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IL-2 normalizes defective suppressor T cell function of patients with systemic lupus erythematosus *in vitro*

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SUMMARY

During autologous mixed lymphocyte reaction (AMLR) both helper and suppressor T cells capable of regulating B cell responses are generated. The proliferative response of T cells as well as the generation of T suppressor cells in the AMLR of patients with active systemic lupus erythematosus (SLE) is diminished. In contrast, the T helper cells generated in the AMLR show a hyperactivity. The diminished HLA-class II antigen expression observed on non-T cells of SLE origin was restored by treatment of the cells with gamma-interferon (γ-IFN). When tested by immunoglobulin secretion, γ-IFN enhanced T helper cell activity but failed to affect T cell proliferation and T suppressor cell generation in the AMLR derived from patients with SLE. Human recombinant interleukin 2 restores both the proliferative response of T cells and the induction of T suppressor cells in AMLR.

Keywords autologous mixed lymphocyte reaction interleukin 2 gamma-interferon-systemic lupus erythematosus

INTRODUCTION

The autologous mixed lymphocyte reaction (AMLR) constitutes activation, proliferation and differentiation of T cells after specific recognition of self MHC-class II determinants on non-T cells (Volk *et al.*, 1984; Romain & Lipsky, 1983). The proliferative response in the AMLR is defective in patients with various autoimmune diseases (Volk & Grunow, 1984) including systemic lupus erythematosus (SLE) (Takada *et al.*, 1985; Sakane, Steinberg & Green, 1978).

Patients with SLE manifest a variety of immune abnormalities: e.g. spontaneous activation of immunoglobulin (Ig)-secreting B cells, diminished HLA-class II antigen expression on monocytes (Shirakawa, Yamashita & Suzuki, 1985; Volk *et al.*, 1985), and defective interleukin 2 (IL-2) production *in vitro* (Linker-Israeli *et al.*, 1983; Volk & v. Baehr, 1984).

In the present study, we compared the immunoregulatory abilities of T cells generated in the AMLR of healthy persons with those of patients with active SLE, and asked whether disfunctions of the regulatory cells derived from AMLR could be reversed by lymphokines, such as human recombinant gamma-interferon (γ-IFN), known to increase HLA-class II antigen expression and human recombinant IL-2, known to be a general signal for T cell proliferation.

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MATERIAL AND METHODS

Patients. Peripheral blood was obtained from 11 adult patients with SLE and 10 healthy adults. All patients studied met the criteria for the classification of SLE by the American rheumatism Association (Tan *et al.*, 1984). All patients had active or moderately active disease at the time of study. Disease activity was assessed on the basis of the following signs and symptoms: serositis, active rash, arthritis, leukopenia, active central nervous system disease, and active renal disease. SLE was considered active if there was current clinical evidence of disease affecting at least one tissue system with corroborative laboratory evidence, including ESR > 30 mm/h, a high antinuclear or anti-DNA antibody titre and a decreased proportion of E rosette-forming cells. Blood was obtained from most patients before any treatment. If patients were on prednisone at the time of the study (four out of the 11 patients), the blood was taken at least 24 h after the last drug administration.

Cell separation. The mononuclear cells were prepared by density gradient centrifugation. For removing cytophilic antibodies and immune complexes the cells were preincubated for 1 h at 37°C in cell culture medium (RPMI-1640, SIFIN, Berlin, GDR) without serum in siliconized glass tubes. Separation of T and non-T cells was performed by the E rosette technique as previously described in detail (Volk *et al.*, 1984). More than 94% of the cells in the rosetting population were reactive with a monoclonal antibody (MoAb) against T cells (VIT-3b). The non-rosetting cell fraction contained 30–55% monocytes, 40–60% B cells, and less than 5% T cells as judged by immunofluorescence technique using the appropriate MoAb. For the helper assay the non-T cell fraction was depleted of monocytes by removing the cells adhering to plastic flasks (Greiner, Nürtingen, FRG). The non-adherent, non-rosetting cell fraction was further treated with the pan-T cell MoAb VIT-3b and pelleted on goat anti-mouse Ig (Medac, Hamburg, FRG)-coated multiwell plates (Greiner). After incubation for 20 min at 4°C, the non-adherent cells were collected. After the panning procedure the B cell fraction contained more than 95% B cells and less than 0·5% of the cells reacted with a cocktail of MoAb against T cells (VIT-4, VIT-8, VIT-3b).

