Clin. exp. Immunol. (1984) **56,** 459–463.

Spontaneous production of human gamma interferon *in vitro* by splenic lymphocytes of patients with idiopathic thrombocytopenic purpura

HU YU-WEN, XUE GUO-XUNG & Y. H. TAN *Department of Medical Biochemistry/Microbiology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada*

(Accepted for publication 14 December 1983)

SUMMARY

Lymphocytes derived from spleens of traffic trauma victims do not appear to produce human interferon (IFN) activity, spontaneously, *in vitro*. However, lymphocytes derived from spleens of four ITP patients were found to produce significant amounts of human IFN activity. The IFN activity produced by the splenic lymphocytes of ITP patients was neutralized by anti-γ-IFN antisera but not anti-α or anti-β antisera. The IFN activity was found to be unstable at pH 2·0 and at 56°C. Thus the human IFN activity of splenic lymphocytes is characterized as human γ-IFN. No human IFN activity was detectable in the serum of the ITP patients and it is not known whether the splenic lymphocytes of ITP patients also produce human γ-IFN *in vivo*. The observations suggest that conditions prevail in the ITP state that predispose the splenic lymphocytes to produce human γ-IFN without stimulation by exogenously added inducer.

Key words human γ-interferon idiopathic thrombocytopenic purpura

INTRODUCTION

Until recently, acceptable immunological methods were not available to unequivocally demonstrate that an anti-platelet antibody causes idiopathic thrombocytopenic purpura (ITP) though patients with ITP have been discovered to have a high frequency of autoantibodies and immunological abnormalities (Conley, 1981; Stuart *et al.*, 1978; Favre, Chatelanat & Miescher, 1979; Huhn & Miller, 1980; Brey, Garner & Wells, 1969; Clancy, Muller & Ward, 1974). Such diseases tend to occur in people with disorders of immune regulation. Since interferon (IFN) is one of the products of lymphoid cells known to regulate immune responses (Ho & Armstrong, 1975; Hooks *et al.*, 1979, 1982; Panem *et al.*, 1982), and is an efficacious anti-viral in immunosuppressed patients (Merigan *et al.*, 1978), we examined the productive capability of splenic lymphocytes of ITP patients and found that splenic lymphocytes of all four ITP patients produced human-γ-IFN constitutively, whereas hardly any IFN activity (< 4 units) was produced by splenic lymphocytes of traffic related trauma victims.

MATERIALS AND METHODS

Isolation and culture of splenic lymphocytes. Fresh, sterile human spleens were obtained from the operating room of the Foothills Hospital (Calgary, Alberta). The spleens were derived from two

Correspondence: Dr Y. H. Tan, Department of Medical Microbiology/Biochemistry, Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 4N1, Canada.

types of patients viz., three trauma victims from traffic accidents (these patients were considered to be normal except for their trauma) and four ITP patients diagnosed according to clinical and haematological criteria. The spleens (50–70 g) were cut into small pieces (2–4 mm) and agitated in RPMI 1640 containing 10% fetal calf serum, penicillin (100 units/ml) and streptomycin (100 μg/ml) to release lymphoid cells from the splenic tissue. Thereafter the suspended cells were centrifuged on Ficoll-paque (Pharmacia, Sweden) according to the procedure provided by Pharmacia. The lymphoid cells were collected at the interface, harvested by a Pasteur pipette, and washed twice with 1 l of RPMI medium to remove excess Ficoll-paque. The cells were pelleted by centrifugation at 800 r/min for 10 min, and diluted in RPMI 1640 to a final density of $2–5 \times 10^6$ cells/ml. A total of 100 ml of cells was cultured in a 850 cm^3 plastic roller bottle (Corning) at 37°C. Aliquots of medium were removed from the roller bottles and assayed for human IFN activity at different times indicated in the Tables and Fig. 1. Similarly, lymphocytes from peripheral blood were prepared by the same procedure.

In a separate experiment, the lymphoid cells ($2–5 \times 10^6$ cells/ml) were induced with 15 μg/ml monoclonal antibodies against T cells viz. OKT3 (a gift from Dr Patrick Kung).

Assay of IFN activity. Human IFN activity was assayed in triplicate accordingly to the procedure of Armstrong (1971) except that the assay cells were human CC123 (a gift from Dr Budd Colby) and the challenge virus was EMC. Each reading of IFN given herein is an average of triplicate assays and standardized to a human α-IFN standard MRC 69/19. Three rabbit antisera against human α-, β-, γ-IFN were used to neutralize IFN activity. The antiserum against β is derived in this laboratory, Tan (1981), anti-α serum was a gift from Dr E. Havell, anti-γ serum was a gift from Dr M. Doyle. Each ml of rabbit anti-α serum neutralized 8,000 units of human α-IFN and each ml of rabbit anti-γ serum neutralized 600 units of human γ-IFN.

