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Interferon and natural killer cells in systemic lupus erythematosus

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SUMMARY

Natural killer (NK) cell activity against several types of target cells was found to be subnormal in patients with systemic lupus erythematosus (SLE). Interferon (IFN) boosted the NK activity of cells from SLE patients to a significantly lesser degree than cells from normal controls. The production of IFN after stimulation of blood cells with Sendai virus was significantly decreased in SLE patients with active disease, and in a substantial proportion of the patients the production of phytohaemagglutinin (PHA)-induced IFN was below normal limits. Although the production of virus-induced IFN was clearly inversely correlated to disease activity no such correlation was observed for PHA-induced IFN. Serum levels of both pH2 stable and pH2 labile IFN were significantly higher in SLE patients than in controls. The findings clearly show that SLE is associated with abnormalities in the NK cell–IFN system but it cannot be stated whether these abnormalities are causally related to the development of disease or are secondary to pathological changes in SLE.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a disease characterized by multiple immunological abnormalities. There is abundant evidence that the function of T cells is disturbed in the diseased patients (Messner, Lindstrom & Williams, 1973; Hahn, 1975) and, in particular, there appears to be a relative lack of T suppressor cell function (Abdou *et al.*, 1976; Bresnihan & Jasin, 1977). Not only T cells but also B cells appear to be abnormal in SLE (Jasin & Ziff, 1975; Nies, Stevens & Louie, 1980) and evidence has also been presented suggesting monocyte and macrophage abnormalities in the disease (Svensson, 1975; Markenson *et al.*, 1978). The function of natural killer (NK) cells, which have been variously claimed to be of macrophage–monocyte or T cell lineage (Herberman, 1981), in SLE has recently been studied by several investigators. We have earlier reported decreased NK cell activity (Strannegård *et al.*, 1980), a finding that has simultaneously been obtained in other studies (Oshimi *et al.*, 1979, 1980; Hoffman, 1980; Goto, Tanimoto & Horiuchi, 1980).

The human NK cell activity is apparently subject to both positive and negative regulatory influences. The main positive regulator appears to be interferon (IFN) (Trinchieri & Santoli, 1978). Thus it may appear paradoxical that SLE seems to be accompanied by both decreased NK cell activity and increased serum levels of IFN (Hooks *et al.*, 1979). The present study was performed in an attempt to further elucidate the role of immunological parameters relating to NK cells and IFN in the pathogenesis of SLE.

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PATIENTS AND METHODS

Patients. Seventeen adult patients, 15 women and two men, between 17 and 55 years old (mean age 39·7), were included in the study. All patients fulfilled the preliminary American Rheumatism Association criteria for SLE. Renal involvement was diagnosed in eight patients. The disease was considered to be 'inactive' in nine patients, who had only slight or moderate symptoms and had not shown a recent rise in the titres of anti-DNA antibodies or a decrease in serum complement factors C3 or C4. Eight patients were treated with azathioprine and prednisolone, and four additional patients were treated with prednisolone only. Only one patient received more than 12·5 mg/day of prednisolone. Control blood specimens were drawn from healthy staff members, (four men and eight women, mean age 37·5). Specimens from one or two patients were in all cases drawn simultaneously with one control specimen and the samples were processed and tested in identical manners.

Assay of natural killer cell activity. Details of the procedure used for NK cell determination have been described elsewhere (Strannegård *et al.*, 1982). Defibrinated blood was separated on Ficoll-Hypaque and the resulting mononuclear cell suspension was treated with carbonyl iron to remove phagocytic-adherent cells. The adherent cell/depleted mononuclear cells were incubated for 1 hr at 37°C in the presence or absence of 300 units/ml of IFN (IFN-α, specific activity 10^7 units/mg, kindly supplied by the Red Cross Service, Helsinki, Finland). After washing three times the cells were mixed with 10^4 ^{51}Cr-labelled target cells (K562 or Chang cells) in microplates wells to give effector target cell ratios of 30:1, 15:1 and 7·5:1 in a final volume of 0·15 ml. All tests were done in sextiplicate. After incubation for 4 hr at 37°C supernatant fluids were collected using a tissue culture fluid collecting system (Titertek, Flow Laboratories, Irvine, Scotland) and assayed for radioactivity. Maximum ^{51}Cr release was determined in cultures treated with Triton X-100. The percentage of cell lysis was determined as 100 × (experimental ^{51}Cr release − spontaneous release)/(maximum release − spontaneous release).

