Clin. exp. Immunol. (1981) **46**, 475–483.

Evaluation of the human interferon system in viral disease

S. LEVIN & THALIA HAHN *John Askin Department of Pediatric Research, Kaplan Hospital, Rehovot, affiliated to the Hebrew University–Hadassah Medical School, Jerusalem, Israel*

(Accepted for publication 1 May 1981)

SUMMARY

The interferon (IFN) system in man regulates viral replication, cell multiplication and immune functions. Its action against viruses takes place in two stages. The first is the production of IFN by cells following stimulation by a variety of IFN inducers including viruses, and the second is the action of this IFN on other cells inducing in them an antiviral state which prevents replication of infecting viruses. A series of assays is described which evaluates these different parameters of the antiviral IFN system. An attempt was made to correlate between IFN production and cell response to IFN on the one hand, and clinical status on the other. Results show that healthy persons have little or no IFN in the blood (mean < 4 units/ml), and that in 94% their PBMC are not in an antiviral state. On the other hand, patients with acute viral diseases have significantly increased levels of IFN in their blood (mean 150 ± 284 units/ml), and in 70% their cells are in an antiviral state. In some seriously ill patients with viral disease, the IFN system was found to be functionally deficient and treatment with human leucocyte IFN rapidly changed this. It is concluded that the examination of several biological parameters of the IFN system including the intracellular antiviral state induced by IFN is necessary in order to better evaluate this antiviral system. This will enable the clinician to obtain optimal pharmacokinetic information for determining which cases are most likely to respond to IFN therapy, and help to monitor the efficacy of this treatment.

INTRODUCTION

Since the discovery in 1957 by Isaacs & Lindenmann of a biological product produced by cells that inhibit replication of virus within cells, hopes have been kindled that this substance called *interferon* (IFN) would eventually become available for clinical use. At present, clinical trials with different types of IFN are being conducted and knowledge about the behaviour of IFN in the human is rapidly accumulating (Dunnick & Galasso, 1979). There are still gaps in this knowledge, and the present study is an attempt to answer several questions of pharmacokinetics relating to the IFN system in the human body which may have clinical significance particularly as related to viral infections.

It is generally believed that the antiviral effects of IFN are expressed by the development of viral resistance at a cellular level which is species-specific but not virus-specific. Most currently available IFN assays involve the determination of IFN activity by its interference with replication of a virus in heterologous tissue culture monolayers (Stewart, 1979a). The present study was designed to detect simultaneously blood IFN levels and *in vitro* IFN production, both 'classical viral' (type I) IFNα and 'immune' (type II) IFNγ, by the peripheral blood mononuclear cells (PBMC) obtained from

Correspondence: Professor S. Levin, Kaplan Hospital, Rehovot 76100, Israel.

0009-9104/81/1200-0475$02.00

normal healthy individuals and compare them to those with viral infections. At the same time a method was developed for determining the antiviral state of PBMC by assessing the ability of these cells to support virus replication *in vitro*, as well as for examining the effect of exogenous human leucocyte interferon (IFNα) on intracellular virus replication.

PATIENTS AND METHODS

Patient population. Blood was obtained from the following groups of subjects. Forty-seven normal healthy children and adults who were free of any clinical viral or bacterial infection when the specimen was obtained following informed consent. The ages ranged from newborns to adulthood, and both sexes were equally represented. All age groups were analysed together as a previous study of ours showed no difference in IFN production and cell protection amongst newborns, children and adults (Handzel *et al.*, 1980). A second group consisted of 76 patients with a mean age of 6 years (range 1 week–28 years) who were suffering from clinically diagnosed viral infections (Table 1). About 80% of the patients could be classified as mildly to moderately ill, and only 20% were seriously ill. Specimens were obtained at onset of the illness, and only cases in which subsequent confirmation of viral infection was obtained were included in the study. Several patients with serious life-threatening viral illness received IFNα* and were extensively studied over a period of several weeks. They included a young pregnant mother with fulminant hepatitis B infection, an adult with untreated leukaemia and extensive pyoderma gangraenosum due to herpes simplex virus, an immunosuppressed young girl with neuroblastoma and spreading herpes simplex infection, and a newborn infant in coma with varicella encephalitis.

