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T cell function in systemic lupus erythematosus: normal production of and responsiveness to interleukin 2

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SUMMARY

To investigate the role of interleukin 2 (IL-2) in systemic lupus erythematosus (SLE) mononuclear cells (MNC) of 68 SLE patients were tested for their ability to produce and also to respond to IL-2. Cells were collected monthly over an one year period. IL-2 production by MNC was measured under various conditions after optimal and suboptimal stimulation. Although we found a large variation in IL-2 production by individual MNC preparations no statistical significant differences were found between normal and SLE cells.

To study IL-2 responsiveness, proliferation of MNC was studied under conditions where endogeneous IL-2 production is limiting. Addition of IL-2 resulted in a four- to eight-fold enhancement of proliferative responses. However also in this respect no differences were found between SLE patients and healthy controls. Thus, in this group of SLE patients no abnormalities in IL-2 production or response could be demonstrated.

Keywords interleukin 2 production interleukin 2 responsiveness systemic lupus erythematosus human T cells

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown etiology, characterized by a variety of immunological dysfunctions including the production of autoantibodies to nuclear constituents (Tan, 1982, Smeenk, Westgeest & Swaak, 1985), T-lymphocytes (Morimoto *et al.*, 1980) and even interferon (Panem *et al.*, 1982). Defects in T cell functions have been described with respect to T-suppressor cells (Fauci *et al.*, 1978; Krakauer *et al.*, 1980; Morimoto, Abe & Homma, 1979; Newman *et al.*, 1979; Sakane *et al.*, 1983), cytotoxic activity (Tsokos & Balow, 1983), autologous mixed lymphocyte reactivity (Sakane, Steinberg & Green, 1978) and interleukin production (Alcocer-Varela & Alarcon-Segovia, 1982; Linker-Israeli *et al.*, 1983; Miyasaka *et al.*, 1984; Nakamura *et al.*, 1983). In general, however, the results are still controversial. Recent findings suggest a normal T-suppressor cell population (Gattringer *et al.*, 1982; Tsokos & Balow, 1983; Draeger & Aarden, 1984), a normal IL-2 production (Sibbit *et al.*, 1984) and a normal IL-2 responsiveness (Miyasaka *et al.*, 1984) of MNC derived from SLE patients.

A central role in all immunoregulatory events is played by interleukin 2 (IL-2). Several models of T cell activation (Smith, 1980; Palacios, 1982) indicate that IL-2 is produced after stimulation of T cells with antigens or mitogens in the presence of the macrophage product interleukin 1 (IL-1) (Larsson, Iscove & Coutinho, 1980; Sando *et al.*, 1981; Neefe, Curi & Woody, 1981). The requirement for the latter factor can be bypassed by a phorbolester (PMA) (Rosenstreich & Mizel, 1979; Fuller-Farrar *et al.*, 1981; Koretzky, Danielle & Nowell, 1982). Stimulated T-lymphocytes express a receptor for IL-2 (Robb, Munck & Smith, 1981; Warren *et al.*, 1982) and after binding to this receptor, IL-2 gives rise to proliferation. Adsorption of IL-2 by IL-2 receptor expressing cells is

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suggested as a mechanism for concanavalin A (Con A)-induced suppression (Palacios & Moller, 1981). Next to its role in T cell activation IL-2 seems to be involved in B cell activation (Zubler *et al.*, 1984; Sauerwein *et al.*, 1985; Miedema *et al.*, 1985). IL-2 receptors are present on leukaemic B cells (Korsmeyr *et al.*, 1983), Epstein-Barr virus (EBV) transformed B cells (Muraguchi *et al.*, 1985) and stimulated normal B cells (Boyd *et al.*, 1985; Miller *et al.*, 1985).

Autoimmune-prone strains of mice (NZB/W, MRL/lpr) are described to be defective in production of and responsiveness to IL-2 (Wofsy *et al.*, 1981; Altmann *et al.*, 1981). However, normal IL-2 production is observed in the presence of PMA (Santoro *et al.*, 1983). Studies concerning human SLE have been carried out on a very limited number of patients. To evaluate the role of IL-2 in the etiology of SLE, we studied MNC of 68 SLE patients and of 20 donors over a period of more than 1 year for their capacity to produce and to respond to IL-2 using various conditions for stimulation. Although large individual variations occur, the results indicate that MNC of SLE patients show a normal IL-2 production as well as a normal response to IL-2.

