Immunology 1988 **64** 1–9

Roles of interferon produced in physiological conditions. A speculative review

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Accepted for publication 7 December 1987

CONTENTS

Introduction

It is well known that mammals and other phyla produce and release interferon (IFN) in the blood stream at the onset of a viral and bacterial infection; this early reaction, by inducing an anti-viral resistance in cells, usually limits the spread of the infection, and has been well documented in human and animal infections (Green *et al.*, 1982). The IFN response during an acute viral infection is probably one of the oldest defensive responses (Stewart, 1979), and today nobody doubts its existence and meaning. On the basis that this response occurs during acute viral infections, autoimmune diseases (Preble & Friedman, 1983), and even in neoplastic patients, several years ago I considered it an acute phase, or emergency response, in order to imply the stringent cause–effect relationship (Bocci, 1981).

The idea that monokines and lymphokines (for the sake of brevity I will refer to them as cytokines), such as IFNs and interleukins (ILs), could be produced in physiological conditions was first considered in depth 8 years ago (Bocci, 1980). The thesis that cytokines are produced in healthy animals throughout life was developed, and the expression of a 'physiological IFN response' was coined in distinction with the acute-phase response.

In the equation health$\rightleftharpoons$disease$\rightarrow$death, health can be considered a dynamic and transient state, in which our defence systems efficiently counteract noxious influences trying to alter homeostasis. Even though exogenous stimuli are potentially pathological, provided they are efficiently counteracted they can be considered as 'physiological' stimuli able to temporarily induce an optimized production of cytokines that help to shift the reaction to the left in order to maintain the animal in a state of health.

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As we shall see, physiological production of cytokines is a rather non-specific process most frequently induced by 'physiological' exogenous or endogenous stimuli. Unfortunately, confusing adjectives such as 'preformed', 'spontaneous', 'endogenous' or 'constitutive' IFN production have been used, because of the lack of conceptual framework to explain apparently different phenomena in a unified way. It is now clear that 'preformed' IFN does not exist (reviewed by Stewart, 1979), and we shall see that most, if not all, of the IFN produced during the physiological response is induced and, therefore, not spontaneous (i.e. uninduced). The use of 'endogenous' does not clarify the process sufficiently, while the adjective 'constitutive' is inappropriate and its use should be restricted to continuous cytokine production by abnormal or neoplastic cells, where either an integrated proviral genome or another promoter favours the transcription of some IFN mRNA (Jameson & Grossberg, 1979; Sugamura *et al.*, 1983; Ymer *et al.*, 1985). In contrast, the physiological response is a miniaturized and desultory process of induction, where mainly monocytes and lymphocytes may occasionally and transiently produce and release some IFN around their pericellular environment. It is proposed that this background production is called 'physiological IFN'.

This article aims to clarify the concept of the response taken as a paradigmatic example (which is by no means restricted to IFN) and to show that it has important practical implications.

Rational basis of the physiological IFN response

Even by using a very sensitive biological assay system (able to detect as little as a few units IFN/ml equivalent to about 10^{-13} M), it is difficult to detect IFN unequivocally in normal animal sera (Chisholm & Cartwright, 1978; Walker *et al.*, 1982). Obviously, this is not clear-cut proof that IFN is not produced but only indicates that either IFN is not present in blood or that

we are unable to detect it. Thus, the generally accepted conclusion that IFN, being absent in blood, is not produced in healthy animals is not warranted.

In fact, there are anatomical, microbiological and immunological observations indicating that normal newborns are sterile and almost virgin of foreign agents, although any semi-allogeneic newborn may have already undergone some immunological interactions while in the uterus. But, obviously, it is only after birth that the newborn becomes fully exposed to the microbial world and endotoxins (LPS), as Erwin Neter wrote: 'host and microorganism seemingly take a marriage vow: for better or for worse, for health or disease' (McGhee, Kiyono & Alley, 1984). Geographical, social, hygienic conditions and breast or artificial milk feeding (Rogers & Synge, 1978) influence the development of the immune system. The profound anatomical, microbiological and immunological differences noted between animals reared either in conventional or in germ-free conditions (Wostmann, 1981; Gustafsson, 1985), suggest that only conventional conditions may allow full expression of the physiological IFN response. It is reasonable to imagine that interactions among bacteria colonizing the gut and other mucosae, besides inducing antibody formation, are taking place with local production of cytokines such as IFNs and ILs. Circulating antibodies passively acquired through the placenta or secretory immunoglobulin A (sIgA), and other factors present in milk (Mata, 1986) with a poorly developed lymphoid system (Nair, Schwartz & Menon, 1985; Wilson *et al.*, 1986), may limit somewhat the physiological response, which reaches full activity after weaning.

The physiological IFN response is envisaged as a localized response mostly limited to the lymphoid system and induced in only a few cells at a single time. In the gut-associated lymphoid tissue (GALT), once immunological equilibrium has been achieved, the fraction of the daily antigen load escaping sIgA binding and able to reach either a membranous cell (Owen, 1977; Sneller & Strober, 1986) or a macrophage, is most probably minimal. Moreover, the presence of suppressor factors (Bland & Warren, 1986) prevents excessive boosting of the immune reaction.

As the response is limited to a few cells, generalized hyporesponsiveness to further induction does not occur, as it does after administration of oral IFN inducers (as a drug) able to involve a far larger number of cells (Wierenga, 1985). On the basis of the lymph flow, a funnel-shaped concentration gradient is likely to be established around stationary cells so that neighbouring cells and cells in transit may be activated by the factor or, in other words, one single secreting cell may activate many thousands of cells passing by.

Hormonal interaction is of paracrine type and most of the secreted cytokine is used locally at short range, and only traces may spill-over into the lymph and into the circulation.

The results of work on the distribution and catabolism of IFN have been instrumental in understanding why it could not be detected in blood (Bocci, 1985b). Lymph has a flow about 3000-fold lower than plasma and contains only a trace of IFN, which disappears altogether on entering the plasma pool where dilution, cell binding and renal filtration take place (Fig. 1). Thus, the inability to detect IFN and other cytokines in blood is due to their modest spill-over in a rapidly turning-over pool. Finally, an important corollary is that activated monocytes and lymphocytes, by virtue of their circulatory ability, can exert and even transfer (Blalock *et al.*, 1982) their biological activities all over the body in the absence of circulating cytokines.

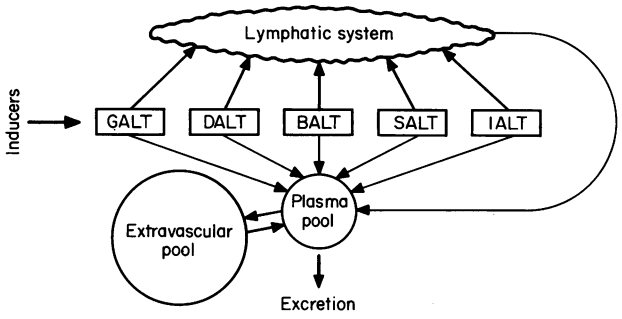

Figure 1. A general scheme of the main sites of cytokine production in physiological condition. Although most of the cytokines are consumed locally, some spill-over occurs in the lymph and in the venous circulation. Cytokine levels do not become apparent in plasma because of extensive dilution, redistribution and excretion. GALT, DALT, BALT, SALT and IALT are abbreviations used to define gut-, duct-, broncho-, skin- and internal-associated lymphoid tissue.

