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Interferon production *in vitro* by leucocytes from patients with systemic lupus erythematosus and rheumatoid arthritis

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SUMMARY

The production of interferons (IFN) by peripheral blood leucocytes from normal donors and patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) has been investigated in response to several IFN inducers *in vitro*. Whereas IFN responses of RA donors did not differ significantly from the normal group, those of SLE patients were significantly reduced, and many of these patients failed to respond at all. Patients with active or acute SLE responded significantly less well than those with inactive disease. There was no apparent effect of steroid therapy on the IFN responses of either SLE or RA patients. These data may indicate a basic immunological defect of the circulating leucocytes of SLE patients, which may be responsible for some of the *in vitro* lymphocyte anomalies reported for this disease.

INTRODUCTION

Interferons (IFN) can inhibit a variety of *in vivo* and *in vitro* immune responses, such as lymphocyte proliferation and antibody synthesis (Epstein, 1977a), and also may be mediators of the suppressor activity exerted by both mitogen- and virus-induced lymphocytes (Kadish *et al.*, 1980; Neighbour & Bloom, 1979). IFN has also been shown to enhance some lymphocyte functions including antibody production (Sonnenfeld, Mandel & Merigan, 1978) and the spontaneous cytotoxicity performed by natural killer (NK) cells (Trinchieri & Santoli, 1978). Many of these same immune responses have been reported to be abnormal in patients with systemic lupus erythematosus (SLE). For example, SLE patients exhibit hypergammaglobulinaemia, autoantibodies and immune complexes in their serum and show diminished levels of mitogen-induced suppressor activity (Bresnihan & Jasin, 1977; Fauci *et al.*, 1978); depressed primary antibody responses (Baum & Ziff, 1969); reduced lymphocyte blastogenesis (Rosenthal & Franklin, 1975); and, most recently, abnormal NK cell activity (Hoffman, 1980). It is possible, therefore, that an imbalance in the IFN-mediated regulation of these responses, caused perhaps by defective IFN production, might be responsible for the observed immunodeficiencies and, thereby, contribute to the immunological pathogenesis of this disease. To investigate this possibility, leucocytes from patients with SLE, rheumatoid arthritis (RA) and normal donors were treated *in vitro* with inducers of both IFN-α (previously termed viral or type I leucocyte IFN) and IFN-γ (immune or type II IFN). Whereas IFN responses of RA donors did not differ significantly from the normal group, those of the SLE patients were significantly reduced, and many of these patients failed to respond at all.

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MATERIALS AND METHODS

Patient selection. Thirty-two SLE patients, 29 women and three men with a mean age of 35, were tested. All patients met at least four ARA criteria for the diagnosis of SLE (Cohen *et al.*, 1971). Thirteen patients were considered to have *active* disease manifested by fever, arthritis, serositis, or an active urinary sediment while five patients, who were hospitalized for acute CNS symptoms, progressive renal disease or thrombocytopenia, were considered to have *acute* disease. The remaining 14 patients, whose current symptoms were limited to rash, arthralgias, Raynaud's phenomenon or proteinuria, were considered *inactive* or suppressed. Patients received either no therapy ($n=5$) or one of the following: less than 20 mg of prednisone per day ($n=9$); 20 mg or more of prednisone per day ($n=11$); or a combination of prednisone and azathioprine ($n=7$).

Fifteen RA patients, 14 women and one man with a mean age of 53, were tested. All patients met the ARA criteria for definite or classical RA (Ropes *et al.*, 1958) and all had a positive test for rheumatoid factor in their serum. Ten patients with two or more objectively warm, tender, swollen joints were considered to have *active* disease. The remaining five patients were considered *inactive* or suppressed. Patients were receiving the following treatment either singly or in combination: gold salts, a non-steroidal anti-inflammatory drug, prednisone (15 mg/day or less), cyclophosphamide, or azathioprine. Nine of the 15 patients were receiving steroids.

Twenty volunteer medical school and laboratory personnel, 11 women and nine men with a mean age of 32, were tested. None of these normal donors showed any apparent signs of clinical disease at the time of testing.