NK cell activity was expressed as calculated percentage lysis at an effector/target cell ratio of 15:1 after computerizing all data obtained.

In experiments using green monkey kidney (GMK) and Burkitt lymphoma (P3HR-1) cells as targets, the tests were performed using mononuclear cells that had not been depleted of adherent cells, and only one effector/target cell ratio, 25:1, was used.

Determination of IFN production. Two millilitres of heparinized blood was mixed with 1 ml RPMI 1640 medium containing 8 mM HEPES, 10% human AB serum, 1% l-glutamine, penicillin and streptomycin in plastic tubes. After addition of 0·5 ml of either phytohaemagglutinin (PHA, Wellcome Laboratories, final concentration 10 μg/ml) or Sendai virus (final concentration 600 haemagglutination units/ml) the tubes were incubated at 37°C in a roller drum for 2 or 5 days (Sendai virus and PHA containing cultures, respectively). After centrifugation, supernatants were collected and stored at −70°C until tested.

Interferon assay. IFN was assayed using either A549 (human lung carcinoma) or MDBK (bovine kidney) cells. The cells were grown in microplates and two-fold dilutions of the test fluid or the reference standard were added to the wells. After incubation for 24 hr the cells were washed three times and virus (Herpes simplex type 2 or vesicular stomatitis virus) was added to each well. After a further incubation for 7–18 hr the cells were washed, treated with glutaraldehyde, and the amount of viral antigen in the cells was determined with an ELISA (enzyme linked immunosorbent) assay. Thus the method used was similar to conventional microtitre IFN assays, except that virus multiplication was assessed by immunoassay instead of by microscopical examination of cytopathic effects. All titres were calculated after comparison with a reference IFN-α preparation. The method, which was found to be reproducible and able to detect IFN concentrations of less than 1 unit/ml, will be described in detail elsewhere.

For measurement of serum IFN, untreated or pH2 treated serum was tested in dilutions of 1:4–1:64. Acid (pH2) treatment was performed by mixing equal parts of serum and 0·2 M HCl and leaving the mixtures in the refrigerator for 4 hr, after which neutral pH was restored by adding 0·2 M NaOH to the tubes.

Statistical methods. Group comparison were performed with Student's *t*-test or, when data were not normally distributed, with Wilcoxon's rank sum test.

RESULTS

NK cell activity in SLE patients and controls

NK cell activity was determined using four different types of target cells. As shown in Fig. 1, NK activity was lower in SLE patients than in controls in all tests at an effector/target cell ratio of 25:1 or 15:1. The differences obtained were statistically significant (Wilcoxon test) only when P3HR-1 and GMK were used as target cells ($P=0{\cdot}02$ and $P=0{\cdot}05$, respectively). The differences were not dependent on the concentration of effector cells used and the dose–response curves obtained were linear when plotted on a semi-logarithmic paper (Fig. 2). There was no evident correlation between disease activity and NK cell activity. Thus, with K562 as target cells, the mean NK activity of seven patients classified as having active disease was 33·0% and eight patients with inactive SLE had a

