Clin. exp. Immunol. (1985) **60,** 267–273.

Interferon deficiency syndrome

S. LEVIN & TALIA HAHN *Pediatric Research Institute, Kaplan Hospital, Rehovot, Israel*

(Accepted for publication 26 November 1984)

SUMMARY

Activation of the interferon (IFN) system is an early defence mechanism against viral infections. The virus stimulates production of IFN by nucleated cells including the peripheral blood mononuclear cells (PBMC), and this IFN in turn activates several IFN-dependent immune mechanisms including the induction of an anti-viral state in cells, which prevents or retards further intracellular viral replication. In an ongoing study of 1,500 individuals of all ages and with various illnesses, we found 15 cases (representing 5% of patients with acute viral disease) in whom the IFN system response during an acute viral illness was absent or grossly deficient. There was no detectable IFN in the blood, PBMC did not produce IFN-α and IFN-γ or produced minimal amounts of one of them *in vitro* following appropriate stimulation, and the patients' PBMC were not in an anti-viral state. These patients had severe progressive or fulminant viral disease, often ending fatally. IFN therapy appears to be beneficial in these cases, as it rapidly induced a cellular antiviral state in most cases, stimulated *in vitro* IFN-α and IFN-γ production by PBMC, and led to rapid recovery in seven of the nine patients who received treatment for at least 3 days. In our opinion IFN replacement therapy should be commenced as early as possible in such cases, and before irreversible cell and organ damage occur.

Keywords interferon peripheral blood mononuclear cells vesicular stomatitis virus poly-inosinic-cytidilic acid phytohaemagglutinin cytopathogenic effect

INTRODUCTION

Activation of the interferon (IFN) system is an important and early defence mechanism against viral infections (Stewart, 1979). There are two phases in this mechanism: during the first phase cells are stimulated by the virus to produce IFN, and this is followed by a phase in which IFN-dependent mechanisms are activated. The blood of normal healthy persons contains minimal amounts of IFN, but many cells have the capacity to produce various types of IFN when appropriately stimulated.

Most viruses are excellent triggers of IFN production by cells. Many mitogens and antigens, including a number of non-viral micro-organisms, can also stimulate IFN production, but generally to a lesser degree than viruses (Stewart, 1979). The reason why a particular molecule, antigen or organism stimulates IFN production is not yet clear. It is known that that IFN levels in the blood may be elevated within hours of the appearance of viraemia, suggesting the importance of IFN in the early immune response against viruses. The IFN molecules act as biological messengers, activating a series of biological mechanisms which are important in maintaining the integrity of the immune system and helping in the defence against non-self antigens or cells.

Deficiencies have been reported of immune mechanisms related to the lymphocyte, granulocyte or complement systems (Stiehm & Fulginiti, 1980); it is thus conceivable that a specific clinical syndrome could result from a defect, either genetically determined or acquired, of the IFN system.

Correspondence: Dr Stanley Levin, Pediatric Research Insitute, Kaplan Hospital, Rehovot-76100, Israel.

In this report we describe the results of a study on IFN in patients suffering from a variety of diseases including virus illnesses, as well as in healthy controls. We also report in more detail on 15 patients, all of whom were critically ill with particularly severe or fulminant viral disease, and in whom the anti-viral IFN system did not respond normally to the presence of a viral infection. Treatment of these patients with natural IFN-α activated the intracellular IFN system in most cases, and this was usually accompanied by a reversal of the progressive course of the disease.

MATERIALS AND METHODS

Patients and assays. Fifteen hundred patients of all ages and with a variety of diseases, as well as a group of 75 normal healthy persons, underwent blood studies of the IFN system following informed consent. In general, heparinized blood (6·0–10·0 ml) was withdrawn under sterile conditions from each individual. Fresh peripheral blood mononuclear cells (PBMC) were separated on a Hypaque-Ficoll gradient and used for all assays requiring cells. The following parameters were assayed as previously described (Levin & Hahn, 1981).

(a) *In vivo* IFN levels in the plasma.

(b) *In vitro* IFN production as expressed by the ability of the PBMC to produce IFN-α (viral IFN) following stimulation with poly-inosinic-cytidilic acid (poly-IC), and IFN-γ (immune IFN) following phytohaemagglutinin (PHA) stimulation.