Sites of the physiological IFN response

Although most of the discussion will be centered on IFN, wherever available pertinent data for IL-1, IL-2 and tumour necrosis factor (TNF) will be reported. So far, attention has been focused on the GALT, on the duct-associated tissue (DALT), on the bronchial-associated lymphoid tissue (BALT), on the skin-associated lymphoid tissue (SALT), on the internal-associated lymphoid tissue (IALT) and on the placenta and its annexes. A seventh site, the central nervous system (CNS) remains to be explored.

As far as the GALT and the liver (which is in close functional relation to the gut) are concerned, most of the potential IFN inducers are of exogenous origin (Bienenstock & Befus, 1980). LPS, derived from the indigenous intestinal microflora (Savage, 1986) can normally be traced in the portal blood but is taken up by the hepatic reticuloendothelial system (RES) so that peripheral blood is practically endotoxin-free (Jacob *et al.*, 1977; Mathison & Ulevitch, 1979; Ruiter *et al.*, 1981). The fraction of LPS present in normal lymph is probably bound to specific antibodies, chylomicrons, lipids and lipoproteins (Ulevitch & Johnston, 1978), but its neutralization by antibodies does not hinder its IFN-induction ability (Borecký, Lackovic & Russ, 1970). An additional route of LPS excretion via the lung has been recognized in the rat where LPS-carrying macrophages migrate to the broncho–alveolar space for over 2 weeks (Freudenberg *et al.*, 1984).

Healthy humans harbour over 400 bacterial species weighing about 1270 g (wet weight), which is almost equivalent to the weight of the liver (Gustafsson, 1985). Of all of these bacteria, about 1000 g (or about 10^4 bacterial cells) are present in the intestines. Thus, it is indeed a marvel to be able to keep at bay such a multitude of foreign cells which easily out-number our own. The amount of LPS absorbed every day is probably an exceedingly small fraction of the total in the gut, but as few as eight molecules of LPS per monocyte can prime it and maintain an optimal oxygen radical response (Pabst, Hedegaard & Johnston, 1982).

LPS is a remarkably good inducer of IFN-α and -γ, IL-1 and TNF (Blanchard *et al.*, 1986; Le *et al.*, 1986; Kirchner, Weyland & Storch, 1986; Beutler & Cerami, 1987). Although LPS acts as a fundamental immunological signal (Vogel & Fertsch, 1987; Gessani *et al.*, 1987) it is not the only one. In fact, examination of the insurgence of Igs in germ-free animals (Wostmann, 1981) reveals that it is not only necessary to remove microbial stimulation but that the dietary antigenicity of sterile solid diets and their endotoxin content must also be eliminated. Even with chemically defined amino acid–glucose 'antigen-free' diets, a limited amount of IgM could become detectable, suggesting that immunological reactivity has not been wholly shut down (Wostmann, Pleasants & Bealmear, 1971). Moreover, muramyl peptides, as breakdown products after macrophagic phagocytosis of bacteria, also can express important immuno-adjuvant effects (Karnovsky, 1986; Masêk, 1986).

From a histological and functional point of view, DALT may be considered as part of GALT (Nair & Schroeder, 1986). DALT is minimal at birth and, unless animals are kept under germ-free conditions, the local microflora (the adult mouth and the vagina contain about 40 g of bacteria) and other exogenous antigenic stimuli bring about its development. Johnson, Petzold & Galask (1985) have shown the presence of at least 54 bacterial species in the vaginal microflora and again, one needs little imagination to realize how the local immune response must be active all the time to control potentially pathogenic micro-organisms and fungi.

The human broncho–alveolar surface is not less than 100 m^2 and is variably exposed to an almost infinite number of vegetable, animal or industrial substances and, more or less directly, to cigarette smoke. House dust contains, among other allergens, mite (Genus *Dermatophagoides*) carcasses and fecal pellets which may act as cytokine inducers, although little research in this specific field has been carried out so far (Tovey, Chapman & Platts Mills, 1981). Fortunately, against the bombardment of so many agents, we normally have a good protection because particles over 1 μ diameter have little chance of reaching the alveolar space and are eliminated by the normally efficient muco-ciliary escalator. Besides the ubiquitous sIgA, the pulmonary surfactant system contains substances able to inhibit bacterial and fungal activity and, while it primes alveolar macrophages, it down-regulates lymphocytes, probably in order to contain the lymphocytic response to the frequent antigenic challenge (Claypool & Fisher, 1983). Briefly, it has been shown that activated alveolar macrophages (Acton & Myrvik, 1966; Koretzky *et al.*, 1983) can release IL-1, TNF and IFN-α, -γ, but most of these cytokines would be used or inactivated locally. However, a small fraction of these scavenger cells re-enters the circulation via the lymph and afterwards their fate is unknown. Similarly, the basal production of cytokines by the BALT would be utilized locally, although traces may be transported away via the lymph (Fig. 1).

The skin is the body's largest organ and, under physiological conditions, contains, among other cells, Langerhans' cells, mast cells, macrophages, keratinocytes and 'homing' T lymphocytes which contribute to make this organ an active element of the immune system (Streilein, 1978). The activity of about 200 g of the indigenous bacterial flora is normally contained by the presence of a thin film of sweat and sebaceous material layered onto the horny layer, but, once again, the environment, the climate and, particularly, sun exposure, as well as parasites and hygiene, may have considerable influence on the activity of exogenous stimuli. Both IL-1 and IFN-α, -γ have been detected (Hauser *et al.*, 1986; Yaar, Palleroni & Gilchrest, 1986) in normal human epidermis and, therefore, it is possible that, besides serving as local immunoregulatory signals, they contribute to maintain the host immune system alert by way of circulatory cells.

The IALT comprises thymus, bone marrow and spleen, and is likely to undergo induction mainly by endogenous stimuli, including autoctonous lectins, cytokines, hormones, growth factors, oxygen radicals and proteinases released by macrophages and neutrophils (Bocci, 1981). The latter compounds are likely to act more efficiently in organs, such as the spleen, where the peculiar blood circulation favours plasma skimming with a strictly localized deficiency of proteinase inhibitors (Bocci, 1976). Moreover, the influence of exogenous inducers cannot be entirely dismissed because a comparative analysis of the spleen and thymus weights between germ-free and conventional animals suggests that foreign antigens or LPS bound to macrophages may reach these organs (Gordon & Wostmann, 1960).

Fowler, Reed & Giron (1980) found IFN-like activity in the murine placenta. This study was followed by somewhat controversial findings in human normal amniotic fluid (AF). While Cesario *et al.* (1981) could not detect IFN but actually found anti-viral antibodies and inactivators against IFN-β, Lebon *et al.* (1982) detected IFN-α in almost all AF samples collected between the 16th and 20th week. A later study (Duc-Goiran *et al.*, 1985) has clarified that the low but consistent amount of IFN can be masked by antagonistic substances.

So far there are no data to indicate that there is physiological production of cytokines in the CNS. There are no lymphatics, and practically no lymphocytes, in the normal brain, and the presence of the blood–brain barrier (BBB) limits the entrance of hydrophilic inducer molecules. However, astrocytes and ameboid microglia can be induced to produce IFN, IL-1 and IL-3-like factors so that, potentially, there may be production at a basal rate (Fontana *et al.*, 1982; Frei *et al.*, 1985; Tedeschi, Barrett & Keane, 1986).

