1995; Riffault, 1997) suggest that localization of the IFN-α/β production to the lymphoid organs could be a general phenomenon in viral infections. Both in spleen and in lymph nodes, the IPCs are in such positions that lymphocytes and other cells are exposed to high IFN-α/β concentrations, and such IFN-α/β transported by blood may even have effects on distant tissues. The ear model described here appears suitable for further studies of the in vivo role of the IPCs and the IFN-α/β in the early immune response to viruses, and perhaps other microorganisms.

Expression of IFN-α/β and IFN-α/β-induced genes in lymphocytic choriomeningitis (Paper V)

The production of IFN-α/β is in most cases beneficial for the recovery from viral infections (see Introduction). Paradoxically, administration of neutralizing antibodies to IFN-α/β to mice infected with lymphocytic choriomeningitis virus (LCMV) protects the animals against the acute and often lethal LCM, but promotes LCMV replication in various organs and establishment of a chronic infection (Pfau et al., 1983; Sandberg et al., 1994). Although the IFN-α/β dramatically can change the course of this viral disease, little is known regarding the function of the IFN-α/β system during the LCMV infection. We therefore infected BALB/c mice by intracerebral injection of LCMV (strain Arm53b), and then examined the localization and kinetics of IFN-α and -β production and LCMV replication in different organs, as well as expression of the IFN-α/β inducible 2′5′-oligoadenylate synthetase (OAS) gene.

The LCMV infected mice demonstrated a significant IFN-α response with significant blood levels of IFN-α determined by DELFIA on day 3 (83 U/ml), but not on the other experimental days, i.e. days 1 and 6. In contrast to the results in Paper III when HSV was injected into C57BL/6 mice, no IFN-β could be detected. This result is surprising considering the fact that the levels of both IFN-α and IFN-β mRNA in e.g. the spleens of the LCMV infected mice were high at both day 1 and 3. Whether this discrepancy is due to an inhibition of translation of IFN-β mRNA, as described in another system (Dehlin et al., 1996) remains to be determined.

In the brain tissue, no IFN-α mRNA could be detected using the sensitive RNase protection assay (RPA) or by in situ hybridization, despite the presence of LCMV infected cells in the choroid plexus and ependyma on days 3 and 6, as well as excessive infiltration of mononuclear leukocytes and inflammation of the meninges. In contrast, a weak expression of IFN-β mRNA was detected by means of RPA on day 3 which further increased on day 6. No IFN-β producing cells could however be detected by in situ hybridization, indicating that the IFN-β production per cell is very low or possibly occurs in very infrequent cells. Attempts were made to demonstrate IFN-mediated effects in the brains by determining levels of mRNA for the IFN-α/β induced OAS and for MHC class I (H2D). Weak and late increases in OAS mRNA, but stronger increases in H2D mRNA were seen using Northern blot hybridization. While both might be due to local IFN-β production, they may also be due to other cytokines or leakage of IFN through the blood-brain barrier which is known to be damaged around day 3 post infection. It can be concluded that the absence of IFN-α and low and delayed production of IFN-β should make the brain vulnerable for virus replication.

In contrast to what was seen in the brain, IFN-α and -β mRNA could be detected in spleens of LCMV-infected mice on days 1 and 3 but not on day 6. By means of in situ hybridization, IFN-α mRNA expressing cells were detected mainly in the marginal zones of the spleen. The same cells appeared to express IFN-β mRNA (unpublished results). Also LCMV-infected cells were seen by in situ hybridization of spleen sections, mostly on day 3, and were localized in the same areas as IFN-α mRNA expressing cells. By analysis of consecutive sections it appeared that many IPC contained LCMV, or at least were very close to LCMV-infected cells.

IFN-α mRNA containing IPCs were also detected in the subcapsular sinus and outer cortex of cervical lymph nodes, that is in a location similar to that described in Paper IV. No IFN-α mRNA was detected in kidney tissue whereas a weak IFN-β signal was visible on day 3 post infection. In contrast, a prolonged expression of IFN-induced OAS mRNA was seen in this organ, perhaps because it is exposed also to circulating IFN-α.

The results in Paper V confirm that also in an infectious viral disease, that is LCM, much of the IFN-α appears to be produced in the lymphoid organs, as previously described using injections of inactivated nonreplicating viruses HSV in mice (Papers II and IV), as well as ADV (Artursson et al., 1995) and the coronavirus TGEV in pigs (Riffault, 1997).This IFN can greatly limit the viral infection by direct antiviral effects and by promoting antiviral immunity (see Introduction). In the case of the LCMV-infection this may not always be beneficial, because the IFN-α/β response allows development in lymphoid organs of antiviral T-cells that subsequently can infiltrate the brain and cause choriomeningitis (Pfau et al., 1983; Sandberg et al., 1994). With regard to at least LCMV, and possibly other infections, it is further interesting that little or no IFN-α/β was produced in the brain, a situation that would promote replication of virus in this organ, and in part contribute to the neurotropism of the virus. In this context, it is surprising that no IFN-α/β producing cells were seen among inflammatory leukocytes of the LCMV-infected brain tissue. Similarly, few IPCs were detected in the skin at sites of intradermal injection of HSV in mice (Paper IV) and ADV in pigs (Artursson et al., 1995). It is therefore possible that the potential IPCs present in e.g. blood rather selectively migrate to lymphoid organs.

Production of IFN-α/β by murine dendritic cell lines (Paper VI)

As discussed in the Introduction, it has been claimed that human DCs produce IFN-α when stimulated by viruses such as HSV-1, SV and HIV-1 (Feldman & Fitzgerald-Bocarsly, 1990; Ferbas et al., 1994; Ghanekar et al., 1996; Perussia et al., 1985). The DCs may be related to, but not necessarily identical to the NIPC which. produce large amounts of IFN-α when stimulated with HSV and bacteria SAC and E. coli (Fitzgerald-Bocarsly, 1993; Rönnblom et al., 1983c; Svensson et al., 1996a). The NIPC are

difficult to study because of for instance their low frequency in blood, and it would therefore be valuable to have access to cell lines that respond to IFN-α/β inducers in the same way as the NIPC.

In order to determine whether DCs could produc e IFN-α/β in a NIPC-like fashion, and to establish an experimental system in which for instance the mechanism of induction of IFN-α/β production and its functional relevance could be studied in more detail, we examined two immortalized DC lines (Girolomoni et al., 1995; Lutz et al., 1994; Sassano et al., 1994) originating from murine spleen (D2SC/1) and fetal skin (FSDC). These DC lines represent rather immature DCs, and have in several other respects been well studied before (Granucci et al., 1994; Lutz et al., 1996; Paglia et al., 1993). We assessed their ability to produce IFN-α/β when stimulated by HSV and SV as well as the bacteria SAC and E. coli. Furthermore, the effects of the cytokines IL-4, IL-10, TNF-α, IFN-γ, IFN-β and GM-CSF on the IFN-α/β production were studied.

We showed that the D2SC/1 cells produced both IFN-α and IFN-β when stimulated by both the viruses and bacteria. This spectrum of reactivity resembles that of human NIPC (Fitzgerald-Bocarsly, 1993; Svensson et al., 1996a), and cells defined as DC can thus produce IFN-α/β in response to widely different microorganisms. The HSV and SV were about equally efficient as IFN-α/β inducers but the IFN-α production was approximately 10 times lower than the IFN-β production. The reason for the low IFN-α production is not known, but one possibility is a lack of costimulatory factors, which has been shown to be important for human NIPC (Cederblad & Alm, 1991) and which may explain why HSV- and SV-stimulated murine leukocytes in vitro produce about equal amounts IFN-α and -β (Paper III). Such costimulatory factors may be cytokines, and in support of this we showed that priming D2SC/1 cells with GM-CSF and IFN-β increased the IFN-α/β responses induced by HSV and bacteria, especially the IFN-α production.

The FSDC cells clearly differed from the D2SC/1 cells in some respects. In particular, they produced much less IFN-β and little or no IFN-α using the various IFN-α/β inducers. The reason for this remains to be determined, and such work could help unravel the requirements for induction of the IFN-α/β response.

At least in man, the HSV stimulates NIPC and the SV stimulates preferentially monocytes (Akerlund et al., 1996; Saksela et al., 1984). These inducers appeared to act differently also on the D2SC/1 cells, because in contrast to HSV-induced IFN-α/β responses, the SV-induced IFN-α/β responses were not increased by priming with IFN-β/GM-CSF and had a longer lag phase before IFN could be detected in culture medium.