(c) Evaluation of an anti-viral state in the PBMC by assaying whether vesiculo-stomatitis virus (VSV) would replicate in these cells in culture.

(d) If no anti-viral state was present (i.e. if the yield of virus was higher than the infecting dose), the integrity of the intracellular anti-viral mechanism was assayed by noting whether and at what dosage an anti-viral state could be induced *in vitro* by extrinisic IFN.

Assays of IFN production. IFN production was studied originally both *in vivo* and *in vitro* by measuring the inhibition of the cytopathogenic effect (CPE) of VSV on cells by IFN in the plasma, and in the supernatant following stimulation of PBMC in culture. At first these assays were carried out using human IFN sensitive fibroblasts from the MDBK bovine continuous cell line (Stewart, 1979), and later on human HeLa (H229) cells in culture. More recently we have employed an ELISA assay (Wallach, 1983), using an antiserum to VSV to measure the amount of viral protein in culture supernatants. The results of each assay are related to an International IFN standard (BRS 69/19) and are designated in units/ml.

Assay of the anti-viral state. This was assayed by measuring the replication efficiency of VSV in PBMC. One hundred thousand PBMC pre-treated with IFN-α at concentrations ranging from 0 to 64 units/ml were infected with 10^3 $TCID^{50}$ of VSV. The virus yield was assayed after 48 h by examining the CPE following the addition of tenfold dilutions of VSV infected PBMC supernatants to indicator fibroblast monolayers, or by measuring the amount of viral protein in culture. A virus yield greater than the original infecting dose of 10^3 $TCID^{50}$ indicated viral replication and absence of anti-viral state in the PBMC examined. Our studies have shown that lymphocytes do not need to be in a stimulated state in order to support viral replication, and that good correlation exists between increased levels of IFN in the blood (>16 units/ml) and the presence of an anti-viral state ($<10^3$ $TCID^{50}$ viral yield) in the PBMC (Levin & Hahn, 1981).

Assay of the integrity of the intracellular anti-viral mechanism. When an anti-viral state was absent in the untreated sample, the integrity of the intracellular anti-viral mechanism was evaluated by noting whether and at what dosage level an anti-viral state could be induced *in vitro* by exogenous IFN-α.

RESULTS

In normal healthy individuals of all ages, including newborn infants, there is virtually no IFN in the serum (mean $\pm$ s.e., $5 \pm 1{\cdot}4$ units/ml), and in only 13% are the cells found to be in an anti-viral state. On the other hand, of all the patients studied about 300 (or 20%) were clinically diagnosed as being

in an acute phase of a viral disease. In this group the mean serum IFN levels (76 ± 10 units/ml) and the presence of a cellular anti-viral state (in 68% of patients) were significantly different from those in healthy persons (Table 1). However, about 20% of the patients in the virus control group had < 16 units/ml of IFN in the blood, and 32% were not in an anti-viral state, usually but not necessarily concurrently. We also found a few patients whose PBMC did not produce IFN-α whilst IFN-γ production was normal, and vice versa. In spite of this partial defect nearly all of these patients showed increased blood IFN levels and/or cells in an anti-viral state concurrently with the acute phase of viral disease. In several cases repeat assays on recovery showed normal production of the previously absent IFN.

Amongst the patients studied were 15 who had acute, severe, progressive viral disease and in whom the total IFN response was defective: there was no IFN in the serum, no anti-viral state and, most important, markedly defective or absent IFN-α and IFN-γ production *in vitro*. In nine of these patients neither type of IFN was produced *in vitro*; in two, less than 10 units/ml of one of them, and in the remaining four, small amounts of either IFN-α or IFN-γ were secreted following appropriate stimulation (Table 1). In five cases, 8 units of exogenous IFN-α was sufficient to induce an anti-viral state *in vitro*, while in eight cases as much as 64 units was required. In one case an anti-viral state could not be induced even by 64 units of exogenous IFN. *In vivo*, an anti-viral state was induced in cells within 2–5 days of instituting IFN therapy, and in several cases *in vitro* IFN-α and IFN-γ production became evident within 1–10 days of injecting IFN-α.

