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Effects of corticosteroids on natural killer cell activity in systemic lupus erythematosus

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SUMMARY

The effects of corticosteroids on the natural killer (NK) cell activity of human peripheral blood lymphocytes were evaluated. When six untreated female patients with systemic lupus erythematosus were compared with fifteen age-matched, corticosteroid-treated female patients, NK activity in the latter was significantly suppressed. Although the administration of high doses seemed to suppress cytotoxicity to a greater extent, there was no close correlation between the daily doses of steroids and NK activity. When cytotoxicity levels were followed before and during corticosteroid therapy in the same patient, NK activity decreased markedly during treatment, particularly in patients on high-dose corticosteroids.

INTRODUCTION

Natural killer (NK) cells, defined as normal unprimed lymphocytes which have cytotoxic activity against various target cells, are suspected of being the mediators of immune surveillance against cancer (reviewed by Welsh, 1978). Whereas interferon and its inducers, viral infections, bacillus Calmette-Guérin and *Corynebacterium parvum* infections, and tumour cells were shown to enhance NK cell activity (Welsh, 1978), corticosteroids, cyclophosphamide and high-dose irradiation were found to inhibit NK cell activity in animals (Shellam, 1977; Oehler & Herberman, 1978; Djeu *et al.*, 1978).

Corticosteroids, which are important therapeutic agents against immunological diseases and various malignant diseases, have profound effects on human immune systems (reviewed by Fauci, Dale & Balow, 1976). The precise way in which their immunosuppressive effects work remains unclear. However, if corticosteroids suppress NK cell activity and NK cells play an important role against cancer, corticosteroids might give rise to aberrations in the immune surveillance system and might subsequently produce conditions favourable to oncogenesis. As far as we know, the effects of corticosteroids on human NK cell activity have been studied only by Parrillo & Fauci (1978), who showed that dexamethasone administered orally to volunteers caused marked inhibition of NK cell activity within 24 hr. A few patients with collagen diseases who were receiving corticosteroids were reported to have very low levels of NK cell activity (Rosenberg *et al.*, 1974), but NK cell activity in untreated patients was not shown. It is not known, however, whether the suppression depends on the daily dose of corticosteroids, the duration of the therapy or individual differences in sensitivity to corticosteroids.

For our study of the effects of corticosteroids, patients with systemic lupus erythematosus (SLE) were used as donors of NK cells, since many of them receive corticosteroid therapy alone and SLE

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shows a variety of immunological abnormalities, including a possible decrease in the number of Tγ cells, i.e. IgG Fc receptor-bearing T cells (Gupta & Good, 1977), by which NK cell activity in T cells is almost exclusively mediated (Gupta *et al.*, 1978). The purpose of the present study was to investigate the suppressive effects of corticosteroids on NK cell activity in SLE and the correlation between the level of suppression and the daily or total dose of corticosteroids.

MATERIALS AND METHODS

Subjects. Normal healthy donors and patients with SLE were studied.

Assay of NK cell activity. Mononuclear cells separated from heparinized whole blood on Ficoll–Conray gradients (Conray 400; sodium iotalamate, Daiichi Seiyaku Company, Tokyo, Japan) were used as effector cells. Incubation of 4×10^5 effector cells was performed with 2×10^4 ^{51}Cr-labelled MOLT-4 target cells (effector:target 20:1) in 0·15 ml medium 199 (Nissui Seiyaku Company, Tokyo) with 10% foetal calf serum in triplicate in microculture wells (Linbro Scientific Incorporated, Hamden, Connecticut, USA, 76-013-05) for 4½ to 5½ hr at 37°C in an incubator containing 5% CO_2. The MOLT-4 cells used as the target cells were a cell line established from a patient with acute lymphoblastic leukaemia (Minowada, Ohnuma & Moore, 1972) and were maintained in Joklik-modified minimum essential medium (Grand Island Biological Company, Grand Island, New York, USA) containing 5% foetal calf serum. For the preparation of the target cells, 2×10^6 MOLT-4 suspended in 1 ml of medium 199 with 10% foetal calf serum were labelled with 100 μCi $Na_2{}^{51}CrO_4$ (Japan Isotope Association) at 37°C for 1½ to 2 hr and washed four times thereafter. At the termination of the ^{51}Cr-release test, supernatants were harvested using the Titertek Supernatant Collection System (Flow Laboratories, Rockville, Maryland, USA) and counted in a gamma well counter. Per cent specific ^{51}Cr release was calculated according to the following formula: % specific ^{51}Cr release = [(experimental release − spontaneous release)/(maximum release − spontaneous release)] × 100, where spontaneous release was the ^{51}Cr release from 2×10^4 labelled MOLT-4 cells incubated alone in medium 199 with 10% foetal calf serum, and maximum release was that from labelled MOLT-4 cells incubated alone in water containing 5% detergent 7X (Linbro Scientific Incorporated). Per cent specific ^{51}Cr release was considered as NK cell activity.