AMLR-proliferation assay. AMLR cultures were established in triplicate by culturing 10^5 T cells with equal numbers of autologous non-T cells treated with mitomycin-C (Serva, Heidelberg, FRG) in a final volume of 200 μl culture medium RPMI-1640, containing 10% heat-inactivated fetal calf serum in 96-well round-bottomed microtitre plates (Greiner). These cultures were incubated for 7 days in a 5% CO_2 air humidified environment. Eighteen hours before the end of incubation, 1 μCi[^{3}H]-thymidine was added to each culture well. [^{3}H]-thymidine incorporation was determined by liquid scintillation spectroscopy. All data are expressed as the mean counts per minute (ct/min).

AMLR-Ig secretion assay. Cells were cultured by using the same conditions as for the proliferation assay except the non-T cells were not treated with mitomycin C. The cell-free supernatants were harvested after 7 days and tested for IgG and IgM amounts by ELISA-technique (Jahn *et al.*, 1986).

Helper and suppressor assay. This followed Kotani *et al.* (1984). To evaluate the ability of T cells activated in AMLR to exert regulatory T cell fractions, AMLR was set 3 or 7 days as described above. These cultured cells were separated by E rosetting. The T cells were subsequently tested for their regulatory activities in a second assay culture system. For the measurement of helper function generated in primary AMLR, 10^5 T cells activated for 3 days in primary AMLR were added to 10^5 autologous, purified B cells which were preactivated with *Staphylococcus* Cowan I (SAC) for 3 days. After 5 days the Ig secretion in the supernatants of the secondary culture was measured. Suppressor activities were measured by adding 5×10^4 T cells, activated for 7 days in primary AMLR, to the secondary assay cultures of 1×10^5 fresh autologous non-T cells with equal numbers of fresh autologous non-T cells. After 7 days the Ig secretion was determined. Controls were performed by adding T cells cultured alone in primary cultures.

Surface immunofluorescence staining. Conventional indirect immunofluorescence assay was performed as described in detail elsewhere (Volk *et al.*, 1984). The percentages of positive cells were estimated by a fluorescence microscope (Orthoplan, Leitz, FRG) in a blind study. The following MoAb were used: VIT-3b (Pan-T cell MoAb, CD-3 cluster), VIT-4 (T helper cells, CD-4 cluster), VIT-8 (T suppressor/cytotoxic cells, CD-8 cluster), VIB-C5 (B cell-specific), VIM-13 (monocyte-

specific), and VID-1 (monomorphic structure of the HLA-class II region). All MoAb were a generous gift from Professor Knapp (Vienna).

Lymphokines. Purified recombinant human IL-2 from *E. coli* (98% IL-2 protein, less than 1 ng endotoxin/mg), kindly provided by Dr Amerding (Sandoz, Vienna), had a specific activity of 10^6 NCI reference units/mg protein. Purified recombinant human γ-IFN, kindly provided by Dr Adolf (Bender & Co. GmbH, Vienna), had a specific activity of 3×10^7 IU/mg protein. Stimulator non-T cells were pretreated with γ-IFN (100 IU/ml) for 20 h at 37°C. Thereafter, the cells were washed twice, treated with mitomycin C for proliferation assays, and added to autologous T cells.

RESULTS

Proliferation in the AMLR. T cells of healthy donors and of SLE patients were cultured in the presence of the respective non-T stimulator cells (AMLR). The proliferative response of SLE-derived T cells was diminished (Fig. 1).