Finally, production of IFN-β_2 (also referred to as 26-K protein, human B-cell differentiation factor, BSF-2, and IL-6) by human diploid fibroblasts has been reported recently (Kohase *et al.*, 1986; Sehgal *et al.*, 1987; Revel *et al.*, 1987). It appears that the expression of IFN-β_2 is not as stringently regulated as that of IFN-β_1 and is promptly inducible by polynucleotides, IL-1, TNF, platelet-derived growth factor and bovine serum. Its normal production *in vivo* remains uncertain but the fact that IFN-β_2 is easily induced by endotoxins and acts as a potent hepatocyte-stimulating factor, raises the interesting possibility that IFN-β_2 may be the factor stimulating the hepatic synthesis of fibrinogen and of acute-phase reactants in normal and emergency conditions, respectively.

Observational results of the response

Direct evidence so far available can be distinguished as follows:

Studies in vitro

Human blood monocytes and bone marrow, rabbit tissues (liver, spleen, bone marrow, Peyers's patches, sacculus rotundus, lungs), rabbit and mouse peritoneal and alveolar macrophages obtained from animals reared in conventional condi-

tions but apparently 'pathogen free', immediately after isolation release in culture some anti-viral activity that, by several criteria, corresponds to IFN-α/β and γ. In general, release of IFN become progressively lower with time, suggesting that IFN production, without further induction, is slowly turned off (Smith & Wagner, 1967; Haller *et al.*, 1979; Blach-Olszewska & Cembrzynska-Nowak, 1979; Fischer & Rubinstein, 1983; Martinez-Maza *et al.*, 1984; Bocci *et al.*, 1984b; Paulesu *et al.*, 1986; Belardelli *et al.*, 1987). On the other hand, peritoneal macrophages obtained from germ-free animals do not release detectable IFN (De Maeyer, Fauve & De Maeyer-Guignard, 1971).

It has already been mentioned that murine and human placentas produce IFN, some of which is of an unusual type and size (Duc-Goiran *et al.*, 1985). Owing to transplacental transfer of protein molecules, IFN present in AF may derive from the mother's blood or, more probably, may be produced at the feto–maternal interface. In attempting to exclude any maternal influence, we have evaluated the IFN production by the human isolated and perfused normal placenta at term, obtained after Caesarean section (Bocci, Paulesu & Ricci, 1985c). With some variability attributed to 'good' or 'poor' responders, all placentas released into the perfusate detectable anti-viral activity which, by the use of IFN antisera, has been attributed to IFN-α and to IFN-β. Whether production of these IFNs is due to an interaction *in vivo* between antigens of the semi-allogeneic conceptus and the maternal immune system, to unusual hormonal levels during pregnancy and/or to a transient derepression of particular IFN genes during fetal life, remains uncertain but these findings and more recent ones (Chard *et al.*, 1986) have shown unequivocally that the placenta is indeed a site of physiological IFN production. However, in comparison with the preceding sites, unusual IFNs (in terms of size and type) are present in the placenta and amniotic fluid, and this may be related either to the anatomical, functional and immunological peculiarities of the placenta, or to the fact that unusual IFN-β have enhanced growth-regulatory or immunosuppressive activities. IL-1-like activity and TNF have also been detected recently during normal pregnancy in murine (Jyonouchi, Voss & Good, 1987) and human amniotic fluids (Jaattela, Kuusela & Saksela, 1987).

Biochemical studies

Traces of mRNA for IFN-α, -β_2 and more rarely -γ have been isolated from several organs of normal mice and individuals (Tovey & Gresser, 1986; Tovey *et al.*, 1987). This approach proves that transcription of IFN genes is taking place *in vivo*, but it neither defines which cells are producing IFN at the time of the test, nor proves that IFN is produced *in vivo* without induction. These uncertainties may eventually be overcome by applying either the method of *in situ* hybridization on tissue slides, or locating IFN-producing cells in human tissues by using enzyme-labelled anti-IFN antibodies.

The circumstantial evidence can be summarized as follows:

Studies aiming to detect typical enzymatic markers. Two enzymes: 2′-5′ oligoadenylate synthetase (2-5A) and protein kinase (p67K), known to be specifically induced with IFN *in vitro* and *in vivo*, show some selective localization *in vivo*. These enzymes markers could also reveal the presence of IFN in its absence (Schattner *et al.*, 1981). The ensemble of the results (Hovanessian *et al.*, 1981; Gresser *et al.*, 1985; Galabru *et al.*, 1985; Proietti *et al.*, 1986; Vogel & Fertsch, 1987) could not support the concept that the physiological IFN response is mainly modulated by exogenous stimuli better: 2-5A and p67K levels are indeed measurable (IFN was not) in circulating cells, tissues and plasma samples of healthy men and mice. Moreover, as predicted (Bocci, 1981), enzyme levels are low in germ-free, pathogen-free, antibiotic-treated and anti-IFN antibody-treated mice.

Studies revealing IFN activities on effector cells. Another lead has been to evaluate whether effector cells obtained from animals kept in conventional conditions could manifest biological activities better than cells drawn from germ-free or conventional animals pre-injected with potent anti-IFN α/β antisera. Here again mouse peritoneal macrophages derived either from normal or anti-IFN antibody-treated mice have been found to restrict or to permit viral multiplication, respectively, implying that macrophages in an anti-viral state have either produced IFN or had a previous contact with it *in vivo* (Belardelli *et al.*, 1984, 1987; Vogel & Fertsch, 1984, 1987; Proietti *et al.*, 1986). It must be noted that macrophages kept in culture progressively lose their viral non-permissiveness, 2-5A activity and other characteristics, in line with the concept that production of IFN is not consitutive but is regulated by inducers. Furthermore, rapid activation of NK cells in mice coincided with the full development of the intestinal microflora and consumption of a conventional diet, suggesting that at weaning, local production of IFN and IL-2 stimulates this immunological function and establishes the usual background activity (Wigzell, 1981; Bartizal, Salkowski & Balish, 1983).

Studies in vivo. As it is suspected that the simple addition of fetal calf serum to cells *in vitro* may in itself cause IFN induction, we have mostly approached the problem *in vivo*, trying to evaluate whether IFN is detectable in rabbits, rats and mice kept in conventional conditions, either in abdominal lymph (Bocci *et al.*, 1984a, 1985a) and intestinal venous blood (Bocci *et al.*, 1986) or in lymph from the hind leg or arterial and peripheral blood. Results show that consistent spill-over of IFN occurs from the GALT: IFN-γ levels are significantly higher in lymph drained from the cisterna chyli and venous (appendix, ileal–cecal valve, mesenteric, portal, splenic) blood. IFN is practically undetectable in peripheral blood. We have also investigated whether in man a sudden activation of lymph production and inflow may cause a transient increase of plasma IFN levels (Bocci *et al.*, 1985b; Viti *et al.*, 1985). A fat-rich meal and vigorous physical exercise can indeed cause a transitory increase of plasma acid-labile IFN-α levels, in keeping with either an enhanced lymph inflow into the plasma during the digestion and/or diminished renal filtration during exercise. Also, IL-1 levels are transiently increased after strenuous exercise (Evans *et al.*, 1986; Cannon *et al.*, 1986).