As mentioned, priming of in particular D2SC/1 cells with IFN-β/GM-CSF increased HSV-induced and some bacterially induced IFN-α/β responses. We further examined if the HSV-induced IFN-α/β production of the DC lines was influenced by preincubating the cells for 2 h or 24 h with cytokines that are known to have effects on maturation and other functions of DCs. The most striking effects were that the IFN-β production by the FSDC cells was stimulated most efficiently by IFN-γ, an effect that may be due to maturation of the cells and perhaps related to the previously observed stimulation of pinocytosis and antigen presenting function in FSDC by IFN-γ (Lutz et al., 1996). In contrast, the most effective stimulation of D2SC/1 was achieved by 2 h priming with IFN-β while the 24 h priming had no stimulatory effect. No IFN-α production by FSDC cells was seen after priming with any of the cytokines.

The IL-4, IL-10, TNF-α or GM-CSF had no significant effects on either cell line. It is somewhat surprising that IL-10 or IL-4 had no effect on the IFN-α/β production, because it has been reported that they inhibit the IL-12 production by natural DC (Koch et al., 1996) and the IFN-α/β response by SV- or HSV-stimulated human PBMC (Gobl & Alm, 1992; Payvandi & Fitzgerald-Bocarsly, 1995). The reason for this lack of response to IL-4 and IL-10 remains to be determined.

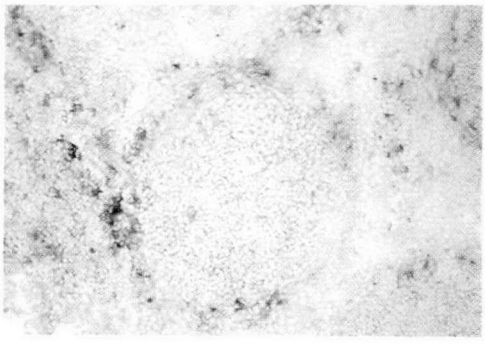
It is concluded that the two DC lines, in particular the D2SC/1 cells, will provide a convenient tool to study the induction and control of the IFN-α/β response induced by virus and bacteria, and perhaps other microorganisms. The FSDC cells may in addition offer possibilities to study differentiation of the ability to produce IFN-α. The DC lines should also provide a good opportunity to study the role of IFN-α/β produced by DCs during immune responses in vitro and in vivo, and how the DCs themselves are affected by IFN-α/β.

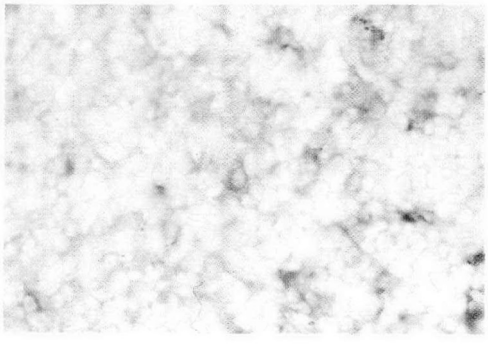
The phenotype of IFN-α/β producing cells in the spleen (Addendum)

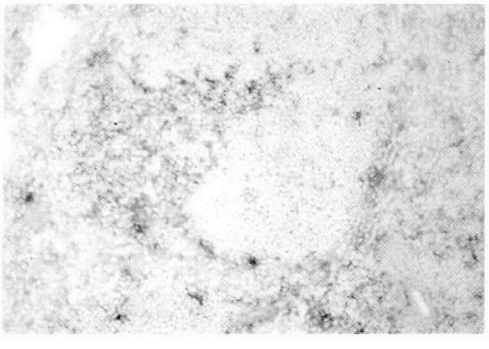
In paper III, we found that the IPCs were almost exclusively lozalized in the marginal zones of the spleens of C57BL/6 mice injected with HSV. As discussed, the marginal zones contain several types of cells that could be the actual IPCs, one good candidate being the DCs, another possible candidate being the metallophilic macrophages. Attempts were therefore made to determine the phenotype of these IPCs by simultaneous histochemical staining for intracellular IFN-α/β and some antigenic markers found on murine DCs (CD11c) and metallophilic macrophages (SER-4) as well as MHC class II ($I\text{-}A^b$).

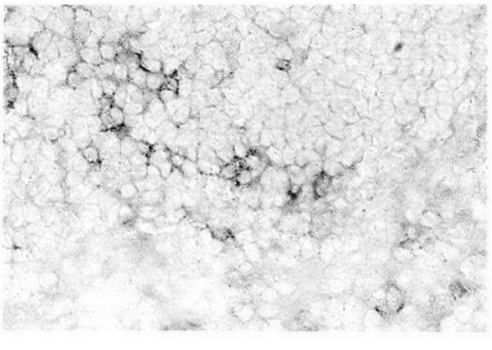
C57BL/6 mice were injected with uv-inactivated HSV as described in Paper III and the spleens were obtained at 6 h and frozen. The 8 μm thick cryosections were dried at room temperature, frozen at -80°C, fixed with ice cold 2% paraformaldehyde for 5 min, rinsed, and finally blocked with 2% normal mouse serum (NMS) and either 2% normal donkey or goat serum. A buffer was used throughout the procedure, consisting of 0.05 M Tris-buffered saline, pH 7.6, containing 0.01 M Hepes and 0.01% human serum albumin. The sections were incubated with sheep anti-IFN-α/β antibodies in the presence of 0.1% saponin and 2% NMS overnight in the cold. After washing, sections were stained with biotinylated donkey anti-sheep antibodies for 1 h at room temperature (RT). Slides were washed and then incubated ABC-AP reagent, that is avidin-biotin complexes (ABC) labelled with alkaline phosphatase (AP), for 30 min at RT, and developed using Vector Blue substrate. After washing, sections were blocked by sequential incubation with avidin and biotin. The sections were then stained for 1 h at RT with either rat anti-SER-4 mAb (reactive with sialoadhesin), or biotinylated rat anti-I-A^b mAb (anti-MHC class II), or hamster anti-CD11c mAb. Slides were washed and incubated with horseradish peroxidase(HRP)-labelled donkey anti-rat Ig (for 1 h), ABC-HRP reagent (for 30 min) or biotinylated goat anti-hamster Ig (for 1 h), respectively. After washing, the first two antibody combinations were developed with diaminobenzidine (DAB) substrate. The hamster anti-CD11c and biotinylated goat anti-hamster Ig combination was incubated with ABC-HRP for 30 min and then developed with DAB. After washing, all slides were mounted in Kaiser's glycerin-gelatin. Controls included sections where primary or secondary antibodies or all antibodies were omitted. Sections from control mice, not injected with HSV, were also used. At least 4 spleens from HSV-injected mice were examined.

Representative results of the immunohistochemical stainings are shown in Figure 1. The localization of the IFN-α/β positive cells were almost exclusively in the marginal zones, as described in paper III. The I-A^b (MHC class II) positive cells were found in the white pulp, with weak staining in the follicular areas and strongly stained cells in especially the PALS (Fig.1 A,B) Marginal zone cells were largely negative, except for areas near the PALS. The IFN-α/β positive cells were uniformly I-A^b negative. The CD11c positive cells were found both in the red pulp and in the PALS of the white pulp (Fig.1 C,D). The marginal zone cells were negative, except for areas near the PALS, as seen with the I-A^b staining. Almost all IFN-α/β positive cells were CD11c negative. Finally, SER-4 positive cells were abundant and almost exclusively localized in the marginal zone, except in the marginal zone areas near the PALS (Fig.1 E,F). In this way, the SER-4 and IFN-α/β positive cells co-localized. The majority of the IFN-α/β positive cells appeared to be positive for SER-4. In many cases, however, the stellate staining patterns of the SER-4 positive cells made identification of double positive cells difficult.