RESULTS

Fluctuations in NK cell activity

NK cell activity was repeatedly assayed in two normal male donors (Fig. 1. The values of NK cell activity assayed in each experiment are connected with solid lines). The results showed that the levels of NK activity fluctuated even in the same individual from day to day and that Donor II always had a higher level of activity than Donor I. This fluctuation was considered to be due mainly to certain factors in the assay process rather than to real fluctuations *in vivo* since the NK levels of these two donors fluctuated in unison. Wide fluctuations have also been noted in other reports where they were attributed to some as yet undefined variables in the NK assay process (Rosenberg *et al.*, 1974; Pross & Baines, 1976).

NK cell activity in normal male and female donors

NK cell activity was compared in eleven normal male and ten normal female donors, 20 to 40 years old, in a single experiment (Fig. 2). Mean NK cell activity in the males was somewhat higher than in the females, although the difference was not significant ($P > 0{\cdot}05$) by Student's *t*-test.

Comparison of NK levels in untreated patients and patients with SLE who were given corticosteroids

NK activity in female patients with SLE, six of whom were untreated (aged 16 to 44, mean 31) and fifteen of whom were on corticosteroid therapy (aged 20 to 44, mean 32), was assessed on different dates for different patients. The disease was active in all untreated patients, both those for whom

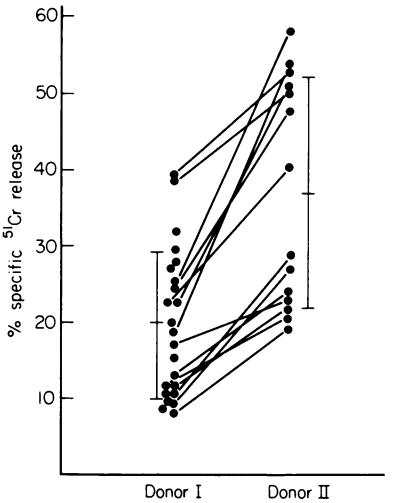

Fig. 1. Fluctuations in NK cell activity. NK cell activity in two normal male donors was assayed repeatedly. The values for each experiment are connected with solid lines.

systemic corticosteroid therapy was necessary and those for whom it was not. Corticosteroid-treated patients included cases in which the disease was active and cases in which it was inactivated by treatment. Since NK activity fluctuated from day to day and this fluctuation seemed to arise from the assay process itself, the values of NK activity were corrected in each experiment according to the following method: the mean levels of the two male controls, Donors I and II, were 19·7 and 37·0 respectively (Fig. 1). Since these two donors were always included as controls in each assay, the

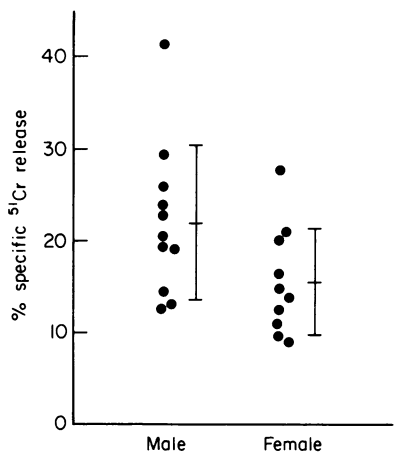

Fig. 2. NK cell activity in normal male and female donors. The NK levels of eleven males and ten females, 20 to 40 years old, were $22{\cdot}0 \pm 8{\cdot}4$ and $15{\cdot}6 \pm 5{\cdot}8$ (mean $\pm$ s.d.) respectively ($P > 0{\cdot}05$).