Leucocyte preparation and interferon production. Leucocytes were obtained from peripheral blood drawn from each donor and prepared for IFN production as described previously (Neighbour, Miller & Bloom, 1981). Briefly, mononuclear cells were separated from heparinized blood by Ficoll–Paque (Pharmacia) density-gradient centrifugation. For the induction of IFN, 2×10^6 cells suspended in 1 ml of RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin (100 units/ml) and streptomycin (100 μg/ml) were incubated either alone or with one of the following: β-propiolactone-inactivated Newcastle disease virus (NDV) at an equivalent multiplicity of 0·5; 20 μg of polyinosinic–polycytidylic acid (Poly I:C; Sigma); β-propiolactone-inactivated measles virus (Edmonston) at an equivalent multiplicity of 0·1; or 10 μg of concanavalin A (Con A, Miles). The culture supernates were harvested after 48 hr of incubation followed by centrifugation at 500 ***g*** for 5 min and were stored at -70°C until assayed for IFN.

Interferon assay. The antiviral activity of each supernate was determined as described previously (Neighbour & Bloom, 1979) by the inhibition of vesicular stomatitis virus cytopathic effect (CPE) on confluent monolayers of human trisomic-21 skin fibroblasts (Detroit 532 cells, American Type Culture Collection) or human foreskin fibroblasts (FS-7 cells, Dr Jan Vilcek, NYU Medical Center) in microtitre wells. IFN titres were calculated as the reciprocal of the highest dilution that inhibited 50% of the viral CPE and are expressed per 0·1 ml in equivalent NIH reference units (NIH human reference IFN G023-901-527).

Statistical analysis. The distribution of IFN titres was skewed because of their derivation from dilution end-point determinations. Therefore, all IFN titres were $\log_{10}$-transformed prior to analysis. IFN responses of each donor group were compared by Student's t-test. *Non-responders* were defined as those donors whose IFN responses were below the 95% confidence limits for the normal population. These limits were calculated as the normal group geometric mean $-t$ (for $N-1$ d.f.) $\times$ 1 s.d. and are presented in Fig. 1. The percentages of non-responders in each group were compared by the χ^2 test. Significance was accepted throughout at the $P<0{\cdot}05$ level.

RESULTS

Leucocytes from normal, SLE and RA donors were treated with NDV, Poly I:C, measles virus and Con A, and the IFN responses were determined by titrating the culture supernates after 48 hr. There was no detectable difference in the viability in culture of leucocytes from SLE patients compared to those from normal donors (data not shown). The geometric mean IFN responses of the SLE and

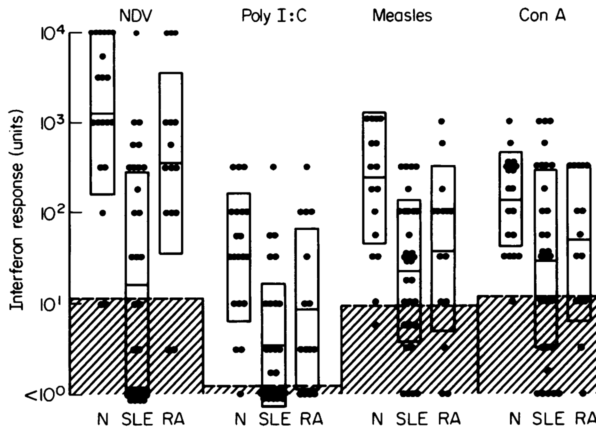

Fig. 1. Individual IFN responses of leucocytes from normal, SLE and RA donors to four IFN inducers. Boxes represent group geometric mean IFN responses ± standard deviation. Shading indicates the area below the lower 95% confidence limit for the normal population and divides 'non-responders' from 'responders' for each inducer.

RA patient groups were consistently lower than those of the normal group for each of the inducers tested (Fig. 1). Many of the SLE and RA patients failed to produce detectable levels of IFN in response to one or more of the inducers, so for the purpose of further analysis it was decided to separate the donors into *responders* and *non-responders*. Ninety-five per cent confidence limits were calculated for the IFN responses to each inducer of the normal group (as indicated in Fig. 1), and donors whose IFN responses were less than the lower limit were considered as non-responders.