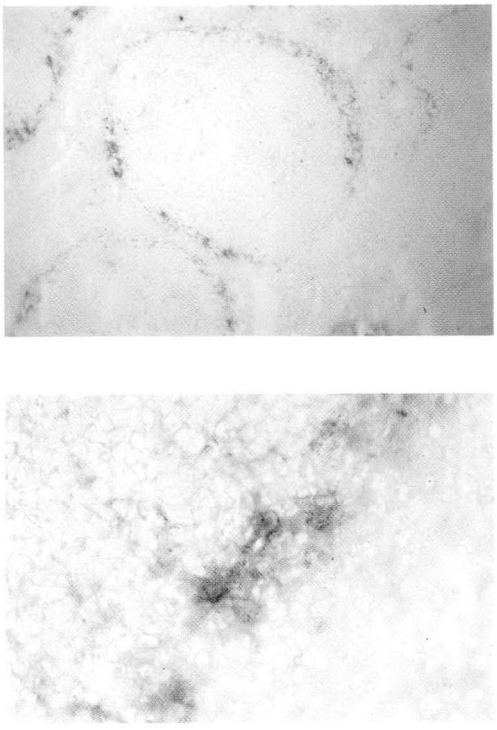


Figure 1. Immunohistochemical double staining of cells for IFN-α/β and the surface markers I-A^b (A-B), CD11c (C-D) and SER-4 (E-F) in cryosections of spleens from mice injected with uv-irradiated HSV. The IFN-α/β containing cells were stained by using alkaline phosphatase-ABC and developed with Vector Blue, while the cells expressing the surface markers were visualized with immunoperoxidase and DAB resulting in brown color. The IFN-α/β containing cells were mainly localized in the marginal zones around the white pulp and were negative for I-A^b (A-B). The great majority of IFN-α/β were negative for CD11c, which was instead strongly expressed by cells in and around the PALS (C-D). The SER-4 and IFN-α/β positive cells co-localized in the marginal zones and the majority of the IFN-α/β containing cells expressed SER-4 (E-F). Original magnifications 160x (A,C,E) and 400x (B,D,F).

Clearly, the majority of the more frequent SER-4 positive cells were still IFN-α/β negative.

The results therefore indicate that the IPCs present in the splenic marginal zones are not typical DCs, because the do not express CD11c which is considered to be present on all DCs, or the I-A antigens which are also expressed by DCs. In addition, the localization of DCs and IPCs differed. These results are somewhat unexpected considering the data obtained especially in man that indicate that the NIPC in blood might be immature DCs (Svensson et al., 1996b) or more general claims that they are DCs (Feldman et al., 1994; Ghanekar et al., 1996; Perussia et al., 1985). The reason for this discrepancy might simply be that the IPCs in man and mouse constitute different cell types. It is also possible that the IPCs present in tissues and in blood are different.

The results indicate further that the IPCs in the spleen could correspond to the metallophilic macrophages, because the two cells show the same localization and most IPCs appeared to expressed SER-4, that is sialoadhesin. The latter has been reported to be expressed only on the metallophilic macrophages and not on for instance marginal zone macrophages (Crocker & Gordon, 1989, Kraal, 1992 #214). The metallophilic macrophages have been reported to be involved in the antibody response to particulate antigens in the spleen (Buiting et al., 1996; Kraal et al., 1988). The sialoadhesin expressed on these cells may be involved in the homing of cells to the white pulp and this cell adhesion molecule can be upregulated by at least IFN-β (Van den Berg et al., 1996). It is therefore important to further elucidate the identity and function of the IPC in the marginal zones, because they may be the cellular basis of a signal system, important in cell migrations and other lymphoid organ functions.

General summary

In this thesis, the IFN-α/β producing leukocytes (IPCs) were studied in vitro and in vivo. A characterization of the IPCs in human blood was performed, as well as an analysis of the IFN-α/β response in mice in vitro and in vivo. The following results were obtained:

1. The phorbolester PMA inhibited the IFN-α/β response induced by SV in human monocytes and by HSV in NIPC. The secretion of IFN-α/β as well as levels of IFN-α/β mRNA were dramatically decreased, even at low PMA concentrations. The inhibitory effects of PMA were due to activation of PKC. At least in the monocytes, PMA decreased transcription of the IFN-α/β genes. In monocytes, the inhibitory effect of PMA on the SV-induced IFN-β gene transcription was dependent on de novo protein synthesis, while this possibility could not be investigated for the IFN-α genes and in NIPC

because the induction of transcription itself is here dependent on de novo protein synthesis. These results point to the existence of a physiological pathway for repression of IFN-α/β genes. Inhibition of IFN-α/β production may also be one mechanism by which PMA and other phorbol esters exert their well known tumor promoting effects.

2. The properties of human NIPC induced by HSV to produce IFN-α/β were compared with defined populations of leukocytes by flow cytometry and sorting. The NIPC were found to reside in a discrete population of cells with relatively high forward light scatter and low to intermediate orthogonal light scatter. They were further found not to express selected markers present on stem cells (CD34), on cells of the myeloid lineage (CD33, CD13, CD11b, CD15, CD14), or on cells of the lymphoid lineage (CD7, CD10, CD19). Together with previous data, the results suggest that the NIPC represent a unique cell population in human blood, not obviously belonging to the lymphoid or myeloid developmental lineages and distinct from NK cells and DCs. However, the possibility that they are immature DCs can not be excluded in light of more recent data.

3. Mononuclear leukocytes from lymphoid organs of mice usually produced about equal proportions of IFN-α and -β when stimulated by HSV in vitro, except for thymic cells that produced predominantly IFN-α. The IFNs were determined by newly developed specific immunoassays. The frequency of cells expressing IFN-α/β mRNA determined by in situ hybridization was generally low, highest among bone marrow cells (15 per 10^4 cells) and spleen cells (4 per 10^4 cells), but each cell produced as much as 0.4 U of IFN. In this way the IPC resembled human NIPC. When mice were injected i.v. with uv-irradiated HSV, high levels of both IFN-α and -β were seen in blood within 3 h and disappeared within 24 h. Frequent cells strongly positive for IFN-α mRNA and IFN-α/β protein were detected almost exclusively in the marginal zone of the spleens, but usually not in other organs, including lymph nodes, bone marrow, liver and kidneys. These IPCs may be the major source of IFN-α/β in the mice and the presumably very high splenic levels of IFN-α/β could have important antiviral and immunoregulatory functions.

The phenotype of the IPC in the splenic marginal zones was partially characterized. It was shown that the IPC were not typical DCs because they did not express the markers CD11c and MHC class II normally present on murine DCs. However, the IPCs co-localized with metallophilic macrophages in the marginal zones, and may possibly be identical with such cells because they expressed sialoadhesin, a cell adhesion molecule present on metallophilic macrophages.

4. When mice were injected with UV-irradiated HSV in one ear, significant levels of IFN-α/β were measured in blood. The IFN-α/β production appeared

to mainly occur in the lymph node draining the ear injected with HSV, because only this lymph node contained frequent IPCs, detected by in situ hybridization or immunohistochemical staining. In contrast, only occasional or no IPCs were detected in injected skin, spleens and contralateral lymph nodes. Further, a marked enlargement of the draining lymph node was seen within 6 h, apparently due to a nonspecific accumulation of all major lymphocyte subsets. This lymph node response could be reduced by approximately 50% by injecting neutralizing anti-IFN-α/β antibodies before HSV-injection, and was therefore at least partially due to the HSV-induced IFN-α/β production. This experimental system appears well suited for further exploration of the immunoregulatory functions of IFN-α/β and other cytokines, including their actions on cell migration.

5. In BALB/c mice infected intracerebrally by LCMV, an IFN-α but not IFN-β response was detected at the blood level. No IFN-α production was detected in the brains, but a weak and slow increase in IFN-β mRNA was seen. A stimulation of IFN-inducible genes in the infected brain was noted, which could be due to locally produced IFN-β, but also IFN-α leaking over the blood-brain barrier, or other cytokines. The low or absent IFN-α/β production in the brain could make this organ vulnerable for virus replication. An IFN-α/β production appeared to occur both in the spleen and lymph nodes, and the localizations of the IPCs were similar to that seen in mice injected with HSV. The IPCs expressed both IFN-α and -β mRNA, and many of them either contained LCMV or co-localized with LCMV-infected cells. This lymphoid organ-associated IFN-α/β production is probably responsible for the previously documented pivotal effects of the IFN-α/β system on the course of the LCMV infection.

6. The murine DC line D2SC/1 produced both IFN-α and -β when stimulated in vitro by the viruses SV and HSV and the bacteria SAC and E. coli. Priming the cells with IFN-β and GM-CSF enhanced IFN-α/β responses, except those induced by SV. The kinetics of the IFN-α/β response induced by SV was also different compared to HSV, suggesting a different induction mechanism for the SV. The D2SC/1 cells therefore resembled to some extent the human NIPC in their responses to at least HSV and bacteria. The FSDC cells differed from D2SC/1 cells because they mostly produced IFN-β, responded poorly or not to the bacteria, and was most efficiently primed by IFN-γ. The cytokines IL-4 and IL-10 did not affect IFN-α/β responses induced by HSV in the DC lines, in contrast to their inhibitory effects on normal monocytes and DCs. These DC lines should be useful in further studies of the induction, control and function of IFN-α/β responses by different microorganisms.