mean of the two, 28·4, was used as a standard control level and, in each experiment, the value of NK cell activity in each patient with SLE was adjusted proportionally to the ratio between the standard level and the mean of the two control levels. The results (Fig. 3) showed that the NK levels of patients on corticosteroid therapy were significantly lower ($P < 0{\cdot}05$) than those of untreated patients according to Student's *t*-test.

Relationship between NK level and dose of corticosteroids

The relationship between the daily dose of corticosteroids and the adjusted NK levels was analysed. Although the NK levels were not correlated closely with the daily dose of steroids (Fig. 4) or with the

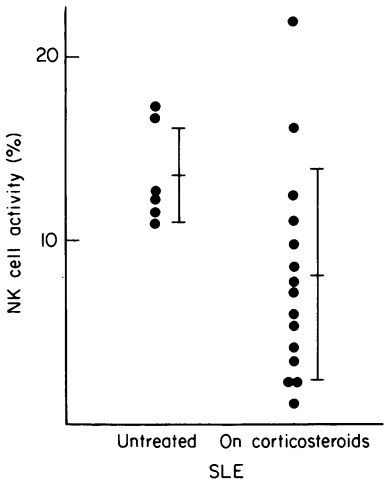

Fig. 3. Comparison between the adjusted NK levels of untreated and corticosteroid-treated female patients with SLE. Means ± s.d. of untreated and treated were 13·6 ± 2·7 and 8·1 ± 5·7 respectively ($P < 0.05$). See text for the method used for adjusting NK levels.

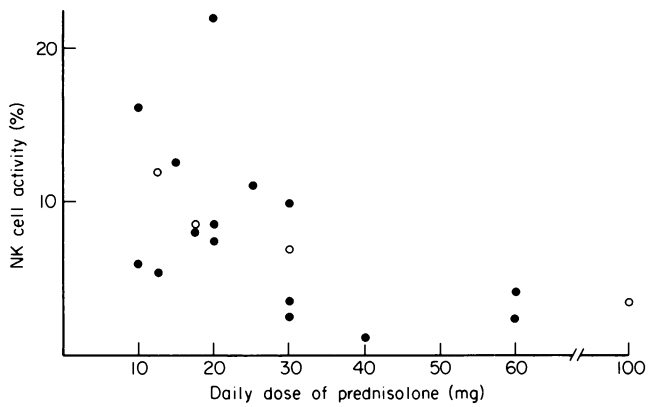

Fig. 4. Relationship between dose and NK levels. The correlation between daily doses of prednisolone and the adjusted NK activity levels in nineteen patients with SLE was analysed: (o) male; (•) female.

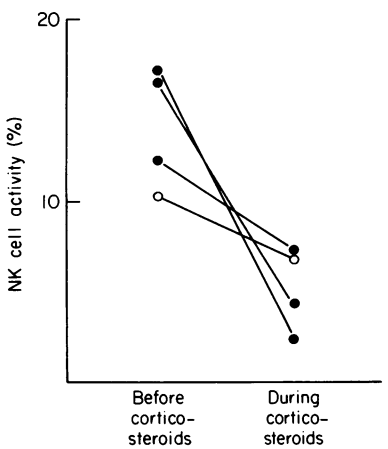

Fig. 5. Comparison of adjusted NK levels before and during corticosteroid therapy in the same patients. Three females (•——•) and one male (o——o).

total dose of steroids (data not shown), NK activity in patients administered with high doses of corticosteroids was generally lower, and NK activity of less than 5% was seen only in patients on more than 30 mg per day. Of these patients, four were active and two were inactive, indicating that while disease activity is not closely correlated to NK activity, treatment may influence NK activity.