Practical implications

By using several approaches, direct and circumstantial evidence has been presented supporting the existence of a basal production of IFN localized at particular sites and taking place more or less continuously in relation to external cues. Other cytokines, such as ILs have been less well investigated but probably follow the IFN pattern, and traces of them have been detected in body fluids (English & Whitehurst, 1984; Spitz *et al.*, 1985; Cannon & Dinarello, 1985). An emerging view is that normal cells do not

produce IFN unless they are induced by exogenous or endogenous stimuli and that production ceases shortly thereafter; it may be resumed later when the cells become responsive and if a new stimulus occurs. On the other hand, continuous (constitutive) production of IFN takes place in transformed cells, but this aspect cannot be related to a truly physiological response and may be harmful to the host.

The following reasons suggest that the physiological response serves to maintain active defence systems essential for survival:

(i) The effect of having a quota of circulating cells in a primed state (Stewart, 1979), the enhancement of the expression of the MHC class I and II antigens on various cells (Capobianchi *et al.*, 1985), the activation of monocytes and macrophages (Steeg *et al.*, 1982; Murray, Spitalny & Nathan, 1985), and the induction of a state of alertness in some of the NK, cytotoxic T cells and neutrophils (Donahoe & Huang, 1976; Shalaby *et al.*, 1985) are examples of the practical usefulness of such a response. It is possible that only an efficient level of the response may prevent infectious diseases and inhibit or delay tumour growth (Gresser *et al.*, 1983) for a long period of the life span. Obviously, rather than IFN alone, a concerted production of cytokines is essential to keep immune defences geared to maintain the state of health.

(ii) Although a paracrine-type secretion seems of paramount importance, limited autocrine and truly endocrine interactions may occur. Nonetheless, only trace levels of cytokines are present in the circulation. This implies that in health, cytokines apparently do not display toxic effects that become evident, either during viral or bacterial infections, or during administration of IFN, IL-2 and TNF through conventional routes yielding high plasma levels. In fact, severe bone marrow depression, fever, cerebral depression and coma, fluid extravasation and disseminated intravascular coagulation are some of the side-effects noted (Scott, 1984; Rosenstein, Ettinghausen & Rosenberg, 1986; Beutler & Cerami, 1987). There are two conclusions to bear in mind: one is that cytokines are not, and should not be considered, circulatory proteins like γ-globulins, and the other is that knowledge of their distribution and mode of action compels consideration of their administration by novel strategies (Bocci, 1985c, 1987).

(iii) There is now little doubt that in the bone marrow trace levels of IFNs and TNF may exert either chalone-like activities and/or differentiation effects to balance colony-stimulating factors and erythropoietin-like activities (Broxmeyer *et al.*, 1983, 1986; Zoumbos *et al.*, 1985; Mamus, Beck-Schroeder & Zanjani, 1985; Trinchieri *et al.*, 1986). IFN is a pleiotropic agent and the activity displayed in this case is the regulation of growth and differentiation of haemopoietic cell lineages. Similarly, IFN-γ and IL-2 appear to play a role in B-cell activation and differentiation (Jelinek, Splawski & Lipsky, 1986).

(iv) Low levels of IFN-β_2, if they are present in the normal animal, may also mediate priming of the IFN system and, more specifically, enhance immunoglobulin secretion or exert autocrine regulation of cell proliferation (Kohase *et al.*, 1986; Revel *et al.*, 1987).

(v) Continuous presence of IFN in strategic sites is probably helpful in inhibiting the integration of viral genomes into cellular DNA (Avery *et al.*, 1980) or, alternatively, could induce repair mechanisms of gene function and reversion to non-malignancy as observed with the C-myc and V-mos oncogenes (Dani *et al.*, 1985; Sergiescu *et al.*, 1986; Newmark, 1987).

(vi) Finally, I would like to entertain the possibility that cytokines produced in some of the envisioned sites may exert regulatory activities on the CNS. As I have mentioned, trace amounts of these hormones are transported through the lymph and into the plasma pool where they disappear. As yet there is little proof that IFN or other ILs are produced at physiological levels in the CNS while, on the other hand, the BBB is practically impermeable to cytokines; however, even a practically invisible cytokine level in the plasma may have physiological effects if these cytokines reach and are bound to strategic sites in the hypothalamus (somnogenic and thermoregulatory centers, neurosecretory nuclei) through particular areas (infundibular recess, median eminence and choroid plexus) which structurally may allow some passage of proteins. This interaction, though limited, could be important and in line with current thinking (Riley, 1981; Besedovsky, Del Rey & Sorkin, 1985) in establishing a link between the immune system and vegetative and neurosecretory sites.

Areas for future progress

Several factors can influence the normal production of 'physiological IFN' and in order to clarify the relevance of the response, it may be worth-while evaluating certain parameters such as genetic background (De Maeyer & De Maeyer-Guignard, 1979; Dreiding, Staeheli & Haller, 1985), sex (Bever, McFarlin & Levy, 1985), presence of inhibitors and antibodies for IFNs and ILs (Lelchuk & Playfair, 1985; Lefkowitz & Fleischmann, 1985; Liao *et al.*, 1985), nutritional status, types of diet, environmental conditions, stress and hormonal changes (Palmblad *et al.*, 1976).

Age is a very important factor and a slow but progressive decay of the response is envisaged in man (Bocci, 1981). Blach-Olszewska, Cembrzynska-Nowak & Kwasniewska (1984) showed that the maximal production of 'physiological IFN' is observed in 8-week-old mice and afterwards declines until it reaches unmeasurable levels in 1-year-old animals. It is remarkable that maximal production of IFN occurs shortly after weaning when the animal bears the brunt of the environmental exposure, and it would be instructive to perform a similar study in man. It appears not coincidental that both background and a prompt and efficient IFN response in young animals is one of the most important factors in determining the outcome of viral infections (Mogensen, 1978; Zawatzky, Engler & Kirchner, 1982). Several questions remain open: Why does production of physiological IFN decrease with age? Is there any relationship with thymic involution? Is there also a derangement of the response in the sense that there is a change in the type and relative production of cytokines? Are thymic hormones or IFN inducers able to restore and correct the response?

As far as the IFN daily cycle is concerned, our studies have indicated that the response has a nyctohemeral rhythm: in rat exposed to the normal light–dark cycle, the IFN peak level is connected somewhat with the intake of food (Paulesu *et al.*, 1985) and in man there is an increase in IFN plasma level throughout the day (Bocci *et al.*, 1985b). It is important to clarify whether daily IFN variations depend upon external cues or internal ones (such as corticosteroids) and if they change throughout the year. So far, bearing in mind the daily cortisol

cycle and its relationship to the lymphocyte circulation, it has been suggested that IFN should be administered at night rather than in the morning (Bocci, 1985a).

Conclusions

For three decades of IFN research, there has not been a clear appreciation of the possible production and role of IFNs and other cytokines in normal individuals. There is now evidence that an array of exogenous and endogenous inducers cause production of a number of cytokines at basal rates in localized micro-environments.