MATERIALS AND METHODS

Patients population. Sixty-eight SLE patients who fulfilled four or more criteria for classification of SLE (Tan *et al.*, 1982) were studied. Fifty-eight patients were female (20–80 years old; mean = 45 ± 16) and 10 were male (52–72 years old; mean = 62 ± 8). Five patients were in an active phase of their disease as defined by the occurrance of major disease symptoms. Disease activity was recorded without knowledge of the serological parameters or by the experiments carried out for the present study and also treatment of the patients was not influenced by this. On standard clinical record sheets, signs of disease activity, medication indication for dosage alterations and routine clinical laboratory results were recorded. Exacerbations were defined by the occurrence of major disease features (Lightfoot & Hughes, 1976; Swaak *et al.*, 1982). Minor symptoms were mainly restricted to the musculoskeletal system and the skin, and at such a stage disease could be controlled by an increase in prednisolone dosage of 5–15 mg per day without the need for admission to hospital. In this study, major features were characterized by at least one of the following: renal impairment, serositis, anaemia (Hb < 7 g/dl) and/or leucopenia (white cell count $< 4 \times 10^9$/l), neurological or psychiatric symptoms. These features always led to hospitilization, and the dosage of prednisolone was increased to more than 20 mg. Episodes in which the features were explained by causes other than SLE were excluded. Patients with pre-existing renal involvement were considered to be inactive when no significant alterations took place in the creatinine values, proteinuria, and urine sediment. An increase in proteinuria or other evidence of further deterioration of renal function, in the absence of other causes (such as pyelonephritis not related to SLE), were recorded as exacerbations of renal disease. All patients were followed on a monthly basis. Venous blood was collected in vacutainer tubes containing 0·1 ml 0·34 M EDTA. MNC were isolated from this blood as described below. All experiments shown were done with freshly prepared MNC.

Twenty healthy volunteers aged between 25 and 48 years were used as donors of normal lymphocytes. Some of them were studied on several occasions.

Lymphocyte isolation and culture conditions. EDTA blood of patients and normal donors was diluted 1:2 in phosphate buffered saline (PBS) (0·15 M NaCl, 0·01 M phosphate, pH 7·4) supplemented with 10% human albumin (pasteurized human plasma solution, this institute) and 0·38% Trisodiumcitrate. MNC were isolated by density centrifugation using Percoll (Pharmacia) d = 1·078. Cells in interphase were harvested, washed and resuspended in culture medium (2×10^5/ml) and cultured in 200 μl wells. As culture medium Iscove's Modified Dulbecco's Medium (IMDM) (GIBCO, Grand Island, NY, USA) was supplemented with Penicillin/Streptomycin (100 U/ml and 100 μg/ml respectively), 5×10^{-5} M 2-mercaptoethanol and 5% heat inactivated human pool serum. If not mentioned otherwise 5 μg ConA/ml (Miles Yeda, Rehovot, Israel) was added for stimulation. Proliferation was measured by a 4 h pulse of ^{3}H-Thymidine (spec. activity 2 Ci/mmol); 0·2 μCi in a volume of 10 μl in saline per well was added. Cultures were harvested on glass-fibre filters (Titertek/Flow Laboratory, Zwanenburg, The Netherlands) and counted in a liquid scintillation counter (Packard). Proliferation is expressed as mean value of counts per minute per triplicate. Standard deviations were less than 5%. For statistical analysis the Mann-Whitney U-test was used.