Table 1. Interferon responses of leucocytes from normal, SLE and RA donors to various inducing agents

Inducing agent	Donor group	No. of donors	Mean IFN response of all donors (units)	No. of non-responders (%)	*P* value	Mean IFN response of responders only: Log10 units	Mean IFN response of responders only: Units	*P* value
NDV	Normal	20	1,296*	2 (10)†	—	3·35±0·6‡	2,224*	—
	SLE	32	17	17 (47)	<0·01§	2·42±0·6	261	<0·001¶
	RA	15	355	2 (13)	>0·05	2·87±0·7	733	>0·05
Poly I:C	Normal	20	34	1 (5)	—	1·61±0·6	40	—
	SLE	32	3	16 (50)	<0·001	1·05±0·6	11	<0·05
	RA	15	9	4 (27)	>0·05	1·27±0·7	19	>0·05
Measles	Normal	20	237	1 (5)	—	2·46±0·6	289	—
	SLE	32	23	9 (28)	<0·05	1·74±0·5	55	<0·001
	RA	15	37	3 (20)	>0·05	1·92±0·6	83	<0·05
Con A	Normal	20	130	1 (5)	—	2·17±0·5	148	—
	SLE	32	29	12 (37)	<0·01	2·05±0·6	112	>0·05
	RA	15	45	5 (33)	>0·05	2·12±0·4	133	>0·05

* Geometric mean.

† Non-responders had IFN responses below the lower 95% confidence limit for the normal group responses as indicated in Fig. 1.

‡ Mean IFN response ±1 s.d.

§ Percentage of non-responders in patient group compared to normal donors; χ^2 test.

¶ IFN responses of patient group responders compared to normal responders; Student's *t*-test performed on log10-transformed IFN titres.

Significantly more of the SLE patients were non-responders to all four inducers when compared with normal donors, while the proportion of RA non-responders did not differ significantly from the normal group (Table 1). Also shown in Table 1, the geometric mean IFN responses of the SLE responders alone to the IFN-α inducers, NDV, Poly I:C and measles were significantly lower than those of normal donor responders. In contrast, apart from a slightly significant reduction in IFN response to measles virus, the RA responders did not produce significantly less IFN than normal donors. Individuals who failed to respond to IFN-α induction by NDV also tended not to respond to the other two IFN-α inducers. The Con A-induced IFN-γ responses of both the SLE and RA patient responders did not differ significantly from those of the normal donors. There was no apparent correlation between IFN-α and IFN-γ responses, since it was observed that many IFN-α non-responders produced significant levels of IFN-γ in response to Con A induction (data not shown).

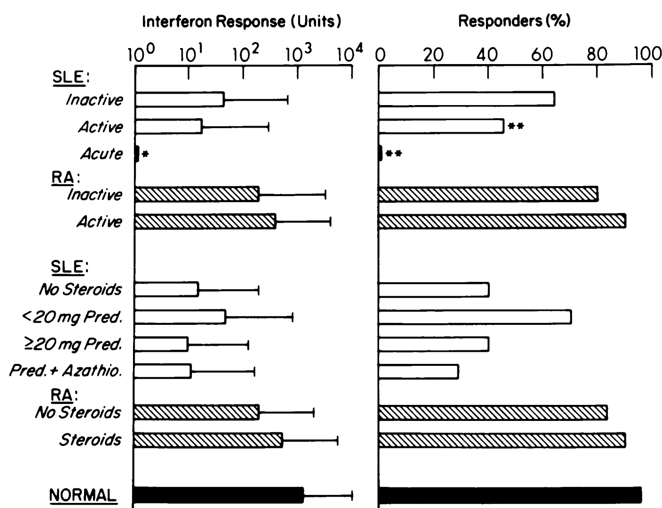

Fig. 2. Effect of disease activity and steroid therapy on the NDV-induced IFN responses and the proportion of responders in patients with SLE and RA. IFN response expressed as the geometric mean ± standard deviation. Significantly different compared to inactive SLE patients: * $P<0.05$; ** $P<0.01$. All other comparisons are not statistically significant ($P>0.05$).