The paracrine-type secretion and local activation of effector cells followed by their continuous circulation throughout the body make the physiological IFN response particularly suited to protect the host against a number of noxae without the disadvantage of significant cytokine levels in the blood. In order to improve the therapeutic index of cytokines (or biological response modifiers), their pharmacological use should simulate the physiological distribution and mode of action. The response has a nyctohemeral rhythm and, on ageing, follows a pattern similar to that observed for thymic hormones. There is now the need to evaluate the response by practical tests and to clarify the role of many factors which may influence it. Considerable work remains to be done in order to make the physiological IFN response useful for predicting immune deficiencies, or derangements, and amenable to pharmacological manipulation for possibly preventing or minimizing viral diseases and tumours in man.

ACKNOWLEDGMENTS

I am very grateful to Professor A. Billiau, Professor V. Borecký, Professor K. Cantell, Dr N. Finter, Dr A. Hovanessian, Professor H. Kirchner and Professor J. Lindenmann for critical reading of the manuscript and helpful comments.

This work was supported by Progetti finalizzati 'Oncologia' and 'Controllo delle malattie da infezione' C.N.R. Roma.

I would like to thank Miss S. Holmes for patiently typing the manuscript several times.

REFERENCES

Acton J. D. & Myrvik Q.N. (1966) Production of interferon by alveolar macrophages. *J. Bact.* **91,** 2300.

Avery R.J., Norton J.D., Jones J.S., Burke D.C. & Morris A.G. (1980) Interferon inhibits transformation by murine sarcoma viruses before integration of provirus. *Nature (Lond.)*, **288,** 93.

Bartizal K.F., Salkowski C. & Balish E. (1983) The influence of a gastrointestinal microflora on natural killer cell activity. *RES.*, **33,** 381.

Belardelli F., Gessani S., Proietti E., Locardi C., Borghi P., Watanabe Y., Kawade Y. & Gresser I. (1987) Studies on the expression of spontaneous and induced interferons in mouse peritoneal macrophages by means of monoclonal antibodies to mouse interferons. *J. gen. Virol.* **68,** 2203.

Belardelli F., Vignaux F., Proietti E. & Gresser I. (1984) Injection of mice with antibody to interferon renders peritoneal macrophages permissive for vesicular stomatitis virus and encephalomyocarditis virus. *Proc. natl. Acad. Sci. U.S.A.* **81,** 602.

Besedovsky H.O., del Rey A.E. & Sorkin E. (1985) Immune-neuroendocrine interactions. *J. Immunol.* **135,** 750s.

Beutler B. & Cerami A. (1987) Cachectin: more than a tumour necrosis factor. *New Engl. J. Med.* **316,** 379.

Bever C.T., McFarlin D.E. & Levy H.B. (1985) A comparison of interferon responses to poly ICLC in males and females. *J. Interferon Res.* **5,** 423.

Bienenstock J. & Befus A.D. (1980) Mucosal immunology. *Immunology*, **41,** 249.

Blach-Olszewska Z. & Cembrzynska-Nowak M. (1979) Synthesis of spontaneous interferon by mouse peritoneal cells *in vitro*. I. Attempts to elucidate the origin of spontaneous interferon. *Acta biol. Med. Germ.* **38,** 765.

Blach-Olszewska Z., Cembrzynska-Nowak M. & Kwasniewska E. (1984) Age-related synthesis of spontaneous interferon in BALB/c mice. In: *Physiology and Pathology of Interferon System* (eds L. Borecky and V. Lackovic), p. 224. Karger, Basel.

Blalock J.E., Baron S., Johnson H.M. & Stanton G.J. (1982) Transmission of IFN-induced activities by cell to cell communication. *Texas Rep. biol. Med.* **41,** 344.

Blanchard D.K., Djeu J.Y., Klein T.W., Friedman H. & Stewart W.E. (1986) Interferon-γ induction by lipopolysaccharide: dependence on interleukin 2 and macrophages. *J. Immunol.* **136,** 963.

Bland P.W. & Warren L.G. (1986) Antigen presentation by epithelial cells of the rat small intestine. II. Selective induction of suppressor T cells. *Immunology*, **58,** 9.

Bocci V. (1976) The role of sialic acid in determining the life-span of circulating cells and glycoproteins. *Experientia*, **32,** 135.

Bocci V. (1980) Is interferon produced in physiologic conditions? *Med. Hypotheses*, **6,** 735.

Bocci V. (1981) Production and role of interferon in physiological conditions. *Biol. Rev.* **56,** 49.

Bocci V. (1985a) Administration of interferon at night may increase its therapeutic index. *Cancer Drug Delivery*, **2,** 313.

Bocci V. (1985b) Distribution, catabolism and pharmacokinetics of interferons. In: *Interferon* (eds N. B. Finter and R. K. Oldham), Vol 4, p. 47. Elsevier Science Publishers B. V., Amsterdam.

Bocci V. (1985c) Immunomodulators as local hormones: new insights regarding their clinical utilization. *J. biol. Response Modifiers*, **4,** 340.

Bocci V. (1987) Metabolism of protein anticancer agents. *Pharmac. Ther.* **34,** 1.

Bocci V., Muscettola M., Paulesu L. & Grasso G. (1984a) The physiological interferon response. II. Interferon is present in lymph but not in plasma of healthy rabbits. *J. gen. Virol.* **65,** 101.

Bocci V., Muscettola M., Paulesu L. & Grasso G. (1985a) The physiological interferon response. V. Antiviral activity present in rat lymph is neutralized by anti-mouse interferon-γ antibodies. *Microbiologica*, **8,** 405.

Bocci V., Paulesu L., Muscettola M., Ricci M.G. & Grasso G. (1984b) The physiological interferon response. III. Preliminary experimental data support its existence. In: *Physiology and Pathology of Interferon System* (eds L. Borecky and V. Lackovic), p. 36. Karger, Basel.

Bocci V., Paulesu L., Muscettola M. & Vanni L. (1986) Presence of interferon in venous blood draining from gut-associated lymphoid tissue. *Immunol. Lett.* **12,** 25.

Bocci V., Paulesu L., Muscettola M. & Viti A. (1985b) The physiologic interferon response. VI. Interferon activity in human plasma after a meal and drinking. *Lymphokine Res.* **4,** 151.

Bocci V., Paulesu L. & Ricci M.G. (1985c) The physiological interferon response: IV. Production of interferon by the perfused human placenta at term. *Proc. Soc. exp. biol. Med.* **180,** 137.

Borecky L., Lackovic V. & Russ G. (1970) Interferon production in leukocytes and the antiviral protection of mice treated with endotoxin. *Ann. N.Y. Acad. Sci.* **173,** 320.

Broxmeyer H.E., Lu L., Platzer E., Feit C., Juliano L. & Rubin B.Y. (1983) Comparative analysis of the influences of human gamma, alpha and beta interferons on human multipotential (CFU-GEMM),

erythroid (BFU-E) and granulocyte-macrophage (CFU-GM) progenitor cells. *J. Immunol.* **131,** 1300.

BROXMEYER H.E., WILLIAMS D.E., LU L., COOPER S., ANDERSON S.L., BEYER G.S., HOFFMAN R. & RUBIN B.Y. (1986) The suppressive influences of human tumor necrosis factors on bone marrow hematopoietic progenitor cells from normal donors and patients with leukemia: synergism of tumor necrosis factor and interferon-γ. *J. Immunol.* **136,** 4487.