Table 1. List of viral diseases in which interferon system studies were performed

Encephalitis		27	(7)
Herpes	8 (2)		
Echo	6 (2)		
Varicella	4 (2)		
Mumps	2 (1)		
Measles	1		
Unspecified	6		
Viral hepatitis		15	(1)
Non A, non B	10 (1)		
A	3		
B	2		
CMV infections	8		
Rubella		6	(1)
Herpes zoster		4	
RSV infections		3	(2)
Infectious mononucleosis		3	
Measles		2	
Congenital rubella		2	(2)
Guillain–Barré		2	(1)
Miscellaneous		4	
Total		76	(14)

Ages: mean = 6 years; range = 1 week–28 years. Figures in parentheses indicate cases in which there was 'spontaneous' production of IFN in culture.

* The human leucocyte IFN used in these studies and in the treatment of patients was produced and kindly supplied to us by Dr Hagai Rosenberg and Tamar Bino of the Israeli Biological Institute, Nes-Ziona, Israel.

Cell preparation and treatment. Plasma and PBMC were separated from heparinized blood by Ficoll–Hypaque (Ficoll–Paque, Pharmacia, Sweden) gradient centrifugation (Böyum, 1968). (a) The plasma was stored at −20°C until tested. (b) The PBMC were washed three times in phosphate-buffered saline, pH 7·2, resuspended and incubated in RPMI 1640 medium (Biolab, Jerusalem) supplemented with 10% fetal calf serum (GIBCO, NY) and gentamicin, 0·04 mg/ml (Teva, Jerusalem), in wells of microtitre test plates (No. 3596 Costar, Cambridge, Massachusetts) as follows: (1) Cells (5×10^4) in 0·1 ml medium, with and without varying concentrations of IFNα, were incubated at 37°C in humidified CO_2 and air for 24 hr and then infected with vesicular stomatitis virus (VSV) at a multiplicity of infection of 0·1 (i.e. approximately 5×10^3 PFU) and incubation was continued for an additional 48 hr. (2) Cells ($1–2 \times 10^6$) in 0·2 ml medium alone or with inducers of IFN production—polyinosinic–cytidilic acid (poly I:C, GIBCO, NY), 100 μg/ml for IFNα, or phytohaemagglutinin (PHA, Wellcome, England), 40 μg/ml for IFNγ—were incubated for 48 hr (Hahn, Levin & Handzel, 1980).

IFN assay. IFN activity was assayed by inhibition of the cytopathic effect (CPE) of VSV on fibroblasts of the MDBK bovine continuous cell line, which have been shown to be sensitive to human IFNα and IFNγ. MDBK cultures, in wells of microtitre test plates, were exposed for 24 hr at 37°C, to serial two-fold dilutions of the previously separated plasma and of supernatants of the PBMC cultured with and without IFN inducers. The fibroblast monolayers were subsequently washed and then infected with 1,000 $TCID_{50}$/ml of VSV. Each assay incorporated similar dilutions of a standard IFN preparation (BRS 69/19, Medical Research Council, London). Virus alone and medium alone served as controls. CPE was determined when the virus control showed confluent CPE. IFN titres were determined by the last dilution of plasma or supernatant to inhibit 50% CPE and compared to that of the standard IFN preparation. IFN titres are expressed in international units (units/ml) using BRS 69/19 as a standard.

Since serum and cell culture supernatants are known to inactivate virus directly at low dilutions (up to 1:8), materials that failed to show antiviral activity at higher dilutions were considered inactive.