RESULTS

Splenic lymphocytes isolated from the four ITP patients secrete substances with human IFN activity into the culture medium even though no IFN inducers was added to stimulate these cells to produce IFN. The amount of human IFN activity spontaneously produced by these lymphocyte cultures is given in Table 1. Such spontaneous production of human IFN was not observed in three other experiments involving lymphocytes from normal spleens of traffic trauma victims or lymphocytes from normal peripheral blood (Table 1 & Fig. 1). The IFN activity spontaneously

Table 1. The spontaneous production of human IFN by ITP splenic lymphocytes

Lymphocytes derived from	IFN (units/ml) produced in response to OKT3 induction	IFN (units/ml) produced spontaneously
ITP spleen (a)	512	128
ITP spleen (b)	256	100
ITP spleen (c)	256	128
ITP spleen (d)	198	128
Normal spleen (e)	256	4
Normal spleen (f)	256	<4
Normal spleen (g)	164	<4
Normal peripheral blood (h)	ND	<4
Normal peripheral blood (i)	ND	<4

The IFN activity was determined from 24 h harvest as described in Materials and Methods.

ND = not done.

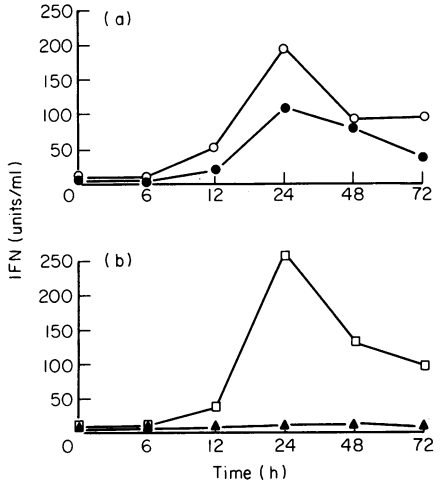

Fig. 1. Kinetics of spontaneous human γ-IFN production (●, ▲) and OKT3-induced human γ-IFN production (○, □) by ITP splenic lymphocytes (a) and traffic trauma splenic lymphocytes (b).

produced by the ITP spleen lymphocytes was neutralized by rabbit antiserum against human γ-IFN but was not affected by rabbit antiserum against human α- or β-IFN (Table 2). No IFN activity (< 4 units/ml) was detectable after pH 2·0 or 56°C treatment.

The ITP splenic lymphocytes also produced γIFN after treatment with OKT3. The induced cells were found to produce about two to four-fold more human γ-IFN than the amount they produced spontaneously (Fig. 1 & Table 1). In either case, no IFN activity was detectable during the first 6 h when the cells were in culture nor in the cell lysates at zero hour. IFN activity was initially detected at 12 h with the peak amount at 24 h. The amount of IFN decreased in the culture fluid at 48 h and 72 h (Fig. 1). This decrease in IFN activity at 48 and 72 h is likely to be due to the instability of γ-IFN at 37°C and is in agreement with the finding that the activity is rapidly inactivated at 56°C. The OKT3-induced human IFN activity produced by the ITP splenic lymphocytes, like the spontaneously produced IFN activity, appears to be gamma type of human IFN (Table 2).

Lymphocytes isolated from normal spleen also produced human γ-IFN after treatment with OKT3 and the amount was comparable to that seen with ITP splenic lymphocytes (Fig. 1 & Table 1).

Table 2. Characterization of interferon activity by antisera to α-, β- and γ-IFNs

IFN	Units IFN/ml after following treatment			
	None	Anti-α	Anti-β	Anti-γ
Spontaneously produced by ITP splenic lymphocytes	128	128	128	⩽16
OKT3-induced ITP splenic lymphocytes	256	256	256	⩽16

The IFN was pooled from samples derived from four ITP patients.

Serum samples were also obtained from the patients from which the spleens were tested in this study. No IFN activity was detected in the serum of these patients.

DISCUSSION

Though the splenic lymphocytes of ITP patients produce human γ-IFN spontaneously *in vitro* (Table 1 & Fig. 1), no IFN activity was detected in the serum of these patients both post- and pre-operatively. In view of this observation, one immediate consideration is that perhaps the splenic lymphocytes do not produce IFN *in vivo*. However, it is also possible that any IFN spontaneously produced *in vivo* is not necessarily released into the blood. The kinetics of spontaneous human IFN production (Fig. 1) show that IFN activity was not present in the culture media during the first 6 h after the cells were cultured. Furthermore, the kinetics of spontaneous IFN production corresponds to those of OKT3-induced IFN production in ITP splenic lymphocytes (Fig. 1). This suggests that ITP splenic lymphocytes did not begin to produce IFN until they were cultured *in vitro*. In contrast, Hooks *et al.* (1979) reported the presence of IFN in serum of patients with autoimmune disease. Since human γ-IFN production is induced in lymphoid cells by antigenic stimuli, it is possible that the high frequency of autoantibodies and/or immunological abnormalities in ITP patients may induce human γ-IFN production in the splenic lymphocytes of these patients. However, the half-life of circulating interferon is short, so that spontaneous production of IFN by the splenic lymphocytes might not lead to detectable serum IFN levels.