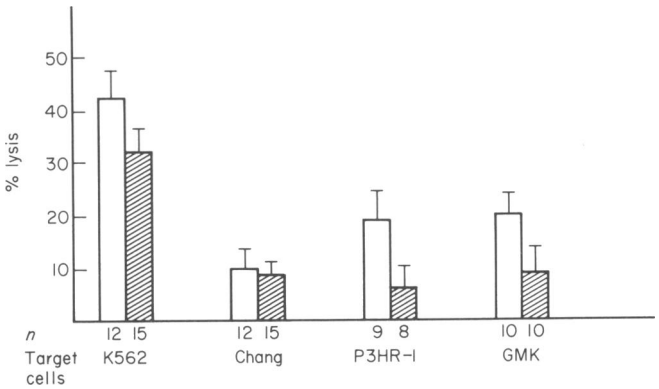

Fig. 1. NK cell activity in SLE patients (▨) and controls (□). Vertical bars represent s.e.m. Percent lysis refers to calculated % killing at an effector/target cell ratio of 15:1 (K562 and Chang cells) or at a ratio of 25:1 (GMK and P3HR-1 cells). The effector cells were depleted of phagocytic-adherent cells in the K562 and Chang cell assays but not in the GMK and P3HR-1 cell assays.

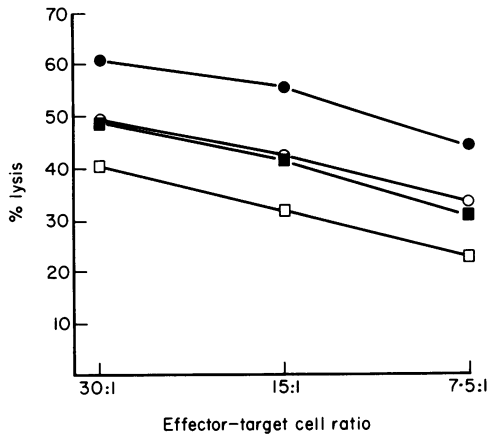

Fig. 2. NK activity of untreated and IFN treated cells at varying effector/target cell ratio. Mean NK activity of untreated (□—□) or IFN-treated (■—■) cells from 17 SLE patients and of untreated (○—○) or IFN-treated (●—●) cells from 12 healthy controls are indicated in the figure. K562 cells were used as targets.

Table 1. Interferon production in whole blood cultures from SLE patients and controls after stimulation with PHA or Sendai virus

Stimulation	Controls	SLE	SLE active	SLE inactive
n	12	17	8	9
PHA	3·02±0·06	2·90±0·13	2·83±0·25	2·96±0·14
Sendai virus	2·82±0·23	2·32±0·22	1·92±0·27*	2·72±0·28†

IFN production is expressed as mean±s.e.m. of $\log_{10}$ IFN units/ml. Numbers in parenthesis refer to number of subjects. Symbols refer to statistical significance (Wilcoxon test) for difference between active SLE patients and controls (*$p < 0.01$) and difference between active and inactive cases of SLE (†$p = 0.05$).

mean activity of 30·5%. Out of 15 patients tested in the K562 assay, five were untreated. All these patients had lower NK activity than simultaneously tested controls ($P < 0.05$).

Interferon production by peripheral blood cells

Production of IFN by Sendai virus stimulated whole blood cell cultures was clearly lower in SLE patients than in controls (Table 1). The IFN produced seemed to be of the α-type as shown by its resistance to pH 2 treatment and by its activity in bovine cells, which are known to be highly selective for IFN-α (Yip *et al.*, 1981). Patients with active SLE produced significantly less IFN than the controls ($P < 0.01$) and than patients with inactive disease ($P = 0.05$). In fact, the overall decrease of IFN production could totally be accounted for by the active SLE cases.

Production of IFN-γ was measured after stimulation of the whole blood cell cultures with PHA (10 μg/ml). Although there was no statistically significant difference between average amounts of IFN produced by patients and controls, seven out of the 17 patients (four with active and three with inactive disease) produced less than 600 units/ml, i.e. the lowest amounts of IFN produced by any of the controls. In accordance with previous results (Yip *et al.*, 1981), PHA-induced IFN appeared to be largely of the γ-type, as revealed by lability at pH 2, inactivation by heat and failure to protect bovine cells against viral infection. There was no correlation between NK cell activity and production of IFN-α or IFN-γ.