Virus titration. Virus titres obtained from the IFN-treated and untreated VSV-infected PBMC cultures were determined by CPE in MDBK monolayers exposed to 10-fold dilutions of those cultures for 24–48 hr. VSV titres are expressed as reciprocals of dilutions causing 50% CPE ($TCID_{50}$) and virus was considered to replicate only when CPE was obtained at dilutions of $1:10^3$ or more. This dilution was chosen in order to eliminate the possibility of CPE caused by residual viral activity of the infecting virus preparation as opposed to that of *de novo* replicated virus.

RESULTS

The results of this simultaneous assay of various parameters of the IFN system are described in Table 2 and can be divided into four major sections. (a) The amount of IFN present in the blood at a given time. (b) The antiviral state of the PBMC evidenced by the ability of VSV to replicate in infected cells. (c) The ability of cells supporting viral replication to be induced into an antiviral state by exogenous IFNα at various concentrations and (d) the ability of the individual's PBMC to produce IFNα or IFNγ in response to appropriate stimuli.

Healthy individuals

(a) Healthy individuals have virtually no IFN in their blood with a mean of < 4 units/ml which is the lowest level of antiviral activity evaluated by us. Only two individuals out of 47 had as much as 16 units/ml in their blood.

(b) PBMC from 94% of this group promoted viral replication to titres equal to or greater than 10^3 $TCID_{50}$/ml after 48 hr and in some instances as high as 10^7 $TCID_{50}$/ml. A control study showed that VSV that had been incubated without cells was inactivated after 48 hr at 37°C. This can be seen in Fig. 1 which shows virus titres obtained in PBMC in the presence and absence of IFN as well as viral inactivation in the absence of cells.

Table 2. Interferon production and protection assay in health and in viral illness

	Plasma IFN (≥16 units/ml)	Viral replication in cells*			IFN production by mononuclear cells (≥16 units/ml)		
		No added IFNα (≥10^3)	100-fold inhibition by added IFNα			Stimulated	
			8 units/ml	128 units/ml	Unstimulated	Poly I:C (IFNα)	PHA (IFNγ)
Healthy control							
(n=47) (%)	5	94	82	100	5	98	87
$\bar{x}\pm$s.d. (units/ml)	<4	$10^{4\cdot8\pm1\cdot7}$			<4	510±638	166±248
Viral illness							
(n=76) (%)	81	30	55	100	20	90	63
$\bar{x}\pm$s.d. (units/ml)	150±284	$10^{1\cdot9\pm2\cdot0}$			22±87	250±375	85±150
P	<0·001	<0·001			<0·05	<0·02	<0·05

* 10^3 virus added to cells 48 hr previously: 10^3 viral growth considered positive replication (no antiviral state).

(c) Preincubation of PBMC with 8 units/ml IFNα diminished viral replication 100-fold or more in 82% and with 128 units/ml IFNα in 100% of assays.

(d) IFNα production by PBMC equal to or more than 16 units/ml was observed in 98% (mean 510 units/ml) and IFNγ in 78% (mean 166 units/ml) of this group. Only two out of 47 cases showed some spontaneous IFN production without stimulation *in vitro*, and this at very low levels. Our results also indicated that there were no significant differences in values between newborns and older individuals.

Patients with viral infections

(a) The mean blood IFN level in this group was 150 units/ml, significantly higher ($P<0\cdot005$) than in the healthy group, and 81% of the patients had 16 units/ml IFN or more.

(b) PBMC promotion of viral replication was considerably lower than in group 1 and in only 30% of cases was a titre of 10^3 $TCID_{50}$/ml or greater attained.

(c) Of the 22 cases that did yield 10^3 $TCID_{50}$/ml or more virus, 11 had 16 units/ml IFN or less in