Daily i.m. treatment with 3×10^6 (or 1×10^5/kg) units IFN-α (prepared from blood bank leucocytes by H. Rosenberg and T. Bino at the Israel Institute for Biological Research, Ness Ziona.

Table 1. IFN assays in 15 cases with IFN deficiency syndrome

Patient No.	Diagnosis (age)	IFN production (units/ml)			Cell protection (virus replication)*		
		In vivo serum IFN	*In vitro* IFN-α	*In vitro* IFN-γ	Natural level	After 8 units IFN	After 64 units IFN
1	Fulminant hepatitis, herpes (18 months)†§	0	0	0	10^5	10^3	0
2	Fulminant hepatitis A (47 years)	0	0	0	10^7	10^6	10^1
3	Hepatitis + malnutrition (4 months)†	0	0	0	10^4	0	0
4	Fulminant hepatitis non-A/non-B (6 years)†	0	0	0	10^4	0	0
5	Herpes + pyoderma gangrenosum (36 years)§	0	0	0	10^7	10^5	0
6	Herpes + Hodgkins (40 years)§	0	0	0	10^4	10^3	0
7	Herpes encephalitis (4 months)†	0	0	0	10^4	10^2	0
8	Herpes encephalitis (18 years)†	0	0	0	10^4	10^3	10^2
9	Herpes encephalitis (24 years)†	0	0	0	10^4	10^4	10^3
10	Toxic varicella (post-measles) (15 months)§	0	8	0	10^7	10^5	10^2
11	Progressive hepatitis A (4 years)§	0	0	27	10^3	10^1	0
12	Fulminant hepatitis non-A/non-B (11 years)§	0	32	0	10^7	10^4	0
13	Fulminant hepatitis B (66 years)†§	0	64	0	10^7	10^6	0
14	Fulminant hepatitis B (23 years)§	0	125	0	10^5	10^1	0
15	AIDS (28 years)†	0	0	5	0	0	0
Normal healthy persons ($n = 75$)		5 ± 1·4‡	382 ± 62	170 ± 26	10^5 (mean)		0
Acute viral infections ($n = 291$)		76 ± 9·7	215 ± 14	165 ± 15	10^2 (mean)		0

* Anti-viral state is present when $< 10^3$ virus is found after 48 h culture of 10^3 VSV on PBMC.
† Died.
‡ Mean ± 1 s.e.
§ Received IFN-α treatment.
n = number of cases.

Israel) to nine of the 15 patients as part of an ongoing clinical trial of IFN-α in acute life threatening viral illnesses (Levin *et al.*, 1982), was followed by recovery in seven. In seven of the treated patients, the intracellular IFN system was activated within 1–5 days of starting IFN therapy. All six untreated patients died.

DISCUSSION

Since the original report of Isaacs & Lindenmann (1957) of an anti-viral substance (IFN) secreted by cells infected by viruses, a number of endogenous IFN have been identified which apparently act as biological messengers, priming or activating a variety of immune and other defence mechanisms. One end result of IFN production is the induction of an anti-viral state in cells, accomplished through the synthesis of intracellular anti-viral proteins which interfere with the translation, assembly and replication of an invading virus (Stewart, 1979). This then allows other defence mechanisms, such as antibody activity or cytotoxicity, to exercise their function in restoring and maintaining homeostasis and in preventing irreversible damage to cells and tissues.

Although the blood of healthy individuals contains virtually no IFN, and their cells (PBMC) are not normally in an anti-viral state, the PBMC are capable of responding *in vitro* to appropriate stimuli by producing both IFN-α and IFN-γ (Levin & Hahn, 1981). In acute viral infections, blood IFN levels are significantly increased and an anti-viral state may be found in about 2/3 of cases (Levin & Hahn, 1981) (Table 1). The importance of the intracellular IFN response in viral illnesses is indicated by the increased amounts of IFN found in the blood within hours of the appearance of viraemia, as well as by the activation of IFN-dependent secondary immune responses within a few hours or days (Saksela, 1981). Our own studies have demonstrated the induction of an anti-viral state in PBMC within 24 h following i.m. injection of IFN in some cases, and in other cases only after 3–4 days or longer. If promptness of the IFN response to viral infection is important, delay in producing endogenous IFN (or an inability to produce it) may lead to uninhibited progression of the viral infection with a serious or fatal outcome.