Preparation and partial purification of IL-2. Human MNC (10^6/ml) were cultured in IMDM with 100 μg/ml polyethylene-glycol (PEG)-4000 and phorbol-myristate acetate (PMA) (1 ng/ml) without serum or other proteins. After culture at 37°C for 24 h, 5 μg/ml Con A (Miles Yeda, Rehovot, Israel) was added. The culture supernatant (containing 500 U IL-2/ml) was harvested after another 48 h and concentrated by ultrafiltration with a hollow fibre device (PM10). The material was fractionated by gel filtration on an AcA54 column (LKB) in a buffer containing 0·15 M NaCl, 100 μgl/ml PEG-4000 and 5 mM HEPES, pH 7·2. The fractions with IL-2 activity (mol. wt ± 15,000) were pooled and concentrated and used to study IL-2 responsiveness of cells.

Measurement of IL-2 production. MNC (4×10^4) in 0·2 ml wells were stimulated with 5 or 20 μg Con A/ml or with 2 μg Con A in the presence of 1 ng PMA/ml. MNC were cultured under proliferative and non-proliferative (serum-free) conditions. In the latter case 100 μg ml PEG-400 was added to prevent IL-2 from sticking to the plastic. Culture supernatants were harvested on various days and stored at −20°C.

Assays for IL-2 activity in the culture supernatants. Quantitation of IL-2 was carried out as described by Gillis *et al.* (1978). Cells of murine IL-2-dependent cytotoxic T cell line (CTLL) were washed, resuspended in culture medium and 5,000 cells were dispensed into microtitre wells. CTLL cells were grown in the presence of various dilutions of putative IL-2 containing culture supernatants. After 20 h proliferation of CTLL cells was measured by a 4 h pulse of ^{3}H-Thymidine. As standard reference a partially purified human IL-2 preparation (40,000 U/ml) was used. The factor concentration which caused half-maximal ^{3}H-Thymidine incorporation was defined as one unit. Con A (< 5 μg/ml), PMA (< 10 ng/ml) and human serum did not influence the test system.

Measurement of IL-2 responsiveness. MNC (5×10^4) stimulated with 5 μg/ml Con A were cultured in 200 μl volume at standard conditions for 5 days. Proliferation was measured by ^{3}H-Thymidine incorporation in the presence or absence of 40 U/ml IL-2 added at the start of the culture.

RESULTS

IL-2 production in healthy individuals and SLE patients

Optimalization of culture conditions. To study the production of IL-2 we investigated the conditions required for optimal IL-2 production. To induce IL-2 production under non-proliferative conditions i.e. in the absence of serum MNC of normal volunteers were stimulated with Con A at various concentrations (1–100 μg/ml; Fig. 1). It turned out that at increasing Con A concentrations, the production of IL-2 increased: at 100 μg Con A/ml, 520 units IL-2 were found per ml culture supernatant. In the presence of PMA (1 ng/ml), a known co-inducer of IL-2 production (Fuller-Farrar *et al.* 1981) maximal IL-2 activity could already be measured with 2 μg Con A/ml. Supernatants of the cultures contained about 2,000 units IL-2/ml. To study the production of IL-2 under proliferative conditions, i.e. in the presence of 5% human pool serum, we measured IL-2 activity in supernatant of cultures performed by stimulating MNC with 2 μg/ml Con A. Maximal

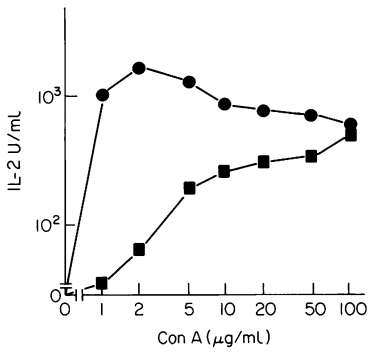

Fig. 1. IL-2 production after stimulation with Con A and PMA is shown. 10^6 MNC/well derived from a normal donor were stimulated with various concentrations of Con A (1–100 μg/ml). Culture supernatants were harvested on day 2 and IL-2 activity in the absence (■) or presence (●) of 1 ng/ml PMA was studied.