CANNON J.G. & DINARELLO C.A. (1985) Increased plasma interleukin-1 activity in women after ovulation. *Science,* **227,** 1247.

CANNON J.G., EVANS W.J., HUGHES V.A., MEREDITH C.N. & DINARELLO C.A. (1986) Physiological mechanisms contributing to increased interleukin-1 secretion. *J. Appl. Physiol.* **61,** 1869.

CAPOBIANCHI M.R., AMEGLIO F., TOSI R. & DOLEI A. (1985) Difference in the expression and release of DR, BR, and DQ molecules in human cells treated with recombinant interferon gamma: comparison to other interferons. *Human Immunol.* **13,** 1.

CESARIO T., GOLDSTEIN A., LINDSEY M., DUMARS K. & TILLES J. (1981) Antiviral activities of amniotic fluid. *Proc. Soc. exp. biol. Med.* **168,** 403.

CHARD T., CRAIG P.H., MENABAWEY M. & LEE C. (1986) Alpha interferon in human pregnancy. *Br. J. Obstet. Gynaecol.* **93,** 1145.

CHISHOLM M. & CARTWRIGHT T. (1978) Interferon production in leukaemia. *Br. J. Haematol.* **40,** 43.

CLAYPOOL W.D. & FISHER A.B. (1983) The immune functions of the lung alveolar lining material. *Surv. Synth. Path. Res.* **2,** 34.

DANI C., MECHTI N., PIECHACZYK M., LEBLEU B., JEANTEUR P. & BLANCHARD J.M. (1985) Increased rate of degradation of c-myc mRNA in interferon-treated Daudi cells. *Proc. natl. Acad. Sci. U.S.A.* **82,** 4896.

DE MAEYER E. & DE MAEYER-GUIGNARD J. (1979) In: *Interferon* (ed. I. Gresser), Vol. 1, p. 75. Academic Press, New York.

DE MAEYER E., FAUVE R.M. & DE MAEYER-GUIGNARD J. (1971) Production d'interféron au niveau du macrophage. *Ann. Inst. Pasteur,* **120,** 438.

DONAHOE R.M. & HUANG K.-Y. (1976) Interferon preparations enhance phagocytosis *in vivo*. *Infect. Immun.* **13,** 1250.

DREIDING P., STAEHELI P. & HALLER O. (1985) Interferon-induced protein Mx accumulates in nuclei of mouse cells expressing resistance to influenza viruses. *Virology,* **140,** 192.

DUC-GOIRAN P., ROBERT-GALLIOT B., LOPEZ J. & CHANY C. (1985) Unusual apparently constitutive interferons and antagonists in human placental blood. *Proc. natl. Acad. Sci. U.S.A.* **82,** 5010.

ENGLISH L.S. & WHITEHURST M. (1984) The production of T-cell growth factor (TCGF) *in vivo* in sheep. *Cell. Immunol.* **85,** 364.

EVANS W.J., MEREDITH C.N., CANNON J.G., DINARELLO C.A., FRONTERA W.R., HUGHES V.A., JONES B.H. & KNUTTGEN H.G. (1986) Metabolic changes following eccentric exercise in trained and untrained men. *J. Appl. Physiol.* **61,** 1864.

FISCHER D.G. & RUBINSTEIN M. (1983) Spontaneous production of interferon-γ and acid-labile interferon-α by subpopulation of human mononuclear cells. *Cell. Immunol.* **81,** 426.

FREI K., BODMER S., SCHWERDEL C. & FONTANA A. (1985) Astrocytes of the brain synthesize interleukin 3-like factors. *J. Immunol.* **135,** 4044.

FONTANA A., KRISTENSEN F., DUBS R., GEMSA D. & WEBER E. (1982) Production of prostaglandin E and an interleukin-1 like factor by cultured astrocytes and C_6 glioma cells. *J. Immunol.* **129,** 2413.

FOWLER A.K., REED C.D. & GIRON D.J. (1980) Identification of an interferon in murine placentas. *Nature (Lond.),* **286,** 266.

FREUDENBERG N., FREUDENBERG M.A., GUZMAN J., MITTERMAYER CH., BANDARA K. & GALANOS C. (1984) Identification of endotoxin-positive cells in the rat lung during shock. *Virchows Arch.* **A 404,** 197.

GALABRU J., ROBERT N., BUFFET-JANVRESSE C., RIVIÈRE Y. & HOVANESSIAN A.G. (1985) Continuous production of interferon in normal mice: effect of anti-interferon globulin, sex, age, strain and environment on the levels of 2-5A synthetase and p67K kinase. *J. gen. Virol.* **66,** 711.

GESSANI S., BELARDELLI F., BORGHI P., BORASCHI D. & GRESSER I. (1987) Correlation between the lipopolysaccharide response of mice and the capacity of mouse peritoneal cells to transfer an antiviral state. Role of endogenous interferon. *J. Immunol.* **139,** 1991.

GORDON H.A. & WOSTMANN B.S. (1960) Morphological studies on the germfree albino rat. *Anat. Rec.* **137,** 65.

GREEN J.A., CHARETTE R.P., YEH T.-J. & SMITH C.B. (1982) Presence of interferon in acute- and convalescent-phase sera of humans with influenza or an influenza-like illness of undetermined etiology. *J. infect. Dis.* **145,** 837.

GRESSER I., BELARDELLI F., MAURY C., MAUNOURY M.-T. & TOVEY M.G. (1983) Injection of mice with antibody to interferon enhances the growth of transplantable murine tumors. *J. exp. Med.* **158,** 2095.

GRESSER I., VIGNAUX F., BELARDELLI F., TOVEY M.G. & MAUNOURY M.-T. (1985) Injection of mice with antibody to mouse interferon α/β decreases the level of 2′-5′ oligoadenylate synthetase in peritoneal macrophages. *J. Virol.* **53,** 221.

GUSTAFSSON B.E. (1985) The future of germfree research. In: *Germfree Research: Microflora Control and Its Application to the Biomedical Sciences,* p. 17. Alan R. Liss, Inc., New York.

HALLER O., ARNHEITER H., GRESSER I. & LINDENMANN J. (1979) Genetically determined, interferon-dependent resistance to influenza virus in mice. *J. exp. Med.* **149,** 601.

HAUSER C., SAURAT J.-H., SCHMITT A., JAUNIN F. & DAYER J.-M. (1986) Interleukin 1 is present in normal human epidermis. *J. Immunol.* **136,** 3317.

HOVANESSIAN A.G., ROLLIN P., RIVIÈRE Y., POUILLART P., SUREAU P. & MONTAGNIER L. (1981) Protein kinase in human plasma analogous to that present in control and interferon-treated HeLa cells. *Biochem. Biophys. Res. Commun.* **103,** 1371.

JÄÄTTELÄ M., KUUSELA P. & SAKSELA E. (1987) Demonstration of tumor necrosis factor in human amniotic fluid and in supernatant of placental tissue. *Immunobiol.* **175,** 111.

JACOB A.I., GOLDBERG P.K., BLOOM N., DEGENSHEIN G.A. & KOZINN P.J. (1977) Endotoxin and bacteria in portal blood. *Gastroenterology,* **72,** 1268.

JAMESON P. & GROSSBERG S.E. (1979) Production of interferon by human tumor cell lines. *Arch. Virol.* **62,** 209.