Acknowledgements

This work was carried out at the Division of Immunology, Department of Veterinary Microbiology, Swedish University of Agricultural Sciences. I would like to express my gratitude to professor Olof Holmberg, Dr Mats Lindahl and Dr Jorge Moreno-Lopez, chairmen of the Department of Veterinary Microbiology, for placing the resources of the Department at my disposition. The projects have been supported by grants from the Swedish Medical Research Council and the Swedish Council for Forestry and Agricultural Research.

For the contributions to this thesis I would especially like to thank:

Gunnar Alm, my supervisor, for his support, great expertise and enthusiasm about immunology. I´m also impressed about his organizing ability during really critical moments.

Caroline Fossum for sharing her knowledge about immunology, human relations and horses with me and of course for Mirre !

Kristian Sandberg, my collaborator and co-supervisor, for his bright mind and "dokument skåpet".

Paola Ricciardi-Castagnoli, Iain Campbell, Sabine Riffault, Bernard Charley, Charles Carrat, Anders Gobl, Anders Johannisson and Mats Lindahl for fruitful collaboration.

Brita Cederblad for valuable discussions and for technical assistance with some minor problems with my computer...

Carina Lindström Backeman for professional assistance, especially filling forms with the type writer among many other things.

Marie Lindersson for nice discussions and company at the lab bench as well as at the China River.

Mrs Lisbet Fuxler and Anne Riesenfeld for support, help and fun.

Katarina Haraldsson for moral support and teaching me how to handle those tiny brown creatures and always willing to help.

Anders Perers, Helena Vallin, and the newest members of the crew Kristina Domeika and Henrik Hedberg for creating a friendly atmosphere at the department.

Lars Rönnblom for being such a positive person and for good anecdotes about G.V. Alm.

Karin Artursson, Per Wallgren and Eva Wattrang for showing that immunological research is also possible to perform in pig scale.

Maria Villacres-Ericsson for collaboration and reagents.

Keiko Funa and Aive Ågren at the Ludwig Institute, for being kind and letting me use their cryostat since many years.

And many others.

The lovely Miss Minni for always chearing me up !

The past and present members of my family for encouragement and finally, Matti for everything.

References

Akerlund, K., Bjork, L., Fehniger, T., Pohl, G., Andersson, J. & Andersson, U. 1996. Sendai virus-induced IFN-alpha production analysed by immunocytochemistry and computerized image analysis. *Scand. J. Immunol. 44*, 345-53.

Artursson, K. 1993. Studies on the interferon-α/β system of pigs. Thesis. Swedish University of Agricultural Sciences.

Artursson, K., Gobl, A., Lindersson, M., Johansson, M. & Alm, G.V. 1992. Molecular cloning of a gene encoding porcine interferon-β. *J. Interferon Res. 12*, 153-60.

Artursson, K., Lindersson, M., Varela, N., Scheynius, A. & Alm, G.V. 1995. Interferon-α production and tissue localization of interferon-α/β producing cells after intradermal administration of Aujeszky´s disease virus-infected cells in pigs. *Scand. J. Immunol. 41*, 121-9.

Babu, N., Raj, K., Cheung, S., Rosztoczy, I. & Pitha, P. 1992. Mouse genotype affects inducible expression of cytokine genes. *J. Immunol. 148*, 1934-40.

Bandyopadhyay, S., Perussia, B., Trinchieri, G., Miller, D.S. & Starr, S.E. 1986. Requirement for HLA-DR+ accessory cells in natural killing of cytomegalovirus-infected fibroblasts. *J. Exp. Med. 164*, 180-95.

Belardelli, F. 1995. Role of interferons and other cytokines in the regulation of the immune response. *APMIS. 103*, 161-79.

Belardelli, F. & Gresser, I. 1996. The neglected role of type I interferon in the T-cell response: implications for its clinical use. *Immunol. Today. 17*, 369-71.

Bhuiya, T.A., Shodell, M., Fitzgerald-Bocarsly, P.A., Murasko, D., Shah, K., Drake, D. & Siegal, F.P. 1994. Interferon-α generation in mice responding to challenge with UV-inactivated Herpes simplex virus. *J. Interferon Res. 14*, 17-24.

Biron, C.A. 1997. Activation and function of natural killer cell responses during viral infections. *Curr. Opin. Immunol. 9*, 24-34.

Boutwell, R.K. 1974. The function and mechanism of promoters of carcinogenesis. *CRC. Crit. Rev. Toxicol. 2*, 419-43.

Boutwell, R.K. 1985. Tumor promoters in human carcinogenesis. In *Important Advances in Oncology* (ed. V.T. DeVita, Jr.), 16-27. Philadelphia:Lippincott.

Brucher, J., Domke, I., Schröder, H. & Kirchner, H. 1984. Experimental infection of inbred mice with Herpes simplex virus. *Arch. Virol. 82*, 83-93.

Buchmeier, M.J., Welsh, R.M., Dutko, F.J. & Oldstone, B.A. 1980. The virology and immunobiology of Lymphocytic choriomeningitis virus infection. *Adv. Immunol. 30*, 275-331.

Buiting, A.M.J., De Rover, Z., Kraal, G. & Van Rooijen, N. 1996. Humoral immune responses against particulate bacterial antigens are dependent on marginal metallophilic macrophages in the spleen. *Scand. J. Immunol. 43*, 398-405.

Campos, M., Godson, D.L., Hughes, H.P.A., Sordillo, L.M. & Babiuk, L.A. (eds.). 1992. Interferon research in veterinary medicine. Galveston, TX: The University of Texas Medical Branch at Galveston, Department of Microbiology.

Capobianchi, M.R., Ankel, H., Ameglio, F., Paganelli, R., Pizzoli, P.M. & Dianzani, F. 1992. Recombinant glycoprotein 120 of human immunodeficiency virus is a potent interferon inducer. *AIDS. Res. Hum. Retroviruses 8*, 575-9.

Cederblad, B. & Alm, G.V. 1990. Infrequent but highly efficient interferon-α producing human mononuclear leukocytes induced by herpes simplex virus in vitro studied by immuno-plaque and limiting dilution assays. *J. Interferon Res. 10*, 65-73.

Cederblad, B. & Alm, G.V. 1991. Interferons and the colony-stimulating factors IL-3 and GM-CSF enhance the IFN-α response in human blood leucocytes induced by Herpes simplex virus. *Scand. J. Immunol. 34*, 549-55.

Cederblad, B., Gobl, A.E. & Alm, G.V. 1991. The induction of interferon-alpha and interferon-beta mRNA in human natural interferon-producing blood leukocytes requires de novo protein synthesis. *J. Interferon Res. 11*, 371-7.

Cella, M., Sallusto, F. & Lanzavecchia, A. 1997. Origin, maturation and antigen presenting function of dendritic cells. *Curr. Opin. Immunol. 9*, 10-6.

Charley, B. & Lavenant, L. 1990. Characterization of blood mononuclear cells producing IFN-α following induction by coronavirus-infected cells (transmissible porcine gastroenteritis virus). *Res. Immunol. 141*, 1-11.

Chehimi, J., Starr, S.E., Kawashima, H., Miller, D.S., Trinchieri, G., Perussia, B. & Bandyopadhyay, S. 1989. Dendritic cells and IFN-α-producing cells are two functionally distinct non-B, non-monocytic HLA-DR+ cell subsets in human peripheral blood. *Immunology 68*, 486-90.

Clavell, L.A. & Bratt, M.A. 1971. Relationship between the ribonucleic acid-synthesizing capacity of ultraviolet-irradiated Newcastle disease virus and its ability to induce interferon. *J. Virol. 8*, 500-8.

Cleary, C.M., Donnelly, R.J., Soh, J., Mariano, T.M. & Pestka, S. 1994. Knockout and reconstitution of a functional human type I interferon receptor complex. *J. Biol. Chem. 269*, 18747-9.

Cousens, L.P., Orange, J.S., Su, H.S. & Biron, C.H. 1997. Interferon α/β inhibition of interleukin 12 and interferon-γ production in vitro and endogenously during viral infection. *Proc. Natl. Acad. Sci. USA. 94*, 634-9.

Crocker, P. & Gordon, S. 1989. Mouse macrophage hemagglutinin (sheep erythrocyte receptor) with specificity for sialylated glycoconjugates characterized by a monoclonal antibody. *J. Exp. Med. 169*, 1333-46.