As compared to normal controls, the proportion of HLA-class II positive non-T cells was significantly lower in SLE patients. That is mainly caused by a decreased proportion of HLA-class II antigen-bearing monocytes in patients (28% vs 68%). Since quantitative variation in HLA-class II antigen expression on antigen-presenting cells plays a central role in immune regulation, a defect in the non-T stimulator cell population may be responsible for the defective AMLR response. In order to test this possibility, normal and SLE non-T cells were preincubated with γ-IFN (100 IU/ml) for 20 h. γ-IFN increased the HLA-class II antigen expression in both groups. (86% in SLE vs. 96% in normals).

γ-IFN treatment increased the proliferative response in AMLR of healthy donors but did not affect the T cell proliferation in the AMLR of SLE patients (Fig. 1).

SLE patient derived lymphocytes are defective in IL-2 production (Linker-Israeli *et al.*, 1983; Takada *et al.*, 1985; Volk *et al.*, 1986). We asked whether the diminished AMLR response can be normalized by addition of pure IL-2. Human recombinant IL-2 reconstituted, at least partially, the impaired AMLR response (Fig. 1). Only two out of 11 patients showed a marginal AMLR response also in presence of IL-2.

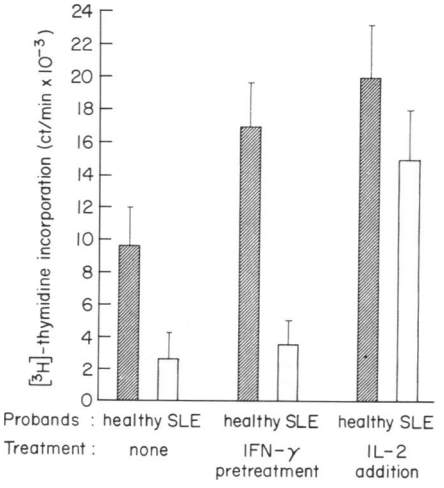

Fig. 1. Proliferation in the AMLR-influence of γ-IFN pretreatment and IL-2 addition. The proliferative response of T cells was measured after 7 days in the AMLR culture. The results are expressed as mean values ($\pm$ s.d.) of 11 patients with SLE and 10 healthy donors. T cells cultured alone incorporated less than 1000 ct/min. The γ-IFN pretreatment was carried out by incubation of non-T stimulator cells for 18 h with recombinant γ-IFN (100 iu/ml). After washing the cells were co-cultured with autologous T cells. In the third group, recombinant IL-2 (50 U/ml) was added to the AMLR cultures.

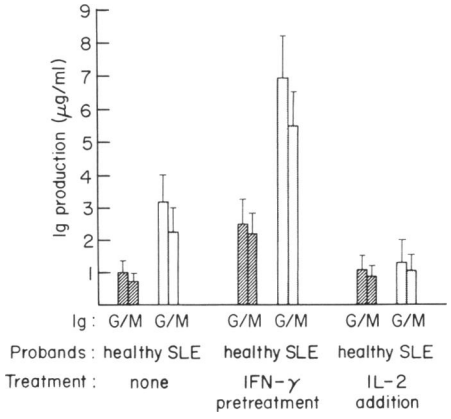

Fig. 2. Immunoglobulin synthesis in the AMLR-influence of IFN-pretreatment and IL-2 addition. The Ig synthesis in the AMLR of six healthy donors and seven patients with SLE was measured by ELISA-technique. The mean values (±s.d.) are expressed. γ-IFN pretreatment and IL-2 addition were carried out as described in Fig. 1. Non-T cells cultured alone produced less than 0·4 μg/ml IgG and 0·3 μg/ml IgM.