JELINEK D.F., SPLAWSKI J.B. & LIPSKY P.E. (1986) The roles of interleukin 2 and interferon-γ in human B cell activation, growth and differentiation. *Eur. J. Immunol.* **16,** 925.

JOHNSON S.R., PETZOLD C.R. & GALASK R.P. (1985) Qualitative and quantitative changes of the vaginal microbial flora during the menstrual cycle. *Am. J. Repro. Immunol. Microbiol.* **9,** 1.

JYONOUCHI H., VOSS R.M. & GOOD R.A. (1987) IL 1-like activities present in murine amniotic fluid. A significantly larger amount of IL-1-like activity is present in the amniotic fluid of autoimmune NZB mice. *J. Immunol.* **138,** 3300.

KARNOVSKY M. (1986) Muramyl peptides in mammalian tissues and their effects at the cellular level. *Fed. Proc.* **45,** 2556.

KIRCHNER H., WEYLAND A. & STORCH E. (1986) Local interferon induction by bacterial lipopolysaccharide in mice after pretreatment with *Corynebacterium parvum*. *J. Interferon Res.* **6,** 483.

KOHASE M., HENRIKSEN-DE STEFANO D., MAY L.T., VILCEK J. & SEHGAL P.B. (1986) Induction of β_2-interferon by tumor necrosis factor: a homeostatic mechanism in the control of cell proliferation. *Cell,* **45,** 659.

KORETZKY G.A., ELIAS J.A., KAY S.L., ROSSMAN M.D., NOWELL P.C. & DANIELE R.P. (1983) Spontaneous production of interleukin-1 by human alveolar macrophages. *Clin. Immunol. Immunopathol.* **29,** 443.

LE J., LIN J.-X., HENRIKSEN-DE STEFANO D. & VILCEK J. (1986) Bacterial lipopolysaccharide-induced interferon-γ production: roles of interleukin 1 and interleukin 2. *J. Immunol.* **136,** 4525.

LEBON P., GIRARD S., THEPOT F. & CHANY C. (1982) The presence of α-interferon in human amniotic fluid. *J. gen. Virol.* **59,** 393.

LEFKOWITZ, E.J. & FLEISCHMANN W.R. (1985) An inhibitor of interferon action: II. Biological properties of the IFN-γ-associated

inhibitor of interferon action. *J. Interferon Res.* **5,** 101.

LELCHUK R. & PLAYFAIR J.H.L. (1985) Serum IL-2 inhibitor in mice. I. Increase during infection. *Immunology*, **56,** 113.

LIAO Z., HAIMOVITZ A., CHEN Y., CHAN J. & ROSENSTREICH D. L. (1985) Characterization of a human interleukin 1 inhibitor. *J. Immunol.* **134,** 3882.

MCGHEE J.R., KIYONO H. & ALLEY C.D. (1984) Gut bacterial endotoxin: influence on gut-associated lymphoreticular tissue and host immune function. *Surv. Immunol. Res.* **3,** 241.

MAMUS S.W., BECK-SCHROEDER S. & ZANJANI E.D. (1985) Suppression on normal human erythropoiesis by gamma interferon *in vitro*. Role of monocytes and T lymphocytes. *J. clin. Invest.* **75,** 1496.

MARTINEZ-MAZA O., ANDERSSON U., ANDERSSON J., BRITTON S. & DE LEY M. (1984) Spontaneous production of interferon-γ in adult and newborn humans. *J. Immunol.* **132,** 251.

MASEK K. (1986) Multiplicity of biological effects of muramyl dipeptide. *Meth. Find. exp. Clin. Pharmacol.* **8,** 97.

MATA L. (1986) Breast-feeding and host defense. In: *Frontiers of Gastrointestinal Research* (ed. P. Rozen), Vol. 132, 119. Karger, Basel.

MATHISON J.C. & ULEVITCH R.J. (1979) The clearance, tissue distribution, and cellular localization of intravenously injected lipopolysaccharide in rabbits. *J. Immunol.* **123,** 2133.

MOGENSEN S.C. (1978) Macrophages and age-dependent resistance to hepatitis induced by herpes simplex virus type 2 in mice. *Infect. Immun.* **19,** 46.

MURRAY H.W., SPITALNY G.L. & NATHAN C.F. (1985) Activation of mouse peritoneal macrophages *in vitro* and *in vivo* by interferon-γ. *J. Immunol.* **134,** 1619.

NAIR M.P.N., SCHWARTZ S.A. & MENON M. (1985) Association of decreased natural and antibody-dependent cellular cytotoxicity and production of natural killer cytotoxic factor and interferon in neonates. *Cell. Immunol.* **94,** 159.

NAIR P.N.R. & SCHROEDER H.E. (1986) Duct-associated lymphoid tissue (DALT) of minor salivary glands and mucosal immunity. *Immunology*, **57,** 171.

NEWMARK P. (1987) Oncogenes and cell growth. *Nature (Lond.)*, **327,** 101.

OWEN R.L. (1977) Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in the normal unobstructed mouse intestine: an ultrastructural study. *Gastroenterology*, **72,** 440.

PABST M.J., HEDEGAARD H.B. & JOHNSTON R.B. (1982) Cultured human monocytes require exposure to bacterial products to maintain an optimal oxygen radical response. *J. Immunol.* **128,** 123.

PALMBLAD J., CANTELL K., STRANDER H., FRÖBERG J., KARLSSON C.-G., LEVI L., GRANSTRÖM M. & UNGER P. (1976) Stressor exposure and immunological response in man: interferon-producing capacity and phagocytosis. *J. Psychosomatic Res.* **20,** 193.

PAULESU L., BOCCI V., LACKOVIC V. & MUSCETTOLA M. (1986) The interferon physiological response as expressed by peritoneal and alveolar macrophages. *IRCS Med. Sci.* **14,** 1053.

PAULESU L., MUSCETTOLA M., BOCCI V. & VITI A. (1985) Daily variations of plasma interferon levels in the rat. *IRCS Med. Sci.* **13,** 993.

PREBLE O.T. & FRIEDMAN R.M. (1983) Biology of disease. Interferon-induced alterations in cells: relevance to viral and nonviral diseases. *Lab. Invest.* **49,** 4.

PROIETTI E., GESSANI S., BELARDELLI F. & GRESSER I. (1986) Mouse peritoneal cells confer an antiviral state on mouse cell monolayers: role of interferon. *J. Virol.* **57,** 456.

REVEL M., ZILBERSTEIN A., RUGGIERI R., CHEN L., GABAI M., MORY Y. & MICHALEVICZ R. (1987) Human IFN-beta-2: a multifunctional, TNF-inducible cytokine. *J. Interferon Res.* **7,** 700.

RILEY V. (1981) Psychoneuroendocrine influences on immunocompetence and neoplasia. *Science*, **212,** 1100.

ROGERS H.J. & SYNGE C. (1978) Bacteriostatic effect of human milk on *Escherichia coli*: the role of IgA. *Immunology*, **34,** 19.

ROSENSTEIN M., ETTINGHAUSEN S.E. & ROSENBERG S.A. (1986) Extravasation of intravascular fluid mediated by the systemic administration of recombinant interleukin 2. *J. Immunol.* **137,** 1735.

