Natural killer cell activity in untreated systemic lupus erythematosus

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SUMMARY With strictly selected controls natural killer cell activity was evaluated in 10 untreated patients with systemic lupus erythematosus. Natural killer levels of the patients were significantly lower than those of the age- and sex-matched normal controls. Natural killer levels, however, did not correlate with disease activity.

Natural killer (NK) cells, defined as normal unprimed lymphocytes which have cytotoxic activity against various target cells, are suspected as the mediators of the first defence mechanism against cancer and viral infections.¹⁻² NK cell activity is found in IgG-Fc receptor-bearing lymphocytes of T and non-T cells in man.³⁻⁴ Because type C viruses may play a possible pathogenetic role⁵⁻⁶ and the number of IgG-Fc receptor-bearing lymphocytes has been reported to be decreased⁷ in systemic lupus erythematosus (SLE), we wanted to know whether or not NK cell activity is decreased in SLE.

The assessment of NK cell function may, however, be accompanied by such difficult problems as variations in NK cell activity from experiment to experiment in the same individual⁸⁻¹² and the influence of age,¹³⁻¹⁴ sex,^{10-11, 14-16} and treatment¹¹⁻¹⁷ on NK cell activity. Thus it is important to obtain strictly selected controls each time in the experiments¹¹⁻¹⁷ and to consider the effects of treatment, particularly of corticosteroids for the exact assessment of NK cell function in diseases. Our preliminary studies¹⁸ showed that, in 4 untreated patients with SLE, NK levels were lower in the patients than in all normal controls. With the use of strictly selected controls we report here the NK levels of 10 untreated patients, including 4 cases previously reported, and present the details of their clinical features. The relationship of NK levels with disease activity is analysed.

Materials and methods

Ten patients with SLE were studied. They were all

females. Details of the clinical findings are shown in Table 1. Cases 1-7 met the preliminary criteria for classification of SLE¹⁹ and cases 8-10, which did not meet the criteria, were diagnosed as SLE from serological data and renal biopsy findings in addition to other clinical findings. All the patients were considered to have active disease according to the criteria of Winfield *et al.*²⁰ No patients were receiving corticosteroids or cytotoxic drugs. NK activity in the patients was assessed on different dates for different patients. In each experiment 5 healthy female controls were used, matched for the patients' ages ± 3 years.

Mononuclear cells separated from heparinised whole blood on Ficoll-Conray gradients (Conray 400; sodium iotalamate, Daiichi Seiyaku Co., Tokyo, Japan) were used as effector cells. For the preparation of the target cells, 1×10^6 K562 cells or 1×10^6 MOLT-4 cells suspended in 0.3 ml of medium 199 (Nissui Seiyaku Co., Tokyo) with 10% fetal calf serum were labelled with $50 \mu\text{Ci Na}_2^{51}\text{CrO}_4$ (Japan Isotope Association, Tokyo) at 37°C for 2 h and washed 4 times thereafter. The K562 cells (erythroleukaemic cell line) and MOLT-4 cells (T cell line) used as the target cells were maintained in RPMI 1640 medium (Grand Island Biological Co., Grand Island, NY) containing 5% fetal calf serum and $5 \times 10^{-8}\text{M}$ 2-mercaptoethanol. Effector cells, 2×10^5 , were incubated with 1×10^4 ^{51}Cr -labelled target cells (effector:target 20:1) in 0.15 ml medium 199 with 10% fetal calf serum in triplicate in microculture wells (76-013-05; Linbro Scientific, Inc., Hamden, Conn) for 5 h at 37°C in an incubator containing 5% CO_2 . At the termination of the ^{51}Cr -release test supernatants were harvested with the Titertek supernatant collection system (Flow Laboratories Inc., Rockville, Md), and radioactivity was counted in a

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Table 1 Clinical findings of the patients and their NK cell activity against K562 target cells

Patient	Age	Sex	Comments	Leucocytes (/mm ³)	ESR (/h)	γ -glob. (g/dl)	CH ₅₀ * (U/ml)	A-DNA (times)	NK (%)	Relative cytotoxic activity [‡]
1	18	F	Discoid rash, alopecia, Raynaud, photosensitivity	4100	13	1.9	26.2	640†	45.4	71.9
2	28	F	Fever, hair loss, photosensitivity, casts	1200	37	1.5	25.1	<80†	12.5	31.3
3	18	F	Butterfly rash, hair loss, fever, casts	2600	73	2.0	<8.0	2560†	33.9	55.9
4	21	F	Butterfly rash, casts	1900	31	1.4	16.3	320†	31.1	61.7
5	28	F	Butterfly rash, alopecia, casts	1700	79	3.1	<8.0	150‡	40.8	87.9
6	40	F	Butterfly rash, alopecia, photo- sensitivity, arthralgia	3100	39	2.1	<8.0	>150‡	21.4	35.3
7	21	F	Fever, arthralgia, Raynaud, hair loss, photosensitivity	5400	130	4.4	<8.0	>150‡	26.2	38.9
8	16	F	Proteinuria, casts	5700	41	1.6	31.8	127‡	25.3	36.7
9	26	F	Nephrotic syndrome, casts, alopecia	5000	33	1.8	31.9	80†	66 ^{§0}	146.0
10	24	F	Butterfly rash, casts, BFP ¹¹	4900	55	1.7	29.7	8‡	27.8	94.2