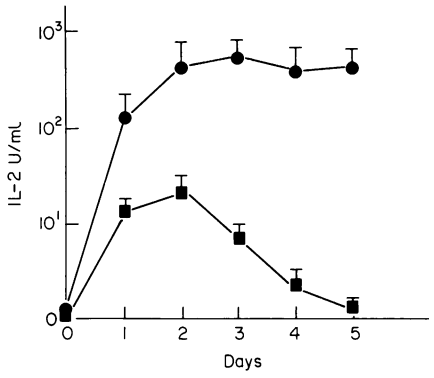

Fig. 2. Kinetics of IL-2 production. Kinetics of IL-2 activity in culture supernatant derived from MNC of normal donors ($n=10$). 5×10^4 MNC in 200 μl wells were stimulated with 2 μg Con A/ml in presence (●) and absence (■) of 1 ng PMA/ml.

IL-2 activity could be detected after 2 days of culture in the absence and in the presence of PMA (1 ng/ml) (Fig. 2). After day 2 the amount of IL-2 in cultures without PMA decreased rapidly which is likely to be due to consumption.

IL-2 production by MNC of SLE patients. IL-2 production *in vitro* by MNC of SLE patients in comparison to normal donors was studied under various conditions. First, IL-2 production was studied under non-proliferative conditions, i.e. in the absence of serum and in the presence of 20 μg/ml Con A. MNC of all individuals, i.e. healthy donors and SLE patients, were able to produce IL-2 under these conditions (Fig. 3). With MNC of SLE patients the mean IL-2 production was 69 ± 21 U/ml. The values for the individual patients ranged from 5 to 525 U/ml. Similar individual differences were, however, obtained with MNC from healthy donors (30 to 300 U/ml). A statistically significant difference between the two groups was not found (Mann-Whitney U-test, $P=0{\cdot}212$). Next, IL-2 production was studied in cultures containing serum. Similar results were obtained with MNC of SLE patients and normal controls: 103 ± 48 U/ml IL-2 (range 0 to 160) for the SLE patients and 122 ± 45 U/ml (range 50 to 235) for the controls. Since differences between patients and normal controls may only show up at suboptimal conditions of stimulation we also

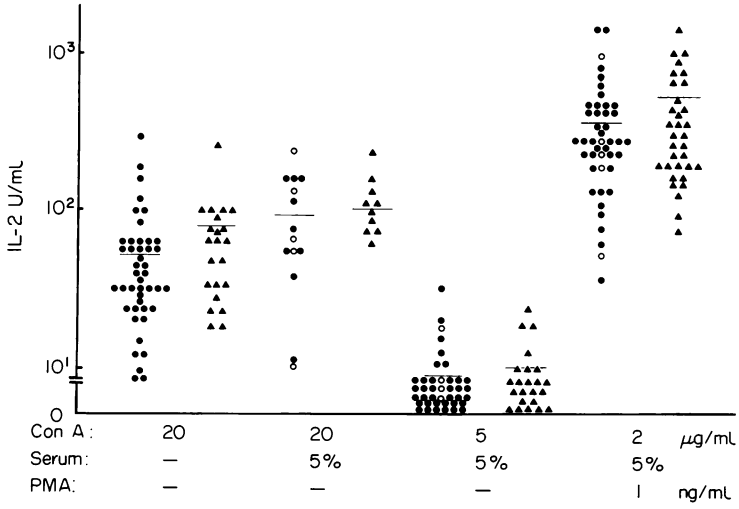

Fig. 3. IL-2 production in MNC: comparison of SLE patients and normal donors. 5×10^4 MNC of inactive SLE patients (●), active SLE patients (○) and normal donors (▲) were stimulated in 200 μl wells under the various conditions as indicated. IL-2 activated in cell-free supernatants was determined after 40 h of culture.

studied IL-2 production under conditions that are less favourable (5% serum; 5 μg/ml Con A). No significant differences between SLE patients (8 ± 11 U IL-2/ml) and healthy donors (12 ± 16 U IL-2/ml) were found in ability of their MNC to secrete IL-2 under these conditions. Finally, we studied whether IL-2 production in the presence of PMA was different in patients and healthy individuals.

Figure 3 demonstrates that for the group of SLE patients the mean IL-2 production in the presence of PMA is increased to 430 ± 130 U/ml. Again a significant difference between MNC of SLE patients and MNC of normal donors was not found ($P = 0{\cdot}49$).