RUITER D.J., VAN DER MEULEN J., BROUWER A., HUMMEL M.J.R., MAUW B.J., VAN DER PLOEG J.C.M. & WISSE E. (1981) Uptake by liver cells of endotoxin following its intravenous injection. *Lab. Invest.* **45,** 38.

SAVAGE D.C. (1986) Gastrointestinal microflora in mammalian nutrition. *Ann. Rev. Nutr.* **6,** 155.

SCHATTNER A., WALLACH D., MERLIN G., HAHN T., LEVIN S. & REVEL M. (1981) Assay of an interferon-induced enzyme in white blood cells as a diagnostic and in viral disease. *Lancet*, **ii,** 497.

SCOTT G.M. (1984) The toxic effects of interferon in man. In: *Interferon* (ed. I. Gresser), Vol. 5, p. 85. Academic Press, New York.

SEHGAL P.B., MAY, L.T., TAMM I. & VILCEK J. (1987) Human β_2 interferon and β-cell differentiation factor BSF-2 are identical. *Science*, **235,** 731.

SERGIESCU D., GERFAUX J., JORET A.-M. & CHANY C. (1986) Persistent expression of v-*mos* oncogene in transformed cells that revert to nonmalignancy after prolonged treatment with interferon. *Proc. natl. Acad. Sci. U.S.A.* **83,** 5764.

SHALABY M.R., AGGARWAL B.B., RINDERKNECHT E., SVEDERSKY L.P., FINKLE B.S. & PALLADINO M.A. (1985) Activation of human polymorphonuclear neutrophil functions by interferon-γ and tumor necrosis factors. *J. Immunol.* **135,** 2069.

SMITH T.J. & WAGNER R.R. (1967) Rabbit macrophage interferons. I. Conditions for biosynthesis by virus-infected and uninfected cells. *J. exp. Med.* **125,** 559.

SNELLER M.C. & STROBER W. (1986) M Cells and host defence. *J. infect. Dis.* **154,** 737.

SPITZ M., GEARING A., CALLUS M., SPITZ L. & THORPE R. (1985) Interleukin-2 *in vivo*: production of and response to interleukin-2 in lymphoid organs undergoing a primary immune response to heterologous erythrocytes. *Immunology*, **54,** 527.

STEEG P.S., MOORE R.N., JOHNSON H.M. & OPPENHEIM J.J. (1982) Regulation of murine macrophage Ia antigen expression by a lymphokine with immune interferon activity. *J. exp. Med.* **156,** 1780.

STEWART W.E. (1979) *The Interferon System.* Springer-Verlag, Wien.

STREILEIN J.W. (1978) Lymphocyte traffic, T-cell malignancies and the skin. *J. Inv. Dermat.* **71,** 167.

SUGAMURA K., MATSUYAMA M., FUJII M., KANNAGI M. & HINUMA Y. (1983) Establishment of human cell lines constitutively producing immune interferon: transformation of normal T cells by a human retrovirus. *J. Immunol.* **131,** 1611.

TEDESCHI B., BARRETT J.N. & KEANE R.W. (1986) Astrocytes produce interferon that enhances the expression of H-2 antigens on a subpopulation of brain cells. *J. Cell Biol.* **102,** 2244.

TOVEY E.R., CHAPMAN M.D. & PLATTS-MILLS T.A.E. (1981) Mite faeces are a major source of house dust allergens. *Nature (Lond.)*, **289,** 592.

TOVEY M.G. & GRESSER I. (1986) Detection of endogenous interferon messenger RNA *in vivo*. In: *The Biology of the Interferon System 1985* (eds W. E. Stewart and H. Schellekens), p. 257. Elsevier Science Publishers B.V., Asterdam.

TOVEY M.G., STREULI M., GRESSER I., GUGENHEIM J., BLANCHARD B., GUYMARHO J., VIGNAUX F. & GIGOU M. (1987) Interferon messenger RNA is produced constitutively in the organs of normal individuals. *Proc. natl. Acad. Sci. U.S.A.* **84,** 5038.

TRINCHIERI G., KOBAYASHI M., ROSEN M., LOUDON R., MURPHY M. & PERUSSIA B. (1986) Tumor necrosis factor and lymphotoxin induce differentiation of human myeloid cell lines in synergy with immune interferon. *J. exp. Med.* **164,** 1206.

ULEVITCH R.J. & JOHNSTON A.R. (1978) The modification of biophysical and endotoxic properties of bacterial lipopolysaccharides by serum. *J. clin. Invest.* **62,** 1313.

VITI A., MUSCETTOLA M., PAULESU L., BOCCI V. & ALMI A. (1985) Effect of exercise on plasma interferon levels. *J. Appl. Physiol.* **59,** 426.

VOGEL S.N. & FERTSCH D. (1984) Endogenous interferon production by endotoxin-responsive macrophages provides an autostimulatory differentiation signal. *Infect. Immun.* **45,** 417.

VOGEL SN. & FERTSCH D. (1987) Macrophages from endotoxin-hyporesponsive (Lps^d) C3H/HeJ mice are permissive for vesicular stomatitis virus because of reduced levels of endogenous interferon: possible mechanism for natural resistance to virus infection. *J. Virol.* **61,** 812.

WALKER J.R., NAGINGTON J., SCOTT G.M. & SECHER D.S. (1982) An immunoradiometric assay of serum interferon using a monoclonal antibody. *J. gen. Virol.* **62,** 181.

WIERENGA W. (1985) Antiviral and other bioactivities of pyrimidinones. *Pharmac. Ther.* **30,** 67.

WIGZELL H. (1981) Regulation of cytotoxic cells by interferon. In: *Miami Winter Symposia* (eds L. W. Mozes, J. Schultz, W. A. Scott and R. Werner), Vol. 18, p. 403. Academic Press, New York.

WILSON C.B., WESTALL J., JOHNSTON L., LEWIS D.B., DOWER S.K. & ALPERT A.R. (1986) Decreased production of interferon-gamma by human neonatal cells. Intrinsic and regulatory deficiencies. *J. clin. Invest.* **77,** 860.

WOSTMANN B.S. (1981) The germfree animal in nutritional studies. *Ann. Rev. Nutr.* **1,** 257.

WOSTMANN B.S., PLEASANTS J.R. & BEALMEAR P. (1971) Dietary stimulation of immune mechanisms. *Fed. Proc.* **30,** 1779.

YAAR M., PALLERONI A.V. & GILCHREST B.A. (1986) Normal human epidermis contains an interferon-like protein. *J. Cell. Biol.* **103,** 1349.

YMER S., TUCKER W.Q.J., SANDERSON C.J., HAPEL A.J., CAMPBELL H.D. & YOUNG I.G. (1985) Constitutive synthesis of interleukin-3 by leukaemia cell line WEHI-3B is due to retroviral insertion near the gene. *Nature (Lond.)*, **317,** 255.

ZAWATZKY R., ENGLER H. & KIRCHNER H. (1982) Experimental infection in inbred mice with herpes simplex virus. III. Comparison between newborn and adult C57BL/6 mice. *J. gen. Virol.* **60,** 25.

ZOUMBOS N.C., GASCON P., DJEU J.Y. & YOUNG N.S. (1985) Interferon is a mediator of hematopoietic suppression in aplastic anemia *in vitro* and possibly *in vivo*. *Proc. natl. Acad. Sci. U.S.A.* **82,** 188.