Serum IFN levels in SLE patients and controls

Serum IFN concentrations were tested using two cell lines, one used to detect IFN of all three types (A549 cells) and the other for selective demonstration of IFN-α (MDBK cells). In both types of assays IFN levels were clearly higher ($P < 0.01$) in SLE patients (Fig 3). In the A549 assay, both untreated and pH2 treated sera were tested. The levels of pH2 stable IFN was significantly higher in SLE patients than in controls ($P < 0.05$). When IFN levels in pH2 treated sera were subtracted from levels in untreated sera, it was evident that the major part of the IFN was of the pH2 labile variety. Similarly to levels of IFN-α, levels of pH2 labile IFN were significantly higher in SLE patients than in controls and the highest levels were observed in patients with active disease (Fig. 3). By contrast, no evident correlation was observed between disease activity and levels of pH stable IFN.

Enhancing effect of IFN-α on NK activity

NK activity in both SLE patients and controls could be boosted by treatment for 1 hr with 300 units of IFN-α. The boosting activity was seen as an increase in percentage lysis, corresponding to about a four-fold increase in lytic units (Figs 2 & 4). SLE cells were significantly ($P < 0.05$) less stimulated by IFN than controls in the Chang cell assay and the K562 assay showed a similar, but not statistically

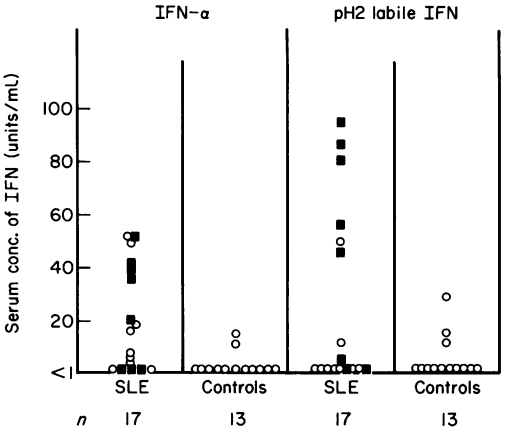

Fig. 3. Serum levels of IFN-α and pH2 labile IFN in SLE patients and controls. IFN-α levels refer to number of units/ml assayed in a bovine kidney cell (MDBK) assay. Units of pH labile IFN was calculated after subtracting number of units of pH2 stable IFN from total number of IFN units measured in a human lung carcinoma cell (A549) assay. Values obtained from active cases of SLE are designated by ■.

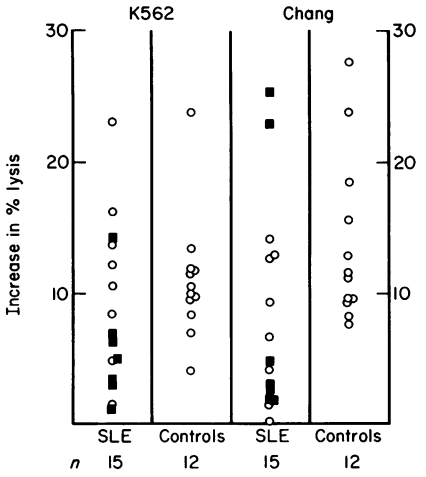

Fig. 4. Augmenting effect of IFN on NK activity of cells from SLE patients and controls. Boosting effect of IFN is expressed as % lysis exerted by effector cells treated with 300 units of IFN-α for 1 hr minus % lysis obtained with untreated effector cells. Values obtained from active cases of SLE are designated by ■.

significant difference. However, when only SLE cases with high activity were considered, the boosting effect of IFN was lower in SLE than in controls also in the K562 assay ($P < 0{\cdot}05$). There was no clear correlation between IFN production or serum IFN levels and sensitivity of the cells to stimulation by IFN.

DISCUSSION

Systemic lupus erythematosus has been associated with abnormal function of T cells (Messner *et al.*, 1973; Hahn, 1975; Rosenthal & Franklin, 1975), B cells (Jasin & Ziff, 1975; Nies *et al.*, 1980) as well as macrophages (Svensson, 1975; Markenson *et al.*, 1978). In the present study, we found evidence of a decreased activity of another cell type in SLE, namely natural killer (NK) cells, which share characteristics with both T cells and monocytes (Herberman, 1981). This did not appear to be a