IL-2 responsiveness in healthy donors and SLE patients

Autoimmune mice have been described to be defective in their response to IL-2. To investigate whether this also holds for SLE patients, we studied the ability of activated MNC to respond to IL-2 by culturing 40,000 MNC with Con A (5 μg/ml) in round bottom microtitre plates in the presence and in the absence of an excess (40 U/ml) of exogenous IL-2 added. When MNC of healthy donors were studied we found that in the absence of exogenous IL-2, optimal proliferation occurred at day 4 (14,000 ct/min). On day 5 and 6, proliferative responses were lower, most likely caused by lack of IL-2. Addition of IL-2 at initiation of the culture resulted in a shift of the optimal response. Then, the peak of optimal ^{3}H-TdR incorporation was measured at day 5 (29,500 ct/min).

Since the effect of IL-2 was most clearly visible at day 5, IL-2 responsiveness of MNC of SLE patients and healthy donors was compared at this day. In the presence of Con A alone, no difference between MNC of SLE patients (7,000 ± 2,900 ct/min) and normal donors (8,400 ± 3,300 ct/min) was found (Fig. 4). Addition of IL-2 in addition to the Con A resulted in a four fold enhancement of the proliferative responses in SLE patients as well as in normal donors (27,000 ± 10,000 ct/min and 28,200 ± 7,000 ct/min respectively). These results suggest that MNC of SLE patients and of normal donors are equally able to respond to IL-2.

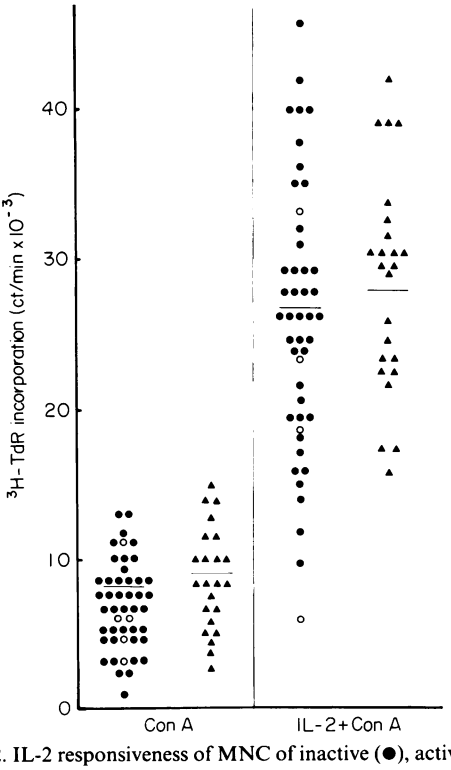

Fig. 4. Responsiveness to IL-2. IL-2 responsiveness of MNC of inactive (●), active (○) and normal donors (▲) was studied by stimulating 4×10^4 MNC with 5 μg/ml Con A in the presence and absence of 40 U IL-2/ml. Proliferation was measured on day 5.

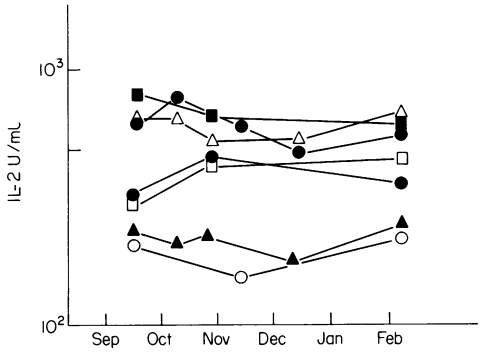

Fig. 5. Longitudinal study of IL-2 production. A longitudinal study of IL-2 production was performed by stimulating 4×10^4 MNC (isolated from peripheral blood collected at different occasions) of SLE patients with 2 μg/ml Con A and 1 ng PMA/ml. IL-2 activity was determined on day 3. Each line represents an individual SLE patient.

Longitudinal studies

From all patients blood was taken at different occasions, in principle on a monthly basis, and MNC were then tested for their ability to produce IL-2. IL-2 production induced under optimal conditions is shown in Fig. 5 for seven SLE patients, none of them in active phase of their disease. Patients with relative low IL-2 production remained low in every test, patients with high IL-2 production reproducibly showed high IL-2 activity. This suggests that the individual differences in IL-2 production were dependent on the individuals and not on variations of culture conditions used.