The spontaneous production of human IFN by ITP splenic lymphocytes has now become an interesting issue, especially with the recent reports that an human IFN activity (not completely neutralized by anti-Hu α-IFN and sometimes pH 2·0 labile) is present in the serum of autoimmune disease patients (Hooks *et al.*, 1979, 1982; Panem *et al.*, 1980). Hooks *et al.* and others, have considered that IFN might play a role in these immunoregulatory disorders. The origin of serum IFN activity is far from being elucidated. In view of our present findings, it is possible that there are factors present in these disease states which induce human IFN production in such cells as the lymphoid cells enabling them to produce α-IFN as found in the sera of the autoimmune patients (Hooks *et al.*, 1979; Panem *et al.*, 1980; Olivia *et al.*, 1982) or human γ-IFN as found in spleen cells of ITP patients, presently reported. Even though, human IFN has been reported to appear in the sera of autoimmune patients, more studies are required to ascertain whether the IFN plays a role, if any, in the development of autoimmune disease.

This work was supported by the MRC of Canada. Hu Yu-Wen and Xue Guo-Xung are Fellows of the Alberta Heritage Trust Fund for Medical Research. Y. H. Tan is the holder of the P.C.H.B. Research Scientist Award.

REFERENCES

ARMSTRONG, J. (1971) Semimicro, dye binding assay for rabbit IFN. *Appl. Microbiol.* **21,** 723.

BREY, O., GARNER, P.R. & WELLS, D. (1969) Cyclic thrombocytopenia associated with multiple autoantibodies. *Br. Med. J.* **3,** 397.

CLANCY, R.L., MULLER, H.K. & WARD, H.A. (1974) Immunodeficiency and grouping with thyrogastric autoimmune disease in patients with chronic idiopathic purpura. *Aust. NZ J. Med.* **4,** 243.

CONLEY, C.L. (1981) Immunologic precursors of autoimmune hematologic disorders. Autoimmune hemolytic anemia and thrombocytopenic purpura. *John Hopkins Med. J.* **149,** 101.

FAVRE, H., CHATELANAT, F. & MIESCHER, P.A. (1979) Autoimmune hematologic diseases associated with infraclinical systemic lupus erythematosus in four patients. A human equivalent of NZB mice. *Am. J. Med.* **66,** 91.

HO, M. & ARMSTRONG, J. (1975) Interferon. *Ann. Rev. Microbiol.* **29,** 131.

HOOKS, J.J., KORDAN, G.W., CUPPS, T., MOUTSOPOULOS, H.M., FANCI, A.S. & NOTKINS, A.L. (1982) Multiple interferons in the circulation of patients with systemic lupus erythematosus and vasculitis. *Arthrit. Rheum.* **25,** 396.

HOOKS, J.J., MOUTSOPOULOS, H.M., GEIS, S.A., STAHL, N.I., DECKER, J.L. & NOTKINS, A.L. (1979) Immune interferon in the circulation of patients with autoimmune disease. *N. Engl. J. Med.* **301,** 5.

HUHN, D. & MILLER, E. (1980) Graves' disease and idiopathic thrombocytopenic purpura. *Arch. Intern. Med.* **140,** 1252.

MERIGAN, T.C., RAND, K.H., POLLARD, R.B., ABDALLAH, P.S., JORDAN, G.W. & FRIED, R.P. (1978) Human leukocyte interferon for the treatment of herpes zoster in patients with cancer. *N. Engl. J. Med.* **298,** 981.

OLIVIA, T.P., BLACK, R.J., FRIEDMAN, R.M., KLIPPEL, J.H. & VILCEK, J. (1982) Systemic lupus erythematosis: presence in human serum of an unusual acid-labile leukocyte interferon. *Science*, **216,** 429.

PANEM, S., CHECK, I.J., HENRIKSEN, D. & VILCEK, J. (1982) Alpha IFN and antibody to alpha IFN in systemic lupus erythematosus. *UCLA Sym. Mol. Cell Biol.* **XXV,** 233.

STUART, M.J., TOMAR, R.H., MILLER, M.L. & DAVEY, F.R. (1978) Chronic idiopathic thrombocytopenic purpura. A familial immunodeficiency syndrome? *JAMA.* **239,** 939.

TAN, Y.H. (1981) Purification of human fibroblast interferon prepared from modified fibroblasts. *Meth. Enzymol.* **78,** 422.