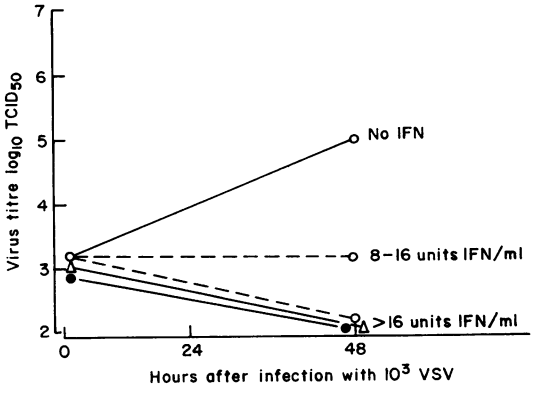

Fig. 1. Control study assaying viral replication on mononuclear cells in relation to amount of interferon present in culture medium. (o——o) Normal healthy donors (blood IFN < 4 units/ml), (o----o) normal donor with added IFN *in vitro*, (Δ——Δ) patient with viral infection (blood IFN > 16 units/ml), (●——●) control VSV without cells.

Table 3. Interferon system assay in four patients receiving interferon

Patient*	Day of treatment	Blood IFN (units/ml)	Virus replication in cells: No added IFNα	Virus replication in cells: Inhibition with IFNα: 8 units/ml	Virus replication in cells: Inhibition with IFNα: 64 units/ml	IFN production: Spontaneous	IFN production: Poly I:C (IFNα)	IFN production: PHA (IFNγ)
1. 23 year-old, 6-month pregnant	−1	0	10^5	10^1	0	0	125	0
woman, comatose, due to rapid	+4	8	10^2	10^1	0	0	500	125
onset fulminant hepatitis B	+6	32	0	0	0	0	64	16
2. 12 year-old with neuroblastoma	−1	32	10^7	10^5	0	0	64	64
who developed extensive herpes	+7	64	10^1	10^1	0	16	0	0
simplex whilst immunosuppressed								
3. 35 year-old male with leukaemia	−1	0	10^7	10^7	10^2	0	0	0
prior to chemotherapy, with	+1	8	10^1	0	0	0	125	0
extensive bullous pyoderma	+3	0	10^1	10^1	0	0	64	0
gangraenosum due to herpes simplex	+8	16	10^1	0	0	0	64	0
4. Newborn infant with varicella	−1	16	10^3	10^2	0	0	1,000	500
and severe encephalitis	+1	125	10^1	10^1	0	0	32	0

* The first three patients received 3×10^6 units IFNα (kindly supplied by Drs Hagai Rosenberg and Tamar Bino of the Israeli Biological Institute, Nes-Ziona) by daily i.m. injection. The infant received 70·000 units/kg i.m. daily. All blood samples were taken prior to the daily IFNα injection. The table indicates selected days following treatment where significant changes occurred.

the blood, and in 50% of them as little as 8 units/ml of added IFNα decreased the viral yield by 100-fold or more.

(d) Both IFNα and IFNγ production by stimulated PBMC was significantly less than in healthy controls. Spontaneous production of IFN occurred in 14 out of 76 cases (18·5%), usually in low titres (16–32 units/ml), although in three cases as much as 500 units/ml IFN was produced (one case each of mumps, encephalitis, varicella encephalitis and RSV infection). In Table 1 we have designated those cases in which IFN was produced by cultured PBMC without exogenous stimulation. We could discern no specific clinical or other reason for this 'spontaneous' production of IFN, although PBMC from both cases of congenital rubella with persistent viral infection produced IFN. Also, 25% of encephalitis cases had some unstimulated IFN production in culture.

(e) Attempts to correlate the results with the age of the patient or the severity of the illness were in general unrewarding, with the exception of the four severely ill patients described below.

Interferon-treated patients

Four severely ill patients with viral infections were treated with IFN and their pretreatment values are included in Table 2. Despite the fact that viral infection had been present for some time in all of them, blood IFN levels prior to IFN therapy were very low or undetectable, and their PBMC-promoted viral replication indicated absence of an antiviral state (Table 3). However, their PBMC were shown to be sensitive to exogenous IFN as, when added *in vitro*, an antiviral state was induced at relatively low dosage levels. IFN production by PBMC was variable. During IFN therapy blood IFN levels taken 24 hr following the previous daily injection remained low in two cases, and slightly increased in two others. IFN assays were performed daily and in some cases several times daily, and in Table 3 we have selected assays on those days where significant changes occurred. What was impressive was the rapid development of an antiviral state in the patient's PBMC concomitant with clinical improvement and evidence of containment of the viral infection.