Some of the patients were found to have no detectable IFN in the blood in spite of being in the acute stage of viral disease. One possible reason for this is that blood IFN levels are known to fluctuate, partly on account of the very short half-life (± 4 h) of circulating IFN. Another possibility is that the infecting virus might be a poor interferonogen, as observed in patients with respiratory syncytial virus infections (Breese Hall, Douglas & Geiman, 1978). The hypothesis that massive viraemia may interfere with the IFN system has not been proved. We also found patients who were in an acute phase of viral disease with relatively low IFN blood levels (16–64 units/ml) and in whom an anti-viral state was absent. This could be explained by the great variability found in the amount of added IFN needed (ranging from 8 to 64 units/ml) to induce an anti-viral state *in vitro*. In cancer patients this relative resistance is quite pronounced (Hahn & Levin, 1982). Once an anti-viral state has been induced in PBMC it tends to persist for several days after IFN levels have become undetectable. We found several patients whose PBMC did not produce either IFN-α or IFN-γ following *in vitro* stimulation with poly-IC or PHA, respectively; however, absence of both IFN-α and IFN-γ production in the same patient is unusual and has been seen, in our experience, only in the 15 cases with a severe prolonged viral illness which in some cases was fatal. It should be noted that not all critically ill patients with acute, unresponsive viral illnesses had defective IFN responses as measured by our assay systems; thus the reason for progressive infection in these latter patients must be sought elsewhere.

As shown in Table 1, the intracellular IFN anti-viral mechanism was intact in all the IFN system deficiency patients but one, as indicated by the induction of an anti-viral state *in vitro* on addition of 8–64 units/ml of IFN. It seems, therefore, that the primary defect in these patients was an inability to produce endogenous IFN on appropriate stimulation. IFN therapy led to rapid induction of an anti-viral state of cells and stimulated production of IFN-α and IFN-γ by PBMC. Clinical improvement usually paralleled the appearance of an anti-viral state of cells in the seven of nine patients that were treated and recovered.

Eight of our patients with IFN system response deficiency had fulminant viral hepatitis. Because of reports in the late 1960s suggesting that IFN levels in viral hepatitis were abnormally low, we

considered the possibility that the IFN system is not activated or is defective in this condition. However, we have shown in a previous study that the response of the IFN system in patients with viral hepatitis is not significantly different from that in other virally infected patients (Levin & Hahn. 1982).

It is unlikely that defective IFN production in any of our patients, other than the infants, was inherited since they had previously handled viral infections normally. Follow-up studies in several patients that survived indicated that their IFN-α and IFN-γ production had returned to normal. Therefore, one can speculate that this acquired defect of IFN production may be due to some transiently produced blocking factor.

We first described an IFN deficiency syndrome in 1980 (Levin & Hahn, 1980). There have since been several reports of other patients with a partial or transient defect of the IFN response to viral infections. One study described deficient production of IFN-α by lymphocytes *in vitro*, and in the nasal secretions *in vivo*, in four young children with recurrent upper and lower respiratory tract rhinovirus infections (Isaacs *et al.*, 1981). In each case IFN-α was produced *in vitro*, and IFN-α was only consistently absent in nasal secretions during infection. Although repeated assays of *in vitro* IFN-α production remained negative over a period of a year in three cases, all four produced normal amounts of IFN-α when retested some years later (Chadda *et al.*, 1984). It is therefore unlikely that the defects were genetically determined. Virelizier & Griscelli (1981) described a 4 year old boy with recurrent bacterial and viral infections in whom *in vitro* IFN-α production was consistently absent and natural killer (NK) cytotoxic activity was profoundly impaired. Treatment with IFN reversed the NK defect, and this was accompanied by clinical improvement. In another report these authors describe a 5 year old girl with severe, persistent Epstein–Barr virus infection and a defect in IFN-γ production (Virelizier, Lenoir & Griscelli, 1978). In a further report they describe four patients with cellular immune deficiencies in whom combined defects of IFN-γ production and low NK activity were observed (Virelizier *et al.*, 1979).