D'Cunha, J., Knight, E., Jr., Haas, A.L., Truitt, R.L. & Borden, E.C. 1996a. Immunoregulatory properties of ISG15, an interferon-induced cytokine. *Proc. Natl. Acad. Sci. U S A. 93*, 211-5.

D'Cunha, J., Ramanujam, S., Wagner, R.J., Witt, P.L., Knight, E., Jr. & Borden, E.C. 1996b. In vitro and in vivo secretion of human ISG15, an IFN-induced immunomodulatory cytokine. *J. Immunol. 157*, 4100-8.

Davis, P.D., Hill, C.H., Keech, E., Lawton, G., Nixon, J.S., Sedgwick, A.D., Wadsworth, J., Westmacott, D. & Wilkinson, S.E. 1989. Potent selective inhibitors of protein kinase C. *FEBS Lett. 259*, 61-3.

De Maeyer, E. & De Maeyer-Guignard, J. Interferons and other regulatory cytokines. John Wiley & Sons: New York, 1988.

De Maeyer Guignard, J., Marcucci, F. & De Maeyer, E. 1988. Identification by in situ hybridization of IFN-beta-producing murine macrophages obtained from high and low interferon producers. *Ann. Inst. Pasteur Virol. 139*, 51-7.

Dehlin, E., von Gabain, A., Alm, G., Dingelmaier, R. & Resnekov, O. 1996. Repression of beta interferon gene expression in virus-infected cells is correlated with a poly(A) tail elongation. *Mol. Cell. Biol. 16*, 468-74.

Derynck, R., Content, J., Declercq, E., Volkaert, G., Tavernier, J., Devos, R. & Fiers, W. 1980. Isolation and structure of a human fibroblast interferon gene. *Nature 285*, 542-7.

Diaz, M.O., Bohlander, S. & Allen, G. 1996. Nomenclature of the human interferon genes. *J. Interferon Cytokine Res. 16*, 179-80.

Dron, M. & Tovey, M.G. 1992. Interferon-α/β, gene structure and regulation. In *Interferon: Principles and medical applications* (ed. S. Baron, D. H. Coppenhaver, F. Dianzani, W. R. Fleischmann Jr, T. K. Hughes Jr, G. R. Klimpel, D. W. Niesel, G. J. Stanton & S. K. Tyring), 33-45. Galveston, TX: The University of Texas Medical Branch at Galveston.

Durbin, J.E., Hackenmiller, R., Simon, M.C. & Levy, D.E. 1996. Targeted disruption of the mouse Stat1 gene results in compromised innate immunity to viral disease. *Cell 84*, 443-50.

Evans, S.S., Collea, R.P., Appenheimer, M.M. & Gollnick, S.O. 1993. Interferon-α induces the expression of the L-Selectin homing receptor in human B lymphoid cells. *J. Cell. Biol. 123*, 1889-98.

Feldman, M. & Fitzgerald-Bocarsly, P. 1990. Sequential enrichment and immunocytochemical visualization of human interferon-α-producing cells. *J. Interferon Res. 10*, 435-46.

Feldman, S.B., Ferraro, M., Zheng, H.-M., Patel, N., Gould-Fogerite, S. & Fitzgerald-Bocarsly, P. 1994. Viral induction of low frequency interferon-α producing cells. *Virology 204*, 1-7.

Feldman, S.B., Milone, M.C., Kloser, P. & Fitzgerald Bocarsly, P. 1995. Functional deficiencies in two distinct interferon alpha-producing cell populations in peripheral blood mononuclear cells from human immunodeficiency virus seropositive patients. *J. Leukoc. Biol. 57*, 214-20.

Ferbas, J.J., Toso, J.F., Logar, A.J., Navratil, J.S. & Rinaldo Jr, C.R. 1994. CD4+ blood dendritic cells are potent producers of IFN-α in response to in vitro HIV-1 infection. *J. Immunol. 152*, 4649-62.

Fiette, L., Aubert, C., Müller, U., Huang, S., Aguet, M., Brahic, M. & Bureau, M.B. 1995. Theiler's virus infection of 129Sv mice that lack the interferon α/β or γ receptors. *J. Exp. Med. 181*, 2069-76.

Finkelman, F.D., Svetic, A., Gresser, I., Snapper, C., Holmes, J., Trotta, P.P., Katona, I.M. & Gause, W.C. 1991. Regulation by interferon α of immunoglobulin isotype selection and lymphokine production in mice. *J. Exp. Med. 174*, 1179-88.

Fitzgerald-Bocarsly, P. 1993. Human natural interferon-α producing cells. *Pharmac. Ther. 60*, 39-62.

Funa, K., Ramstedt, U., Rönnblom, L. & Alm, G.V. 1985. Human peripheral blood null lymphocytes stimulated by Staphylococcus aureus Cowan I produce atypical acid-labile interferon in vitro. *Scand. J. Immunol. 21*, 55-62.

Ghanekar, S., Zheng, L., Logar, A., Navratil, J., Borowski, L., Gupta, P. & Rinaldo, C. 1996. Cytokine expression by human peripheral blood dendritic cells stimulated in vitro with HIV-1 and herpes simplex virus. *J. Immunol. 157*, 4028-36.

Girolomoni, G., Lutz, M.B., Pastore, S., Abmann, C.U., Cavani, A. & Ricciardi-Castagnoli, P. 1995. Establishment of a cell line with features of early dendritic cell precursors from fetal mouse skin. *Eur. J. Immunol. 25*, 2163-9.

Gobl, A.E. & Alm, G.V. 1992. Interleukin-4 down-regulates Sendai virus-induced production of interferon-α and -β in human peripheral blood monocytes. *Scand. J. Immunol. 35*, 167-75.

Gobl, A.E., Cederblad, B., Sandberg, K. & Alm, G.V. 1992. Interferon-alpha but not -beta genes require de novo protein synthesis for efficient expression in human monocytes. *Scand . J. Immunol. 35*, 177-85.

Gobl, A.E., Funa, K. & Alm, G.V. 1988. Different induction patterns of mRNA for IFN-α and IFN-β in human mononuclear leukocytes after in vitro stimulation with Herpes simplex virus-infected fibroblasts and Sendai virus. *J. Immunol. 10*, 3605-9.

Granucci, F., Girolomoni, G., Lutz, M.B., Foti, M., Marconi, G., Gnocci, P., Nolli, L. & Ricciardi-Castagnoli, P. 1994. Modulation of cytokine expression in mouse dendritic cell clones. *Eur. J. Immunol. 24*, 2522-6.

Gray, D. 1996. Viral immunity: interferons jog old T-cell memories. *Curr. Biol. 6*, 1254-5.

Gresser, I. 1984. Role of interferon in resistance to viral infection in vivo. In *Interferons and the immune system* (ed. J. Vilcek and E. De Maeyer), 222-47. Amsterdam: Elsevier.

Gresser, I., Guy-Grand, D., Maury, C. & Maunoury, M.-T. 1981. Interferon induces peripheral lymphadenopathy in mice. *J. Immunol. 127*, 1569-75.

Hallum, J.V., Younger, J.S. & Stinebring, W.R. 1965. Interferon activity associated with high molecular weight proteins in the circulation of mice injected with endotoxin or bacteria. *Virology 27*, 429-31.

Harada, H., Fujita, T., Miyamoto, M., Kimura, Y., Maruyama, M., Furia, A., Miyata, T. & Taniguchi, T. 1989. Structurally similar but functionally distinct factors, IRF-1 and IRF-2, bind to the same regulatory elements of IFN and IFN-inducible genes. *Cell 58*, 729-39.

Hein, W.R. & Supersaxo, A. 1988. Effect of interferon-alpha-2a on the output of recirculation lymphocytes from single lymph node. *Immunology 64*, 469-74.

Heufler, C., Koch, F., Stanzl, U., Topar, G., Wysocka, M., Trinchieri, G., Enk, A., Steinmann, R.M., Romani, N. & Schuler, G. 1996. Interleukin-12 is produced by dendritic cells and mediates T helper 1 development as well as interferon-γ production by T helper 1 cells. *Eur. J. Immunol. 26*, 659-68.

Higashi, Y., Sokawa, Y., Watanabe, Y., Kawade, Y., Ohno, S., Takaoka, C. & Taniguchi, T. 1983. Structure and expression of a cloned cDNA for mouse interferon-beta. *J. Biol. Chem. 258*, 9522-9.

Hiscott, J., Nguyen, H. & Lin, R. 1995. Molecular mechanisms of interferon beta gene induction. *Semin. Virol. 6*, 161-73.