DISCUSSION

Some two decades following the original description of a biological substance which causes interference with intracellular viral replication, a highly complex IFN system is being unravelled which is involved in several biological activities in the body. Besides its effect of protecting cells against viruses, IFN also inhibits cell multiplication and is an immune modulator (Baron & Dianzani, 1977). The mechanism for inducing viral resistance involves two phases—IFN production followed by the induction of an antiviral state in cells. The first phase of antiviral IFN activity begins when a virus or other inducer enters a cell or even attaches to its membrane, leading to the derepression of the IFN genome on the 5th or 9th chromosome permitting the formation of a messenger RNA which is translated by cytoplasmic ribosomes into IFN. Actual synthesis begins a few hours after induction, and IFN molecules are rapidly secreted into the surrounding areas. IFN synthesis in the cell is short-lived (from 1 to 4 days) and is followed by a refractory state lasting from 5 to 13 days during which further IFN is not secreted (Stewart, 1979b). Phase two begins when IFN molecules bind to receptors on the cell membrane (Besancon, Ankel & Basu, 1976). This signals the production in the cell of precursor antiviral proteins or enzymes which are initially inactive (Epstein, 1979). These soluble precursors are activated when a virus or viral genetic material enters the cell, and selectively prevents translation of early viral mRNA thereby preventing viral replication. IFN itself has no known direct effect on viruses.

The assays described in this study are based on this concept and, using peripheral blood plasma and mononuclear cells, an attempt was made to answer several questions which arise concerning the IFN system in patients with viral illnesses.

(a) In the presence of viral infection is the level of IFN in the blood increased indicating effective *in vivo* cell stimulation and production?
(b) Has IFN produced an antiviral state in cells *in vivo*?
(c) If not, is the intracellular antiviral IFN mechanism intact and can the cells be primed to prevent viral replication *in vitro* by exogenous IFN, and at what dosage levels?

(d) Are the PBMC capable of producing IFNα and IFNγ when appropriately stimulated? (We did not examine for IFNβ production.)

(e) Do the cells produce IFN *in vitro* without external stimulation indicating possible intrinsic (viral?) cellular stimulation?

In some cases of viral infection with or without viraemia, IFN levels may vary from day to day. Our studies, as well as those of others, have shown this variability in blood IFN levels which may be partly due to the short half-life of IFN of about 4 hr or less (Stewart, 1979a). The presence of low IFN levels therefore may not always be a true indication of a defective IFN response in a patient with viral illness, whereas high values obviously have significance. Our results show that there is a significant and discriminatory difference between blood IFN levels in healthy persons as a group compared to those with viral illnesses. Only two out of 47 healthy individuals had as much as 16 units/ml IFN in the blood, with the majority having undetectable amounts. On the other hand, only 14 out of 76 patients with viral illnesses had less than 16 units/ml, whilst the majority had values that ranged between 16 and 2,000 units/ml with a mean of 150 units/ml.

When blood IFN levels are elevated, it is obvious that cells are producing IFN. However, if IFN is not present or present only in small amounts, the question arises as to whether the cells are functionally capable of producing IFN if appropriately stimulated. This may be ascertained *in vitro* by assessing IFN production by stimulated PBMC which are known to produce both IFNα and IFNγ depending on the inducing agent. We have previously reported that PBMC produce IFNα when stimulated by poly I:C and IFNγ when stimulated by PHA, as characterized by stability to pH 2·0 (Hahn *et al.*, 1980). Our study shows that normal healthy individuals are capable of producing both types of IFN while lower levels were produced by patients with viral illnesses. Whether or not this is due to the immune-suppressive effect of the IFN present *in vivo* or directly to the viral infection itself remains to be clarified. No correlation was observed in individuals between the production of both types of IFN, and in some cases where little or no IFNα was produced, high levels of IFNγ could be found, and vice versa. In all healthy individuals it was possible to stimulate the production of at least 16 units/ml of one or both types of IFN in this test system, whereas in three cases with viral infection no IFN was present in the blood nor could the PBMC be stimulated to produce it. Whether the results obtained using PBMC can be extrapolated to IFN production by other body cells has yet to be proven.