DISCUSSION

In SLE, extensive studies of lymphocytes with an immunoregulatory function have been performed to get more insight into the etiology of this disease. It has been suggested that a defect in T cell function may occur in SLE, based on findings in a murine model for SLE, *viz.* aged MRL/lpr mice. In these mice, a defect in IL-2 production (Wofsy *et al.*, 1981) as well as lack of the ability to express an IL-2 receptor (Altman *et al.*, 1984) has been reported. These phenomena may be a consequence of impaired IL-1 production because PMA, a substitute for IL-1, can abrogate the deficiency in these mice to produce IL-2 (Santoro *et al.*, 1983). In accordance with this murine model, several investigators have reported a defective IL-2 production of lymphocytes derived from SLE patients. Miyasaka *et al.* (1984) tested IL-2 activity in supernatants of MNC cultures upon stimulation with phytohaemagglutin (PHA). Low IL-2 activity was found in 13 out of 22 SLE patients, compared to five out of 19 healthy controls. Similarly, Linker-Israeli *et al.* (1983) found that T cells from 19 SLE patients and 12 control donors were able to produce IL-2 upon stimulation with PHA, but that the amounts of IL-2 produced were reduced in SLE patients with respect to healthy controls. However, no correlation with disease activity was found. Other authors suggest an impaired macrophage function in SLE patients. Sibbitt *et al.* (1984) could restore a defective IL-2 production by the addition of PMA *in vitro*; Faucal *et al.* (1984) on the other hand found normal IL-2 production in SLE patients after adding indomethacin to the cultures. Controversial results may be the consequence of different culture conditions used for stimulation of IL-2 production and may be dependent upon the size and selection of the group of SLE patients. Our current study was undertaken to establish whether T cells of SLE patients are able to produce IL-2 and/or to respond to it under different culture conditions, varying from suboptimal to supra-optimal. This was done with a large group of patients ($n = 68$) over a period of more than 1 year.

In order to compare IL-2 production in SLE patients with that in healthy donors, conditions for IL-2 production were optimalized using MNC of normal donors (Figs 1 & 2). As might be expected, the amounts of IL-2 produced differ with various stimulation conditions. Under optimal conditions we found that all patients were able to produce IL-2 (Fig. 3). Addition of PMA to Con A stimulated MNC resulted in four- to eight-fold enhancement of IL-2 production in MNC of SLE patients.

However, no statistically significant differences with the group of healthy donors were found with either of these conditions. When individual donors were compared with respect to the amount of IL-2 produced, we found a wide range between individuals (healthy as well as SLE). During longitudinal studies of individual SLE patients, however, IL-2 production of each patient was more or less constant (Fig. 5).

From these experiments, we conclude that these 68 SLE patients have lymphocytes capable of producing IL-2. To investigate the ability of MNC of SLE patients to respond to IL-2, we chose for culture conditions where IL-2 was limiting. Addition of an excess of IL-2 to Con A stimulated MNC resulted in a strong enhancing effect of the proliferative T cell response in SLE patients as well as healthy controls (Fig. 4). Again, no significant differences between SLE patients and controls occurred. These results indicate a normal responsiveness to IL-2 of MNC from SLE patients. Similar results have been found for inactive patients by others (Linker-Israeli *et al.*, 1983; Miyasaka *et al.*, 1984; Nakamura *et al.*, 1983). In contrast it has been reported that T cells of active patients have a decreased ability to respond to IL-2 (Alcocer-Varela & Alarcon-Segovia, 1982).

Altogether our data suggest that neither production of IL-2 nor the response to IL-2 by MNC of SLE patients is different from lymphocytes of normal donors. Our data do not exclude the possibility that SLE patients undergoing an exacerbation are different in this respect, because only five of such patients were present in this prospective study. Moreover, we did not have the opportunity to test these active patients at different occasions. We think that because of the large individual variability in the production of IL-2, only longitudinal studies of individual patients may give an answer to whether an eventual defect in IL-2 production is related to clinical manifestations in SLE and what the eventual role of medication is in this process.

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