Ho, M. 1984. Induction and inducers of interferon. In *Interferon 1: General and applied aspects* (ed. A. Billiau), 79-124. Amsterdam: Elsevier.

Hughes, A.L. 1995. The evolution of type I interferon gene family in mammals. *J. Mol. Evol. 41*, 539-48.

Humphrey, J.H. & Grennan, D. 1981. Different macrophage populations distinguished by means of fluorescent polysaccharides. Recognition and properties of marginal-zone macrophages. *Eur. J. Immunol. 11*, 221-8.

Isaacs, A. & Lindenmann, J. 1957. Virus interference. I. The interferon. *Proc. R. Soc. B. 147*, 258-67.

Isaacs, A., Lindenmann, J. & Valentine, R.C. 1957. Virus interference. II some properties of interferon. *Proc. R. Soc. B. 147*, 268-73.

Ishikawa, R. & Biron, C.A. 1993. IFN induction and associated changes in splenic leukocyte distribution. *J. Immunol. 150*, 3713-27.

Ito, Y., Nishiyama, Y., Shimokata, K., Nagata, J., Takeyama, H. & Kunii, A. 1978a. The mechanism of interferon induction in mouse spleen cells stimulated with HVJ. *Virology 88*, 128-37.

Ito, Y., Nishiyama, Y., Shimokata, K., Takeyama, H. & Kunii, A. 1978b. Active component of HVJ (Sendai virus) for interferon induction in mice. *Nature 274*, 801-2.

Jestin, V. & Cherbonnel, M. 1991. Interferon-induction in mouse spleen cells by the Newcastle disease virus (NDV) HN protein. *Ann. Rech. Vet. 22*, 365-72.

Kaibuchi, K., Takai, Y., Sawamura, M., Hoshijima, M., Fujikura, T. & Nishizuka, Y. 1983. Synergistic functions of protein phosphorylation and calcium mobilization in platelet activation. *J. Biol. Chem. 258*, 6701-4.

Kalaaji, A.N., Abernethy, N.J., McCullough, K. & Hay, J.B. 1988. Recombinant bovine interferon-α inhibits the migrations of lymphocytes from lymph nodes but not into lymph nodes. *Reg. Immunol. 1*, 56-61.

Ke, Y.H., Ho, M. & Merigan, T.C. 1966. Heterogeneity of rabbit serum interferons. *Nature 211*, 541-2.

Kierszenbaum, F. & Sonnenfeld, G. 1982. Characterization of the antiviral activity produced during Trypanosoma cruzi infection and protective effects of exogenous interferon against experimental Chagas' disease. *J. Parasitol. 68*, 194-8.

Kikkawa, U., Kishimoto, A. & Nishizuka, Y. 1989. The protein kinase C family: heterogeneity and its implications. *Annu. Rev. Biochem. 58*, 31-44.

Kingsbury, D.W. 1990. Paramyxoviridae and their replication. In *Fields Virology* (ed. B. Fields and D. Knipe), 945-62. New York: Raven Press.

Kirchner, H., Engler, H., Schröder, C.H., Zawatsky, R. & Storch, E. 1983. Herpes Simplex Virus Type 1-induced Interferon Production and Activation of Natural Killer Cells in Mice. *J.gen.Virol. 64*, 437-41.

Kirchner, H., Keyssner, K., Zawatsky, R. & Hilfenhaus, J. 1980. Studies of the producer cell of Herpes simplex virus induced interferon in mouse spleen cell cultures. *Immunobiol. 157*, 401-6.

Knight, E., Jr., Hunkapiller, M.W., Korant, B.D., Hardy, R.W. & Hood, L.E. 1980. Human fibroblast interferon: amino acid analysis and amino terminal amino acid sequence. *Science 207*, 525-6.

Koch, F., Stanzl, U., Jannewein, P., Janke, K., Heufler, C., Kämpgen, E., Romani, N. & Schuler, G. 1996. High level IL-12 production by murine dendritic cells : upregulation via MHC class II and CD40 molecules and downregulation by IL-4 and IL-10. *J. Exp. Med. 184*, 741-6.

Kraal, G. 1992. Cells in the marginal zone of the spleen. *Int. Rev. Cytol. 132*, 31-74.

Kraal, G., Janse, M. & Claassen, E. 1988. Marginal metallophilic macrophages in the mouse spleen: effects of neonatal injections of MOMA-1 antibody on the humoral immune response. *Immunol. Letters 17*, 139-44.

Laude, H., Gelfi, J., Lavenant, L. & Charley, B. 1992. Single amino acid changes in the viral glycoprotein M affect induction of alpha interferon by the coronavirus transmissible gastroenteritis virus. *J. Virol. 66*, 743-9.

Leaman, D.W., Cross, J.C. & Roberts, R.M. 1992. Genes for the trophoblast interferons and their distribution among mammals. *Reprod. Fertil. Dev. 4*, 349-53.

Lebon, P. 1985. Inhibition of herpes simplex virus type 1-induced interferon synthesis by monoclonal antibodies against viral glycoprotein D and by lysosomotropic drugs. *J gen. Virol. 66*, 2781-6.

Lebon, P., Bernard, A. & Boumsell, L. 1982. Identification of lymphocyte populations producing alpha-interferon by monoclonal antibodies. *C. R. Seances Acad. Sci. III. 295*, 79-82.

Lebon, P., Commoy Chevalier, M.J., Robert Galliot, B., Morin, A. & Chany, C. 1980. [Production of human type I interferon by lymphocytes in contact with cells infected by herpesvirus and fixed with glutaraldehyde]. *C R Seances Acad Sci D. 290*, 37-40.

Lefevre, F. & Boulay, V. 1993. A novel and atypical type one interferon gene expressed by trophoblast during early pregnancy. *J. Biol. Chem. 268*, 19760-8.

Leung, D.W., Capon, D.J. & Goeddel, D.V. 1984. The structure and bacterial expression of three distinct bovine interferon beta genes. *Biotechnology 2*, 458-.

Levy, D.E. 1995. Interferon induction of gene expression through the Jak-Stat pathway. *Semin. Virol. 6*, 181-9.

Levy, D.E., Kessler, D.S., Pine, R. & Darnell, J.E., Jr. 1989. Cytoplasmic activation of ISGF3, the positive regulator of interferon-alpha-stimulated transcription, reconstituted in vitro. *Genes Dev. 3*, 1362-71.

Levy, D.E., Kessler, D.S., Pine, R., Reich, N. & Darnell, J.E., Jr. 1988. Interferon-induced nuclear factors that bind a shared promoter element correlate with positive and negative transcriptional control. *Genes Dev. 2*, 383-93.

Levy, H.B. & Salazar, A.M. 1992 Interferon inducers. In *Interferon: Principles and medical applications* (ed. S. Baron, D. H. Coppenhaver, F. Dianzani, W. R. Fleischmann Jr, T. K. Hughes Jr, G. R. Klimpel, D. W. Niesel, G. J. Stanton & S. K. Tyring), 65-76. Galveston, TX: The University of Texas Medical Branch at Galveston.

Li, Q., Feldman, M., Harmon, C. & Fitzgerald-Bocarsly, P. 1996. Role of tyrosine kinases, protein kinase C, and protein kinase A in the regulation of interferon-alpha production induced by herpes simplex virus type 1. *J. Interferon Cytokine. Res. 16*, 109-18.

Lutz, M.B., Abmann, C.U., Girolomoni, G. & Ricciardi-Castagnoli, P. 1996. Different cytokines regulate antigen uptake and presentation of a precursor dendritic cell line. *Eur. J. Immunol. 26*, 586-94.

Lutz, M.B., Granucci, F., Winzler, C., Marconi, G., Paglia, P., Foti, M., Abmann, C.U., Cairns, L., Rescigno, M. & Ricciardi-Castagnoli, P. 1994. Retroviral immortalization of phagocytic and dendritic cell clones as a tool to investigate functional heterogeneity. *J. Immunol. Methods 174*, 269-79.

Lyons, A.B. & Parish, C.R. 1995. Are murine marginal-zone macrophages the splenic white pulp analogue of high endothelial venules ? *Eur. J. Immunol. 25*, 3165-72.

Meraz, M.A., White, J.M., Sheehan, K.C., Bach, E.A., Rodig, S.J., Dighe, A.S. et al. 1996. Targeted disruption of the Stat1 gene in mice reveals unexpected physiologic specificity in the JAK-STAT signalling pathway. *Cell 84*, 431-42.