*Normal value of CH₅₀ is 24.7–39.5 U. †A-DNA antibody titre by passive haemagglutinin assay (normal, less than 80 times). ‡A-DNA antibody titre by ¹²⁵I-DNA binding assay (normal, less than 10 U/ml). §The percentage ratio between the patient's NK activity and the mean of the 5 controls' NK activity. ¹¹Biological false positive.

gamma well counter. The percent specific ⁵¹Cr release was calculated according to the following formula: [(experimental release – spontaneous release)/(maximum release – spontaneous release)] × 100, where the spontaneous release was the ⁵¹Cr release from 1 × 10⁴ labelled cells incubated alone in

medium 199 with 10% fetal calf serum, and the maximum release was that from the labelled target cells incubated alone in water containing 5% 7X detergent (Linbro Scientific Inc.). The percentage specific ⁵¹Cr release was considered as NK cell activity.

Fig. 1 NK cell activity in SLE. In each experiment the NK level of an untreated patient (○) was compared with those of age- and sex-matched 5 normal control donors (●). K562 (upper) and MOLT-4 (lower) were used as target cells.

Fig. 2 NK levels and disease activity. Relative cytotoxic activity of the patients' NK cells was compared with total haemolytic complement (CH_{50}). $r=0.38$. A CH_{50} of less than 8 was plotted as 8.

Results

To find the optimal effector:target (E:T) ratio in a ^{51}Cr -release assay varying numbers of effector cells (1×10^5 , 2×10^5 , and 4×10^5) obtained from case 1 and five control donors were incubated with 1×10^4 K562 or MOLT-4 target cells, and the specific ^{51}Cr release values obtained were plotted versus the different E:T ratios. In most of the donors the curves were linear between these E:T ratios (data, not shown). Thus, an E:T ratio of 20:1, a median of the 3 different E:T ratios, was used for the following assays.

NK cell activity in the patients and normal controls is depicted in Fig. 1. In 7 of the 10 patients NK activity was lower than all the controls, in 2 patients NK activity was within normal control values, and in one the level was higher than the controls. With Welch's test NK activity of the patients was significantly lower than that of the controls ($\alpha = 0.005$ and 0.01 in the experiments in which K562 and MOLT-4 were used as target cells respectively). Almost same results were obtained in the experiments of both K562 and MOLT-4 target cells, and an individual who had high NK activity against one target had high NK activity against the other target.

To assess the relationship between NK function and disease activity the patients' NK levels were compared with several laboratory values—leucocyte count, lymphocyte count, ESR, gamma globulin, CH_{50} , C3, C4, and anti-DNA titre. Since NK levels of the same individual fluctuate from experiment to

experiment, relative cytotoxic activity (the percentage ratio between the patients' NK activity and the mean of the 5 controls' NK activity) was used for the statistical analysis. The results showed that none of the above laboratory values correlated with the relative cytotoxic activity. Neither did the patients' unmodified NK levels correlate with the laboratory values. Fig. 2 compares the relative cytotoxic activity with CH_{50} levels. The correlation coefficient was $r=0.38$.

Discussion

Several points must be considered in the assessment of NK cell function, namely, fluctuations in NK cell activity from experiment to experiment in the same individual, and the influence of age, sex, and treatment on NK cell activity. Fluctuations of NK activity in the same individual were observed in our experiments as shown elsewhere.^{11, 12} Our data and those of others⁸⁻¹⁰ suggest that fluctuations in NK levels depend mainly on some as yet undefined variables in the NK assay process rather than the real fluctuations in vivo. With regard to age, we do not know whether age has a major effect on NK cell activity when K562 or MOLT-4 is used as target cells. With regard to sex difference, mean NK level in males was somewhat higher than in age-matched females when MOLT-4 was used for target cells.¹¹ On the effects of corticosteroids, Parrillo and Fauci¹⁷ showed that dexamethasone administered to volunteers caused marked inhibition of NK cell activity, and our data also suggested that corticosteroids could suppress NK cell function in SLE patients.¹¹

In addition to our preliminary report¹⁸ Hoffman²¹ and Penschow and Mackay¹⁴ indicated that NK cell function was impaired in SLE. However, Hoffman did not mention the age of patients or control donors, nor did he give details of the fluctuation of NK activity. In Penschow and Mackay's report most of the patients were on prednisolone. With strictly selected controls we have shown that the NK levels were lower in untreated patients with SLE than in age- and sex-matched controls and that the NK levels did not correlate with disease activity. The conclusion that all the patients did not have low NK activity (Fig. 1) might suggest that impaired NK function is not the primary cause of the disease but rather the result of the disease.

The mechanism by which NK cell function is impaired in SLE is unknown. Can immune complexes impair the NK cell function by virtue of their attachment to the surface of NK cells? This possibility may be small because 48 hours' incubation of patients' lymphocytes did not restore the NK cell function (data not shown). The presence of suppressor cells seems unlikely, because the addition of

patients' lymphocytes to those of normal donors did not inhibit the NK cell function of the normal donors (data not shown). One of the authors (N. Gonda) has recently found that the treatment of normal lymphocytes with IgG fraction of patients' serum plus complement markedly suppressed their NK cell function (manuscript in preparation). Although this result must be confirmed, it is suggested that patients with SLE might have antibody reactive to NK cells.

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