Of interest is the spontaneous production of IFN by cultured PBMC from patients with viral illness without extrinsic stimulation. Three out of 76 patients produced more than 250 units/ml IFN and 11 others produced 16–32 units/ml of IFN. Only two normal individuals produced as much as 16 units/ml. Possibly IFN production by these cells in culture without extrinsic stimulation could indicate the presence of some intrinsic inducer such as an intracellular viral genome, as in the two cases of congenital rubella.

The assay of the antiviral state of PBMC described here has proven most interesting. Although it is likely that there are other mechanisms involved in the prevention of intracellular virus replication, such as changes induced by the patient's virus infection altering PBMC infectivity (e.g. change in a lymphocyte subset) (Denman *et al.*, 1976) or induction of a cell population (e.g. natural killer cells) which limits VSV infection (Herberman *et al.*, 1978), it is possible that the IFN system is a determining factor and our results seem to indicate this. If IFN has been produced and secreted in sufficient amounts *in vivo*, then theoretically it should have come into contact with IFN receptors on the cell membranes of non-infected cells, thereby inducing a potential antiviral state in the cell. Viruses should not replicate in these cells. Conversely, when intracellular viral replication does occur *in vitro*, absence of the antiviral state is assumed which may be due either to absence of IFN in the blood as is found in healthy persons, or to the production of defective IFN, or to a deficient intracellular IFN system. The latter can be ascertained by attempting to trigger the intracellular antiviral state in cells promoting good viral replication by *in vitro* preincubation with extrinsic IFNα. Our results indicate that in 94% of healthy individuals an antiviral state does not exist, and replication of 10^3 $TCID_{50}$/ml or more VSV could be obtained. There is a significant inverse correlation in this group of persons between viral replication in PBMC and the amount of IFN in the blood. Moreover, preincubation of these cells *in vitro* with 8 units/ml of IFNα reduced viral

replication at least 100-fold, and in most cases preincubation with 32 or 64 units/ml completely inhibited viral replication, indicating an intact intracellular IFN-dependent antiviral mechanism.

In patients with viral illness viral replication in PBMC was greatly diminished, and in the majority no viral proliferation whatsoever was observed in infected PBMC. This antiviral state correlated well with the elevated blood IFN levels and it was mainly in those few patients with viral illness in which blood levels were undetectable or very low that high titres of virus could be found. Here again preincubation with as little as 8 units/ml of IFNα reduced viral growth 100-fold in most cases and 128 units/ml eliminated viral replication in all cases. In several cases, the IFN assay was performed at the onset of viral illness, and repeated 10–14 days later following clinical recovery, when the stimulated IFN system generally had returned to 'normal'.

Several investigators have claimed that VSV proliferation occurs on monocytes and not on unstimulated lymphocytes (Edelman & Wheelock, 1967). We examined this in our study and found that although our PBMC populations consisted of a mixture of these cells, there was no correlation between the proportion of monocytes and the levels of virus titres obtained.

Pharmacokinetic studies have been performed on four patients with life-threatening viral disease who received treatment with IFNα (Table 3). Three of the four showed evidence at the onset of therapy of a deficient IFN system as indicated by low blood IFN levels and the absence of an intracellular antiviral state despite the presence of viral infection of some days' duration. Case 4 was of more acute onset and showed evidence of *in vivo* IFN response. Preincubation of the patient's cells with IFNα induced an antiviral state in all cases. Following IFNα therapy, blood IFN levels were variable and not too helpful clinically, particularly in the first three cases where blood samples were taken as often as 6-hourly. However, an *in vivo* antiviral state was rapidly induced in PBMC in all the cases concomitant with marked clinical improvement and evidence that the virus infection was under control. How soon after onset of therapy the antiviral state is induced, and how long it persists is under investigation. The presence of a deficiency of the IFN system as noted in three of these cases, and its relationship to severity of viral illness has been reported elsewhere (Levin & Hahn, 1980).