Metlay, J.P., Witmer-Pack, M.D., Agget, R., Crowley, M.T., Lawles, D. & Steinmann, R.M. 1990. The distinct leukocyte integrins of mouse spleen dendritic cells as identified with new hamster monoclonal antibodies. *J. Exp. Med. 171*, 1753-71.

Milone, M.C. & Fitzgerald-Bocarsly, P. 1996. The macrophage mannose receptor plays a role in the induction of interferon-α synthesis by natural interferon-α producing cells. *J. Interferon Cytokine Res. 7*, 613.

Moskophidis, D., Battegay, M., Bruendler, M.-A., Laine, E., Gresser, I. & Zinkernagel, R.M. 1994. Resistance of lymphocytic choriomeningitis virus to alpha/beta interferon and to gamma interferon. *J. Virol. 68*, 1951-5.

Mufson, R.A., Myers, C., Turpin, J.A. & Meltzer, M. 1992. Phorbol ester reduces constitutive nuclear NF kappa B and inhibits HIV-1 production in mature human monocytic cells. *J. Leukoc. Biol. 52*, 637-44.

Müller, U., Steinhoff, U., Reis, L., Hemmi, S., Pavlovic, J., Zinkernagel, R.M. & Aguet, M. 1994. Functional role of type I and type II interferons in antiviral defence. *Science 264*, 1918-21.

Nagata, S., Taira, H., Hall, A., Johnsrud, L., Streuli, M., Ecsodi, J., Boll, W., Cantell, K. & Weissmann, C. 1980. Synthesis in E. coli of a polypeptide with human leukocyte interferon activity. *Nature 284*, 316-20.

Nakajima, H., Nakao, A., Watanabe, Y., Yoshida, S. & Iwamoto, I. 1994. IFN-alpha inhibits antigen-induced eosinophil and CD4+ T cell recruitment into tissue. *J Immunol. 153*, 1264-70.

Nash, A.A. & Cambouropoulus, P. 1993. The immune response to herpes simplex virus. *Semin. Virol. 4*, 181-6.

Nash, A.A., Quartey-Papafio, R. & Wildy, P. 1980. Cell-mediated immunity in Herpes simplex virus-infected mice: Functional analysis of lymph node cells during periods of acute and latent infection, with reference to cytotoxic and memory cells. *J. gen. Virol. 49*, 309-17.

Nickolaus, P. & Zawatsky, R. 1994. Inhibition by Interleukin-4 of constitutive beta interferon synthesis in mouse macrophages. *J. Virol. 68*, 6763-6.

Niesel, D.W. & Klimpel, G.R. 1992. Interferon as a mediator of host defence against bacteria. In *Interferon: Principles and medical applications* (ed. S. Baron, D. H. Coppenhaver, F. Dianzani, W. R. Fleischmann Jr, T. K. Hughes Jr, G. R. Klimpel, D. W. Niesel, G. J. Stanton & S. K. Tyring), 311-328. Galveston, TX: The University of Texas Medical Branch at Galveston.

Nishizuka, Y. 1984. The role of protein kinase C in cell surface signal transduction and tumour promotion. *Nature 308*, 693-7.

Novick, D., Cohen, B. & Rubinstein, M. 1994. The human interferon alpha/beta receptor: characterization and molecular cloning. *Cell 77*, 391-400.

Nowacki, W. & Charley, B. 1993. Enrichment of coronavirus-induced interferon-producing blood leukocytes increases the interferon yield per cell: a study with pig leukocytes. *Res. Immunol. 144*, 111-20.

Ojo Amaize, E.A., Salimonu, L.S., Williams, A.I., Akinwolere, O.A., Shabo, R., Alm, G.V. & Wigzell, H. 1981. Positive correlation between degree of parasitemia, interferon titers, and natural killer cell activity in Plasmodium falciparum-infected children. *J. Immunol. 127*, 2296-300.

Paglia, P., Girolomoni, G., Robbiati, F., Granucci, F. & Ricciardi-Castagnoli, P. 1993. Immortalized dendritic cell line fully competent in antigen presentation initiates primary T cell responses in vivo. *J. Exp. Med. 178*, 1893-901.

Pavlovic, J., Schroder, A., Blank, A., Pitossi, F. & Staeheli, P. 1993. Mx proteins: GTPases involved in the interferon-induced antiviral state. *Ciba Found. Symp. 176*, 233-43.

Pavlovic, J. & Staeheli, P. 1991. The antiviral potentials of Mx proteins. *J. Interferon Res. 11*, 215-9.

Payvandi, F. & Fitzgerald-Bocarsly, P. 1995. IL-10 inhibits production of IFN-α by PBMC induced with Herpes simplex virus or Sendai virus. *J. Interferon Res. 15*, 165.

Perussia, B., Fanning, V. & Trinchieri, G. 1985. A leukocyte subset bearing HLA-DR antigens is responsible for in vitro alpha interferon production in response to viruses. *Nat. Immun. Cell Growth Regul. 4*, 120-37.

Peters, J.H., Gieseler, R., Thiele, B. & Steinbach, F. 1996. Dendritic cells: from ontogenetic orphans to myelomonocytic descendants. *Immunol. Today. 17*, 273-7.

Pfau, C.J., Gresser, I. & Hunt, K.D. 1983. Lethal role of interferon in lymphocytic choriomeningitis virus-induced encephalitis. *J. gen. Virol. 64*, 1827-30.

Pfau, C.J., Valenti, J.K., Jacobsen, S. & Pevear, D.C. 1982. Cytotoxic T cells are induced in mice infected with Lymphocytic choriomeningitis virus strains of markedly different pathogenicities. *Infection Immunity 36*, 598-602.

Pitha, P.M. & Au, W.-C. 1995. Induction of interferon alpha genes expression. *Semin. Virol. 6*, 151-9.

Rawls, W.E. 1985. Herpes simplex virus. In *Virology* (ed. B. N. Fields), 527-61. New York: Raven Press.

Recht, M., Borden, E.C. & Knight, E., Jr. 1991. A human 15-kDa IFN-induced protein induces the secretion of IFN-gamma. *J Immunol. 147*, 2617-23.

Reis, L.F., Ruffner, H., Stark, G., Aguet, M. & Weissmann, C. 1994. Mice devoid of interferon regulatory factor 1 (IRF-1) show normal expression of type I interferon genes. *Embo J. 13*, 4798-806.

Riffault, S. 1997. Induction virale d'interferon-α/β: Etude *in vivo* des cellules productrices dans les tissus lymphoides et des modifications localement induites par l'interféron. Thesis. Institut National Agronomique Paris-Grignon.

Roberts, R.M., Cross, J.C. & Leaman, D.W. 1991. Unique features of the trophoblast interferons. *Pharmacol. Ther. 51*, 329-45.

Rogge, L., Barberis-Maino, L., Biffi, M., Passini, N., Presky, D.H., Gubler, U. & Sinigaglia, F. 1997. Selective expression of an interleukin-12 receptor component by human T helper 1 cells. *J. Exp. Med. 5*, 825-31.

Romagnani, S. 1992. Induction of TH1 and TH2 responses: a key role for the "natural" immune response? *Immunol. Today. 13*, 379-81.

Romagnani, S. 1996. Understanding the role of Th1/Th2 cells in infection. *Trends Microbiol. 4*, 470-3.

Rosen, S.D. & Bertozzi, C.R. 1994. The selectins and their ligands. *Curr. Opin. Cell Biol. 6*, 663-73.

Rosztoczy, I. & Pitha, P.P. 1993. Priming does not change promoter sequence requirements for IFN induction or correlate with the expression of IFN regulatory factor-1. *J. Immunol. 151*, 1303-11.

Ruffner, H., Reis, L.F., Naf, D. & Weissmann, C. 1993. Induction of type I interferon genes and interferon-inducible genes in embryonal stem cells devoid of interferon regulatory factor 1. *Proc. Natl. Acad. Sci. U S A. 90*, 11503-7.

Rönnblom, L., Cederblad, B., Sandberg, K. & Alm, G.V. 1988. Determination of Herpes simplex virus-induced alpha interferon-secreting human blood leucocytes by a filter immuno-plaque assay. *Scand. J. Immunol. 27*, 165-70.

Rönnblom, L., Forsgren, A. & Alm, G.V. 1983a. Characterization of interferons induced by bacteria and interferon-producing leukocytes in human peripheral blood. *Infection Immunity 40*, 126-32.