Stiehm (1982) reviewed the conditions in which a deficiency of either IFN-α or IFN-γ production has been reported. Defects have been described in normal neonates, congenital viral infections, Down's syndrome, certain primary immune and secondary immune deficiencies, such as those found in uraemia, malnutrition and chronic hepatitis, as well as in immune suppressed patients and some with lymphatic malignancies. However, some of these findings are contradicted by other reports: for example a normal IFN system in newborn infants was described in our previous study (Hahn, Levin & Handzel, 1980). Many of the studies cited in this review were based on a single assay, and in none were attempts made to include more comprehensive evaluations involving *in vivo* IFN production and cell protection from viral infections as well as IFN-α and IFN-γ production *in vitro*.

There appears to be no direct correlation between defects of the humoral or cellular immune systems and those of the IFN system. In some cases of IgA deficiency a defective T cell IFN-γ response has been found, but in other B or T system immunodeficient patients no IFN defects were observed (Epstein & Ammann, 1974). Normal IFN responses have been found in patients with thymic dysplasia (Miller & Hummeler, 1967) and with ataxia-telangiectasis (Ray & Starkey, 1970). In patients with Down's syndrome, in which there is evidence of thymic deficiency, we found diminished IFN-α and IFN-γ production *in vitro* (Levin *et al.*, 1979), whereas others have found normal production (Strander *et al.*, 1970). The IFN response has been shown to be relatively resistant to immunosuppression by drugs, irradiation or anti-lymphocyte serum (Stiehm, 1982). Our own studies have not shown any significant suppression of the IFN response in patients receiving steroids.

Defective IFN production by the PBMC has been reported in patients with pre-leukaemia or acute non-lymphocytic leukaemia with acquired abnormalities of chromosome 5 (Pedersen-Bjergaard *et al.*, 1980) and in chronic lymphatic leukaemia (Strander *et al.*, 1970; Ludwig, 1979). Patients with systemic lupus erythematosus, particularly during the active stage, have shown defective *in vitro* IFN-α production but normal IFN-γ production in response to a number of viral stimulants (Neighbour & Grayzel, 1981). A different type of IFN deficiency has been reported in patients with multiple sclerosis, namely the inability of these patients to produce IFN-α *in vitro* in response to a specific measles viral antigen (Neighbour & Bloom, 1979).

In conclusion it appears that only by using appropriate IFN system assays are we able to diagnose a treatable condition of defective IFN system response to a viral infection in which the viral disease runs a progressively downhill course. This IFN deficiency syndrome is characterized by inability to produce IFN *in vivo* and IFN-α and IFN-γ by PBMC *in vitro*, and non-induction of an anti-viral state in cells; the latter being a functional indicator of the integrity of the IFN system in viral infections. The prevalence of IFN deficiency syndrome among the patients diagnosed as having acute viral disease in this series was 5%. We believe that specific replacement therapy with IFN is essential in these cases, and this should be commenced as early as possible and before irreversible cell and organ damage occur.

The technical assistance of Valentina Bregman and Naomi Barzilai, who performed many of the assays, is gratefully acknowledged. The help of many physicians in various Israeli hospitals who referred patients to us and co-operated in this study is appreciated. The IFN-α used in the treatment of patients was produced by Dr Hagai Rosenberg and Tamar Bino of the Israel Institute for Biological Research, Ness Ziona.

Supported in part by the BMFT, FRG and the Council for Research and Development, Israel Ministry of Energy.

REFERENCES

Breese Hall, C., Douglas, R.L. & Geiman, J.M. (1978) Interferon production in children with respiratory syncytial, influenza and parainfluenza infections. *J. Pediat.* **93,** 28.

Chadda, H., Brenner, M.K., Valman, H.B. & Webster, A.D.B. (1984) Production of leucocyte interferon *in vitro* in children with recurrent respiratory infections. *Lancet*, **i,** 1247.

Epstein, L.B. & Ammann, A.J. (1974) Evaluation of T lympocyte effector function in immunodeficiency disease: abnormality in mitogen-stimulated interferon in patients with selective IgA deficiency. *J. Immunol.* **112,** 617.

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.