In conclusion it appears from our studies that this combined assay should be of particular assistance to the clinician evaluating a patient's resistance to a viral infection and facing the dilemma of deciding which case would benefit from IFN therapy. Our experience indicates that the evaluation of the antiviral state of the patient's mononuclear cells is more relevant to the resistance quotient to viral disease and response to IFN therapy than the IFN blood levels. Further work in progress will determine whether the *in vitro* viral replication inhibition assay will prove useful in determining dosage requirements and optimal blood IFN levels for successful IFN therapy in viral illness.

This work was supported in part by a grant from the National Council for Research and Development, Israel, and GSF, München, Germany.

We gratefully acknowledge the technical assistance of Mrs Valentina Bergman and the constructive criticism of this study by Professor Pearay Orgra of the Buffalo Childrens Hospital, Buffalo, New York.

REFERENCES

BARON, S. & DIANZANI, F. (1977) General considerations of the interferon system. *Tex. Rep. Biol. Med.* **35,** 1.

BESANCON, F., ANKEL, H. & BASU, S. (1976). Specificity and reversibility of interferon ganglioside interaction. *Nature*, **259,** 576.

BÖYUM, A. (1968) Separation of leukocytes from blood and bone marrow. *Scand. J. clin. Lab. Invest.* **21** (Suppl. 97), 1.

DENMAN, A.M., PELTON, B.K., APPLEFORD, D. & KINSLEY, M. (1976) Virus infections of lymphoreticular cells and autoimmune disease. *Transplant. Rev.* **31,** 79.

DUNNICK, J.K. & GALASSO, G.J. (1979) Clinical trials with exogenous interferon. Summary of a meeting. *J. infect. Dis.* **139,** 109.

EDELMAN, R. & WHEELOCK, E.F. (1967) Specific role of each human leucocyte type in viral infections. I. Monocyte as host cell for VSV replication *in vitro*. *J. Virol.* **1,** 1134.

EPSTEIN, L.B. (1979) The comparative biology of immune and classical interferons. In *Biology of the Lymphokines* (ed. by S. Cohen, E. Pick and J. J. Oppenheim), p. 443. Academic Press, New York.

HAHN, T., LEVIN, S. & HANDZEL, Z.T. (1980) Production of immune and viral interferon by lymphocytes of newborn infants. *Isr. J. Med. Sci.* **16,** 33.

HANDZEL, Z.T., LEVIN, S., DOLPHIN, Z., SCHLESINGER,

M., HAHN, T., ALTMAN, Y., SCHECHTER, B., SCHNEYOUR, A. & TRAININ, N. (1980) Immune competence of newborn lymphocytes. *Pediatrics*, **65**, 491.

HERBERMAN, R.B., DJEU, JY., ORTALDO, J.R., HOLDEN, H.T., WEST, W.H. & BONNARD, G.D. (1978) Role of interferon in augmentation of natural and antibody-dependent cell-mediated cytotoxicity. *Cancer Treat. Rep.* **62**, 1893.

ISAACS, A. & LINDENMANN, J. (1957) Virus interference. I. The interferons. *Proc. R. Soc. Ser. B*, **147**, 258.

LEVIN, S. & HAHN, T. (1980) The interferon system in immunodeficiency, and deficiency of the interferon system. In *Primary Immunodeficiencies* (ed. by M. Seligman and W. H. Hitzig), p. 465. Elsevier/North-Holland, Amsterdam.

STEWART, W.E., II (1979a) Interferon assays. In *The Interferon System* (ed. by W. F. Stewart II), p. 13. Springer-Verlag, New York.

STEWART, W.E., II (1979b) Pharmacokinetics of interferon. In *The Interferon System* (ed. by W. E. Stewart II), p. 257. Springer-Verlag, New York.