Rönnblom, L., Ojo-Amaize, E.A., Franzén, L., Wigzell, H. & Alm, G.V. 1983b. Plasmodium falsiparum parasites induce interferon production in human peripheral blood "null" cells in vitro. *Parasite Immunol. 5*, 165-72.

Rönnblom, L., Ramstedt, U. & Alm, G.V. 1983c. Properties of human natural interferon-producing cells stimulated by tumour cell lines. *Eur. J. Immunol. 13*, 471-6.

Saksela, E., Virtanen, I., Hovi, T., Secher, D.S. & Cantell, K. 1984. Monocyte is the main producer of human alpha interferons following Sendai virus induction. *Prog. Med. Virol. 30*, 78.

Salazar-Mather, T.P., Ishikawa, R. & Biron, C.A. 1996. NK cell trafficking and cytokine expression in splenic compartments after IFN induction and viral infections. *J. Immunol. 157*, 3054-64.

Sandberg, K., Gobl, A., Funa, K. & Alm, G.V. 1989. Characterization of the blood mononuclear leucocytes producing alpha interferon after stimulation with Herpes simplex virus in vitro by means of combined immunohistochemical staining and in situ RNA-RNA hybridization. *Scand. J. Immunol. 29*, 651-8.

Sandberg, K., Kemper, P., Stalder, A., Zhang, J., Hobbs, M.V., Whitton, L.J. & Campbell, I.L. 1994. Altered tissue distribution of viral replication and T cell spreading is pivotal in the protection against fatal lymphocytic choriomeningitis in mice after neutralization of IFN-α/β. *J. Immunol. 153*, 220-31.

Sandberg, K., Matsson, P. & Alm, G.V. 1990. A distinct population of nonphagocytic and low level CD4+ null lymphocytes produce IFN-α after stimulation by Herpes simplex virus-infected cells. *J. Immunol. 145*, 1015-20.

Sassano, M., Granucci, F., Seveso, M., Marconi, G., Foti, M. & Ricciardi-Castagnoli, P. 1994. Molecular cloning of a recombinant retrovirus carrying a mutated env^{AKR}-myc^{MH2} fusion gene immortalizing cells of the monocytic-macrophage lineage. *Oncogene 9*, 1473-7.

Schattner, A., Meshorer, A. & Wallach, D. 1983. Involvement of interferon in virus-induced lymphopenia. *Cell. Immunol. 79*, 11-25.

Sekellick, M.J., Ferrandino, A.F., Hopkins, D.A. & Marcus, P.I. 1994. Chicken interferon gene: cloning, expression, and analysis. *J. Interferon Res. 14*, 71-9.

Sen, G.C. & Ransohoff, R.M. 1993. Interferon-induced antiviral actions and their regulation. *Adv. Virus Res. 42*, 57-102.

Shoshkes Reiss, C., Liu, L.L.M. & Mowshowitz, S.L. 1984. Interferon production by cultured murine splenocytes in response to Influenza virus-infected cells. *J. Interferon. Res. 4*, 81-9.

Sonehara, K., Saito, H., Kuramoto, E., Yamamoto, S., Yamamoto, T. & Tokunaga, T. 1996. Hexamer palindromic oligonucleotides with 5´-CG-3´motif(s) induce production of interferon. *J. Interferon Cytokine Res. 16*, 799-803.

Springer, T.A. 1995. Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration. *Annu. Rev. Physiol. 57*, 827-72.

Staeheli, P. 1990. Interferon-induced proteins and the antiviral state. *Adv. Virus Res. 38*, 147-200.

Steinman, R.M. 1991. The dendritic cell system and its role in immunogenicity. *Annu. Rev. Immunol. 9*, 271-96.

Stewart, W.E. 1979. Varied biologic effects of interferon. In *Interferon1* (ed. I. Gresser), 29-51. London: Academic Press.

Stewart, W.E. 1980. Interferon nomenclature. *Nature 286*, 110.

Svensson, H., Cederblad, B., Lindahl, M. & Alm, G.V. 1996a. Stimulation of natural interferon-α/β producing cells by Staphylococcus aureus. *J. Interferon Res. 16*, 7-16.

Svensson, H., Johannisson, A., Nikkilä, T., Alm, G.V. & Cederblad, B. 1996b. The cell surface phenotype of human natural interferon-α producing cells as determined by flow cytometry. *Scand. J. Immunol. 44*, 164-72.

Szabo, S.J., Dighe, A., Gubler, U. & Murphy, K.M. 1997. Regulation of the interleukin (IL)-12Rb2 subunit expression in developing T helper 1 (Th1) and Th2 cells. *J. Exp. Med. 185*, 817-24.

Taira, H., Broeze, R.J., Jayaram, B.M., Lengyel, P., Hunkapiller, M.W. & Hood, L.E. 1980. Mouse interferons: amino terminal amino acid sequences of various species. *Science 207*, 528-30.

Tamai, T., Shirahata, S., Noguchi, T., Sato, N., Kimura, S. & Murakami, H. 1993. Cloning and expression of flatfish (Paralichthys olivaceus) interferon cDNA. *Biochim. Biophys. Acta 1174*, 182-6.

Taniguchi, T., Ohno, S., Fujii-Kuriyama, Y. & Muramatsu, M. 1980. The nucleotide sequence of human fibroblast interferon cDNA. *Gene. 10*, 11-5.

Tough, D.F., Borrow, P. & Sprent, J. 1996. Induction of bystander T cell proliferation by viruses and type I interferon in vivo. *Science 272*, 1947-50.

Uzé, G., Lutfalla, G. & Mogensen, K.E. 1995. α and β interferons and their receptors and their friends and relations. *J. Interferon Cytokine Res. 15*, 3-26.

Van den Berg, T.K., Brevé, J.J.P., Damoiseaux, J.G.M.C., Döpp, E.A., Kelm, S. & Crocker, P.R. 1992. Sialoadhesin on macrophages. Its identification as a lymphocyte adhesion molecule. *J. Exp. Med. 176*, 647-55.

Van den Berg, T.K., van Die, I., de Lavalette, C.R., Döpp, E.A., Smit, L.D., van der Meide, P.H., Tilders, F.J.H., Crocker, P.R. & Dijkstra, C.D. 1996. Regulation of sialoadhesin expression on rat macrophages. Induction by glucocorticoids and enhancement by IFN-β, IFN-γ, IL-4 and lipopolysaccharide. *J. Immunol. 157*, 3130-8.

Van den Broek, M.F., Müller, U., Huang, S., Aguet, M. & Zinkernagel, R.M. 1995. Antiviral defence in mice lacking both alpha/beta and gamma interferon receptors. *J. Virol. 69*, 4792-6.

Wattrang, E., Wallgren, P., Fuxler, B., Lindersson, M. & Fossum, C. 1997. Tissue chambers - a useful model for in vivo studies of cytokine production in the pig. *Vet. Immunol. Immunopathol.* In press.

Weissmann, C. & Weber, H. 1986. The interferon genes. *Prog. Nucleic Acid Res. Mol. Biol. 33*, 251-300.

Wenner, A.C., Güler, M.L., Macatonia, S.E., O'Garra, A. & Murphy, K.M. 1996. Roles of IFN-γ and IFN-α in IL-12 induced T helper cell-1 development. *J. Immunol. 156*, 1442-7.

Wheelock, E.F. 1965. Interferon-like virus-inhibitor induced in human leukocytes by phytohemagglutinin. *Science 149*, 310-1.

Williams, B.R.G. 1995. The role of the dsRNA-activated kinase, PKR, in signal transduction. *Semin. Virol. 6*, 191-202.

Yamamoto, S., Yamamoto, T., Kataoka, T., Kuramoto, E., Yano, O. & Tokunaga, T. 1992. Unique palindromic sequences in synthetic oligonucleotides are required to induce IFN and augment IFN-mediated natural killer activity. *J.Immunol. 148*, 4072-6.

Zoon, K.C., Smith, M.E., Bridgen, P.J., Anfinsen, C.B., Hunkapiller, M.W. & Hood, L.E. 1980. Amino terminal sequence of the major component of human lymphoblastoid interferon. *Science 207*, 527-8.

Acta Universitatis Agriculturae Sueciae
presents doctoral theses from the faculty of agriculture, landscape architecture and horticulture, the faculty of forestry and the faculty of veterinary medicine at the Swedish University of Agricultural Sciences. Each faculty has its own subseries, Agraria, Silvestria and Veterinaria, respectively, with separate numbering.

ISSN 1401-6257
ISBN 91-576-5400-X

REPRO PRINT AB
Stockholm 1997