Hahn, T. & Levin, S. (1982) The interferon system in patients with malignant disease. *J. Interferon Res.* **2,** 97.

Isaacs, A. & Lindenmann, J. (1957) Virus interference. I. The interferons. *Proc. R. Soc. Lond. (Biol).* **147,** 258.

Isaacs, D., Tyrrel, D.A.S., Clarke, J.R., Webster, A.D.B. & Valman. H.B. (1981) Deficient production of leucocyte interferon *in vitro* and *in vivo* in children with recurrent respiratory tract infections. *Lancet*, **ii,** 950.

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

Levin, S. & Hahn, T. (1981) Evaluation of the interferon system in viral disease. *Clin. exp. Immunol.* **46,** 475.

Levin, S. & Hahn, T. (1982) Interferon system in acute viral hepatitis. *Lancet*, **i,** 592.

Levin, S., Hahn, T., Rosenberg, H. & Bino, T. (1982) Treatment of life-threatening viral infections with interferon-alpha: pharmacokinetic studies in a clinical trial. *Isr. J. med. Sci.* **18,** 439.

Levin, S., Schlesinger, M., Handzel, Z.T. & Hahn, T. (1979) Thymic deficiency in Down's syndrome. *Pediatrics*, **63,** 80.

Ludwig, H. (1979) Absent serum interferon in chronic lymphatic leukemia. *N. Engl. J. Med.* **301,** 1007.

Miller, M.E. & Hummeler, K. (1967) Thymic dysplasia (Swiss agammaglobulinemia). II. Morphologic and functional observations. *J. Pediat.* **70,** 737.

Neighbour, P.A. & Bloom, B.R. (1979) Absence of virus-induced lymphocyte suppression and interferon production in multiple sclerosis. *Proc. Natn. Acad. Sci. USA.* **76,** 476.

Neighbour, P.A. & Grayzel, A.L. (1981) Interferon-production *in vitro* by leucocytes from patients with systemic lupus erythematosus and rheumatoid arthritis. *Clin. exp. Immunol.* **45,** 576.

Pedersen-Bjergaard, J., Haahr, S., Philip, P., Thomsen, M., Jensen, G. Ersboll, J. & Nissen, N.I. (1980) Abolished production of interferon by lymphocytes of patients with the acquired cytogenic abnormalities 5q-or-5 in secondary and de-novo acute non-lymphocytic leukaemia. *Br. J. Haemat.* **46,** 211.

Ray, C.G. & Starkey, D.D. (1970) Leucocyte interferon production in immunological deficiency diseases. *Lancet*, **i,** 312.

Saksela, E. (1981) Interferon and natural killer cells. In *Interferon 3* (ed. by I. Gresser) p. 45. Academic Press, London.

Stewart, W.E. (1979) *The interferon system.* Springer-Verlag, New York.

Stiehm E.R. (1982 Interferon: Immunobiology and clinical significance. UCLA conference. *Ann. intern. Med.* **96,** 80.

Stiehm, E.R. & Fulginiti, V.A. (1980) *Immunological disorders in infant and children*, 2nd edn. W.B. Saunders, Philadelphia.

Strander, H., Cantell, K., Leisti, J. & Nikkila, E. (1970) Interferon response of lymphocytes in disorders with decreased resistance to infections. *Clin. exp. Immunol.* **6,** 263.

VIRELIZIER, J.L. & GRISCELLI, C. (1981) Selective defect of interferon secretion associated with impaired natural killing activity. *Arch. Fr. Pediat.* **38,** 77.

VIRELIZIER, J.L., LENOIR, G. & GRISCELLI, C. (1978) Persistent EBV infection in a child with hypergammaglobulinaemia and immunoblastic proliferation associated with a selective defect in immune interferon secretion. *Lancet*, **ii,** 231.

VIRELIZIER, J.L., LIPINSKI, M., TURSZ, T. & GRISCELLI, C. (1979) Defects of immune interferon secretion and natural killer activity in patients with immunological disorders. *Lancet*, **ii,** 696.

WALLACH, D. (1983) Quantitation of the anti-viral effect of interferon by immunoassay of viral proteins. *J. gen. Virol.* **64,** 2221.