Leucocytes from less than 5% of the donors in each group were found to release low levels of IFN (< 10 units) spontaneously when cultured alone for 48 hr (data not shown). However, no significant differences were observed in spontaneous IFN production between the three donor groups, and there was no significant correlation between the production of spontaneous IFN and responsiveness to any of the IFN inducers.

Some of the anomalies which have been described for SLE patient lymphocyte responses appear to fluctuate according to clinical stage (Bresnihan & Jasin, 1977; Rosenthal & Franklin, 1975). Therefore, the NDV-induced IFN responses of SLE and RA patients were analysed according to the disease activity of each donor. There was a significant decrease in both the mean IFN response and the proportion of responders as disease activity increased in the SLE patient group (Fig. 2). In contrast, patients with active RA responded to NDV no differently from those with inactive disease. The IFN responses to Poly I:C, measles and Con A varied accordingly (data not shown).

To determine whether the therapeutic use of corticosteroids may have influenced IFN responsiveness, particularly in those patients with active and acute disease, the NDV-induced IFN responses of SLE and RA patients were analysed according to the level of steroids received by each donor. The IFN responses of SLE patients treated with low or high doses of prednisone or with a combination of prednisone and azathioprine were not significantly lower than those SLE patients who had not received steroid therapy (Fig. 2). In fact, it was noted that two patients who had been diagnosed recently with active SLE were found to be non-responders to NDV prior to their

receiving any steroid therapy. In contrast, the three SLE patients who produced the highest levels of IFN in response to NDV-induction were all receiving prednisone at the time of testing.

A group of 10 SLE patients were tested a second time up to 9 months after the initial testing. Two of the patients who had been responders to each of the inducers were found to have remained as responders when subsequently tested. Of the other eight donors who had been non-responders initially, only one was found to respond significantly upon retesting. Interestingly, the clinical status of this patient changed from active to inactive disease during the 3 months between tests.

DISCUSSION

Leucocytes of patients with various diseases, including Hodgkin's disease, acute or chronic lymphocytic leukaemia, infectious mononucleosis and multiple sclerosis have been shown to produce abnormally low levels of both IFN-α and IFN-γ *in vitro* in response to appropriate inducers (Neighbour & Bloom, 1979; Neighbour, Miller & Bloom, 1981; Rassiga-Pidot & McIntyre, 1975; Lee, Ozere & van Rooyen, 1966; Rassiga-Pidot, Maurer & McIntyre, 1973). In the present study, we report that SLE patients exhibit a highly significant defect in their IFN-α response to NDV, Poly I:C and measles virus and IFN-γ response to Con A. Almost half of the SLE patients tested failed to produce detectable levels of IFN-α to each of the inducers, and those who did respond produced only very low levels. In contrast, while a significant proportion of the SLE patients also failed to respond to Con A, the remainder produced normal levels of IFN-γ. Of particular interest was the finding that patients with RA, another chronic autoimmune disease, did not demonstrate similar anomalies. Apart from a slightly depressed IFN-α response to measles virus alone, RA patients responded normally to both IFN-α and IFN-γ inducers.

In an earlier study of IFN production by SLE leucocytes in response to Sendai virus, it was reported that the patients produced similar levels of IFN to normal controls (Alarcón-Segovia *et al.*, 1974). The reasons for this discrepancy are not clear. However, in that study, Sendai virus induced only low levels of IFN in both donor groups, and this may have obscured any significant differences between them.

It should be noted that because of the pronounced leucopenia exhibited by the majority of SLE patients in this study, fewer leucocytes were recovered by Ficoll-separation from these patients compared to normal and RA donors. Thus incubation of an equal number of cells from each donor with each of the IFN inducers did not accurately reflect the IFN-producing capacity of circulating leucocytes *in vivo*. Recalculation of these data per ml of whole blood after correction for leucocyte cell number further emphasizes the IFN deficiency in SLE patients, with approximately 75% of SLE donors appearing as non-responders for IFN production.