Comparison of adjusted NK activity levels before and during corticosteroid therapy

NK activity was followed in three of the above female and one male patient before and during corticosteroid therapy (Fig. 5). The NK levels of all four patients fell during steroid therapy. Two patients showed a marked decrease in NK activity compared with the other two patients. Following assays taken before treatment in all four cases, further assays were taken after the former two had been given 60 mg of prednisolone daily for 4 and 7 weeks respectively, and the latter two 30 mg daily for 6 weeks and 20 mg daily for 12 weeks respectively. Although the time lapse between the two assays could have led to changes in other critical factors influencing NK activity, the results seem to indicate an association between NK activity and the daily dose, rather than between NK activity and the total dose of corticosteroids in each individual.

DISCUSSION

The data presented in this paper strongly suggest that corticosteroids suppress NK cell activity. Corticosteroids are known to influence various immune reactions (Fauci *et al.*, 1976); monocyte–macrophage function seems to be particularly sensitive to corticosteroids, and their administration causes transient leucocytopenia in all detectable lymphocyte subpopulations, particularly recirculating T cells. Our present study and that of Parrillo & Fauci (1978) demonstrate the suppressive effects of corticosteroids on human NK cell function. The precise mechanism of the suppression is not known. Corticosteroids seem to act directly on NK cells to lyse them or to suppress their function rather than to change the distribution of circulating NK cells, since the addition of corticosteroids to *in vitro* cultures also suppresses their activity (unpublished observation). As seen from Fig. 5, the suppression of NK activity seems to correlate with the daily dose rather than with the total dose of corticosteroids. This observation is supported by an *in vitro* culture study in which corticosteroids were added in various concentrations to *in vitro* cultures which were observed after 2 days; the greater the concentration of corticosteroids, the higher the degree of suppression of NK cell activity. This *in vitro* study also revealed individual differences in NK cell sensitivity to corticosteroids (manuscripts in preparation).

The application of these assessments of NK cell function to clinical studies is accompanied by such difficult problems as variations in NK cell activity from experiment to experiment in the same individual and the influence of age and sex on NK cell activity. Our present data and that of others (Rosenberg *et al.*, 1974; Pross & Baines, 1976) suggest that fluctuations in NK levels depend mainly on some as yet undefined variables in the NK assay process. Until these variables are eliminated from the assay process, it is necessary to express experimental data relative to the control NK activity.

We have not studied the influence of age on NK cell activity. Takasugi, Mickey & Terasaki (1973) and Oldham *et al.* (1975) found no significant relationship between cytotoxicity and the ages of the normal donors. In contrast, Rosenberg *et al.* (1972) found that donors under the age of 16 had a considerably lower incidence of cytotoxicity. The cause of this discrepancy is not known, though the data of Takasugi *et al.* (1973) suggested that it depended on the type of target cells used in the assays. Since it is not known whether age has a major effect on NK cell activity when MOLT-4 is used for the target cells, our studies were done between age-matched untreated (aged 16 to 44, mean 31) and treated (aged 20 to 44, mean 32) patients with SLE (Fig. 3).

With regard to sex differences, Santoli *et al.* (1976) reported that NK cell activity in male donors was twice that in female donors using non-lymphoid adherent cells as target cells, and Ono, Amos & Koren (1977) showed that males were higher in reactivity than females when thymocytes were used as target cells; on the other hand, there were no significant differences when T and B cell lines were used. Our data (Fig. 2) revealed that normal male donors had somewhat, though not significant,

higher reactivity than age-matched normal females, and this observation made it necessary to compare NK levels within the same sexes.

Because of the reasons stated above, it is necessary to select adequate controls when NK cell activity is to be assayed in patients. The precise level of NK cell activity in the six untreated female patients with SLE was not determined in comparison with normal females since neither female nor age-matched donors were used as controls in this study and in most of the other studies conducted by us (as yet unreported). However, we should mention that, using strictly selected donors as controls, i.e. of the same sex and the same age as the patient, we studied NK cell activity in four untreated patients with active SLE, in which the level of NK cell activity was lower than in all the control donors (data not shown). Although Santoli & Koprowski (1979) briefly mentioned similar findings in which one patient with SLE had low NK activity, it is necessary to make further studies to ascertain whether NK levels in SLE are, in fact, low.

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