consequence of treatment of the patients and was neither correlated with disease activity. The finding of low NK cell activity in SLE confirms previous observations by us and by others (Strannegård *et al.*, 1980; Oshimi *et al.*, 1979; Hoffman, 1980; Goto *et al.*, 1980). The cause of the decreased NK cell activity is unknown. There is evidence for occurrence of anti-NK cell antibodies in SLE (Goto *et al.*, 1980) but immune complexes or suppressor cells have not been related to the depression of NK activity (Hoffman, 1980). The observations by us and by Fitzharris *et al.*, (1982) that NK cells from patients with active SLE have a decreased sensitivity to the augmenting effect of IFN, suggest that the low NK activity is not due solely to a reduction in numbers of NK cells. Rather, the results suggest a functional abnormality of the NK cells, or that there is reduced number of NK cells together with a shift in the balance between IFN inducible pre-NK cells and activated NK cells. The findings of increased serum IFN levels in SLE would certainly be compatible with the latter possibility.

Our findings of decreased virus-induced IFN production of blood cells from SLE patients is at variance with some studies (Alarcon-Segovia *et al.*, 1974; Suzuki, 1978) but agrees with our earlier results on a different population of patients (Strannegård *et al.*, 1980). These discrepancies may be explained by differences in the methodologies used or the patient groups studied. The virus-induced IFN in our study appeared to be of the α variety since it was pH2 stable and active in bovine cells. A decreased production of IFN-α should be expected in SLE, since the activity of NK cells, which probably produce IFN-α (Saksela & Timonen, 1980), is subnormal.

The average production of mitogen-induced IFN, which is mainly of the γ-type (Yip *et al.*, 1981) was not significantly subnormal in our cases. However, the variation in IFN-γ production was considerable, some patients producing very high amounts of IFN, and seven out of 17 producing less IFN than any of the controls. Since levels of serum IFN are increased in SLE, the decreased in vitro production of IFN-γ, seen in some patients in this study and in a previous one by Suzuki (1980), may be a reflection of prior excessive stimulation and exhaustion of IFN-γ producing cells *in vivo*. An alternative interpretation would be that decreased production of IFN-γ reflects a basic defect in the T cells. Several subpopulations of T cells appear to have the ability to produce IFN-γ (Epstein & Gupta, 1981) and the proliferative response to T cell mitogens like PHA is decreased in SLE (Rosenthal & Franklin, 1975). In NZB/NZW mice, which have a disease similar to SLE in humans, a decreased production of mitogen-induced IFN has been observed (Menchaca *et al.*, 1979).

In agreement with a previous study (Hooks *et al.*, 1979) we found increased serum levels of pH2 labile IFN in SLE, especially in cases with active disease. In addition, we found levels of pH2 stable IFN, apparently of the α-type, to be increased. High IFN levels might be a sign of chronic viral infection, but several other explanations are possible. For instance, since IFN appears to be a positive regulator of both NK and suppressor cells, deficiencies in these populations might secondarily lead to abnormalities in the IFN producing system. Irrespective of the cause of increased serum IFN levels there are possible pathogenetic implications of the findings. Several studies have shown that administration of IFN-γ accelerates development of autoimmunity in NZB/NZW mice (Heremans, Billiau & Colombatti; 1978; Engleman *et al.*, 1979, 1981). In particular IFN-γ may be a very potent stimulator of antibody production (Sonnenfeld, Mandel & Merigan, 1978). Therefore, although some of the reported effects of IFN, e.g. suppressor cell activation (Attallah *et al.*, 1981; Kadish *et al.*, 1980) might be beneficial in SLE, the therapeutic use of IFN in this disease (Siniachenko, 1980; Alekberova *et al.*, 1980) should be regarded with scepticism.

In summary, the present studies have confirmed earlier findings of decreased NK cell activity, decreased sensitivity of NK cells to stimulation by IFN, and increased serum levels of pH2 labile IFN in SLE. In addition we have found increased levels of pH2 stable IFN and obtained evidence for a decreased production by peripheral blood cells of IFN-α and in certain cases, also of IFN-γ. The interpretation of these findings and elucidation of their possible role in the pathogenesis of SLE will require further studies.

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