The defects in IFN production appeared to be significantly greater in those SLE patients with more active disease, to the extent that none of the five patients with acute symptoms produced any detectable IFN in response to NDV, normally a highly potent inducer of IFN-α. The activity of so-called mitogen-induced suppressor cells has been extensively studied in patients with SLE and found to be depressed, particularly during active and acute stages of the disease (Bresnihan & Jasin, 1977; Fauci *et al.*, 1978). The use of antibodies to IFN has strongly suggested that IFN is a probable mediator of this suppressor activity (Kadish *et al.*, 1980; Neighbour & Bloom, 1979). The data presented here support both of these observations and possibly explain the findings of reduced suppressor activity in this and other chronic diseases.

The lack of significant correlation between steroid therapy and the IFN response in both SLE and RA groups strongly indicates that drug-induced immunosuppression was not the underlying cause of the observed anomalies. Perhaps the most likely explanation of the present observations is that there may be an imbalance in the proportion or a total absence of the IFN-producing cells in the circulation. While the majority of leucocyte subpopulations are known to be capable of synthesizing IFN after appropriate stimulation (Epstein, 1977b), it is currently believed that null lymphocytes are the predominant cell type responsible for IFN-α production (Trinchieri *et al.*, 1978; Yamaguchi *et al.*, 1977), and T cells are the major source of IFN-γ (Epstein, 1977b). The relative proportions of the major subclasses of lymphocytes, and particularly of T cells and T cell subsets

(such as, for example, Tγ cells), appear to vary in SLE (Fauci *et al.*, 1978; Moretta *et al.*, 1979). However, until the exact identity of the IFN-producing cells has been determined, the relevance of these findings to the present study remains unclear. Interferon appears to be closely associated with spontaneous cytotoxicity mediated by NK cells (Trinchieri & Santoli, 1978; Trinchieri *et al.*, 1978; Santoli & Koprowski, 1979). We (manuscript in preparation) and others (Hoffman, 1980; Santoli & Koprowski, 1979) have found marked anomalies in the NK cell activity of SLE patients. Whether this deficiency is the cause or the effect of defective IFN production is currently under investigation in our laboratory.

Hooks *et al.* (1979) have reported the detection of IFN in the serum of patients with autoimmune diseases of the collagen-vascular system including SLE and RA. In the present study, we were unable to detect any significant spontaneous release of IFN from the peripheral leucocytes of these patients. This suggests that the IFN found in the serum is not produced by leucocytes in the circulation, but originates from stimulated lymphocytes located either within a lymphoid organ, or more likely, at a localized site of lymphocyte infiltration and activation. It has yet to be determined whether the serum IFN is responsible for the lymphocyte hyporeactivity of these patients.

Because the underlying mechanism of the observed defects has yet to be determined, we feel at present that it is premature to speculate whether decreased IFN production in many SLE patients predisposes to their disease or is a secondary consequence of disease activity. However, we believe that our results indicate a basic immunodeficiency of the circulating leucocytes of SLE patients, which may explain some of the *in vitro* lymphocyte anomalies that have been described for this disease.

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REFERENCES

Alarcón-Segovia, D., Ruíz-Gomez, J., Fishbein, E. & Bustamante, M.E. (1974) Interferon production by lymphocytes from patients with systemic lupus erythematosus. *Arthritis Rheum.* **17**, 590.

Baum, J. & Ziff, M. (1969) Decreased 19S antibody response to bacterial antigens in systemic lupus erythematosus. *J. clin. Invest.* **48**, 758.

Bresnihan, B. & Jasin, H.E. (1977) Suppressor function of peripheral blood mononuclear cells in normal individuals and in patients with SLE. *J. clin. Invest.* **59**, 106.

Cohen, A.S., Reynolds, W.E., Franklin, E.C., Kulka, J.P., Ropes, M.W. & Shulman, L.E. (1971) Preliminary criteria for the classification of systemic lupus erythematosus. *Bull. Rheum. Dis.* **21**, 643.

Epstein, L.B. (1977a) The effects of interferons on the immune response *in vitro* and *in vivo*. In *Interferons and Their Actions* (ed. by W. E. Stewart, II), p. 91. CRC Press, Cleveland.

Epstein, L.B. (1977b) Mitogen and antigen induction of interferon *in vitro* and *in vivo*. *Tex. Rep. Biol. Med.* **35**, 42.

Fauci, A.S., Steinberg, A.D., Haynes, B.F. & Whalen, G. (1978) Immunoregulatory aberrations in systemic lupus erythematosus. *J. Immunol.* **121**, 1473.

Hoffman, T. (1980) Natural killer function in systemic lupus erythematosus. *Arthritis Rheum.* **23**, 30.

Hooks, J.J., Moutsopoulos, H.M., Geis, S.G., 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.

Kadish, A.S., Tansey, F.A., Yu, G.S.M., Doyle, A.T. & Bloom, B.R. (1980) Interferon as a mediator of human lymphocyte suppression. *J. exp. Med.* **151**, 637.

Lee, S.H.S., Ozere, R.L. & van Rooyen, C.E. (1966) Interferon production by human leukocytes *in vitro*. Reduced levels in lymphatic leukemia. *Proc. Soc. exp. Biol. Med.* **122**, 32.

Moretta, A., Mingari, M.C., Santoli, D., Perlmann, P. & Moretta, L. (1979) Human T-lymphocyte subpopulations: alterations in systemic lupus erythematosus. *Scand. J. Immunol.* **10**, 223.

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

Neighbour, P.A., Miller, A.E. & Bloom, B.R. (1981) Interferon responses of leukocytes in multiple sclerosis. *Neurology (Minneap.)*. (In press.)

Rassiga-Pidot, A.L. & McIntyre, O.R. (1975) *In vitro* leukocyte interferon production in patients with Hodgkin's disease. *Cancer Res.* **34**, 2995.

Rassiga-Pidot, A.L., Maurer, L.H. & McIntyre, O.R. (1973) Leukocyte interferon production, RNA synthesis and PHA responses in patients with infectious mononucleosis. *Blood*, **42**, 175.

Ropes, M.W., Bennett, G.A., Cobb, S., Jocox, R.F.

& JESSAR, R.A. (1958) 1958 revision of the diagnostic criteria for rheumatoid arthritis. *Bull. Rheum. Dis.* **9,** 175.

ROSENTHAL, C.J. & FRANKLIN, E.C. (1975) Depression of cellular-mediated immunity in systemic lupus erythematosus. Relation to disease activity. *Arthritis Rheum.* **18,** 207.

SANTOLI, D. & KOPROWSKI, H. (1979) Mechanisms of activation of human natural killer cells against tumor and virus-infected cells. *Immunol. Rev.* **44,** 125.

SONNENFELD, G., MANDEL, A.D. & MERIGAN, T.C. (1978) Time and dosage dependence of immunoenhancement of murine type II interferon preparations. *Cell. Immunol.* **40,** 285.

TRINCHIERI, G. & SANTOLI, D. (1978) Anti-viral activity induced by culturing lymphocytes with tumor-derived or virus-transformed cells. Enhancement of human natural killer cell activity by interferon and antagonistic inhibition of susceptibility of target cells to lysis. *J. exp. Med.* **147,** 1314.

TRINCHIERI, G., SANTOLI, D., DEE, R.R. & KNOWLES, B. (1978) Anti-viral activity induced by culturing lymphocytes with tumor-derived or virus-transformed cells. Identification of the anti-viral activity as interferon and characterization of the human effector lymphocyte subpopulation. *J. exp. Med.* **147,** 1299.

YAMAGUCHI, T., HANDA, K., SHIMIZU, Y., ABO, T. & KUMAGAI, K. (1977) Target cells for interferon production in human leukocytes stimulated by Sendai virus. *J. Immunol.* **118,** 1931.