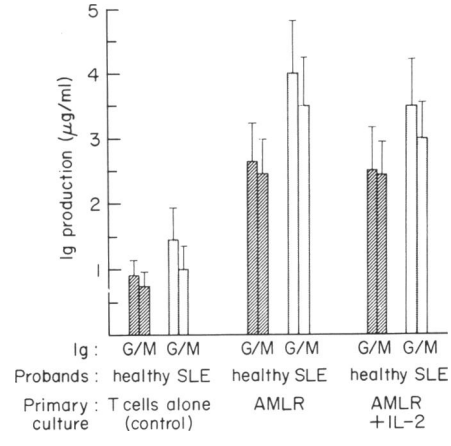

Fig. 3. Helper cell assay. T cells were activated in a primary AMLR culture in presence or absence of IL-2 (50 U/ml) for 3 days and separated by E rosetting. 10^5 activated T cells (T act.) were added to 10^5 autologous, pure B cells preactivated with *Staphylococcus* Cowan I for 3 days. The Ig synthesis in the secondary AMLR culture was measured after a further 5 days of culture. The mean values (±s.d.) of five patients with SLE and four healthy donors are shown.

Immunoglobulin secretion in the AMLR. It has been reported that spontaneous Ig secretion is enhanced in AMLR of SLE patients (Cohen, Litvin & Winfield, 1982; Sakane *et al.*, 1983). We asked whether γ-IFN or IL-2 do normalize the enhanced Ig secretion in patients' AMLR cultures. Pretreatment of stimulator non-T cells with γ-IFN induced an increase in the Ig secretion in both groups, but particularly in the SLE group. On the other hand, IL-2 had no effect on the Ig secretion in cultures of normals but normalized the enhance Ig production in AMLR from SLE-patients (Fig. 2).

Helper assay. To evaluate the ability of T cells activated in the AMLR (primary culture) to exert T helper cell activity activated T cells, derived from primary 3-day AMLR cultures, were incubated with SAC-preactivated, autologous, purified B lymphocytes for 5 days (secondary culture). In the supernatant of these cultures, IgM and IgG amounts were determined using ELISA-technique.

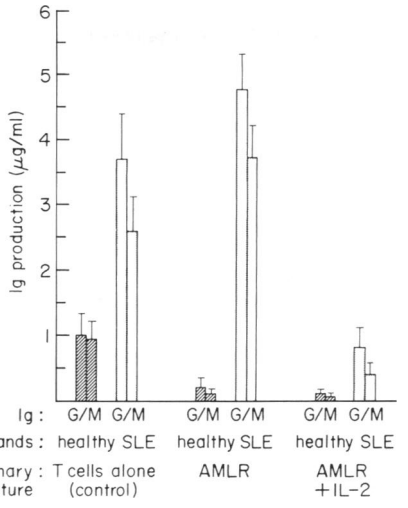

Fig. 4. Suppressor cell assay. T cells were activated in a primary AMLR culture in presence or absence of IL-2 (50 U/ml) for 7 days and then separated by E rosetting. 5×10^4 activated T cells (T act.) were added to fresh, autologous T cells (10^5) and non-T cells (10^5). Following 7 days of culture the Ig synthesis was measured. The mean values (±s.d.) of five patients with SLE and four healthy donors are shown.

T cells derived from AMLR of SLE patients were more active in inducing SAC-preactivated B cells to secret Ig of both subclasses than those of controls (Fig. 3). IL-2 did not affect generation of helper activity in the primary cultures (Fig. 3).

Suppressor assay. We asked whether the effect of IL-2 on the enhanced Ig secretion in AMLR cultures derived from SLE patients (Fig. 2) is due to normalization of a defective suppressor activity. In order to test this possibility, human recombinant IL-2 was added to primary cultures and the suppressive activity generated (7 day culture period) was tested in a secondary culture for Ig secretion inhibition activity. As shown in Fig. 4, in contrast to normal cells, T cells from SLE patients, when cultured in a primary AMLR for 7 days, showed no suppressive activity. Moreover, in SLE patient derived cultures some helper activity was detectable (Fig. 4). IL-2 restored the defective capacity of cells derived from SLE patients to generate suppressor cells in the primary AMLR (Fig. 4).

DISCUSSION

The results continue and extend previous observations by others (Linker-Israeli *et al.*, 1983; Takada *et al.*, 1985) and us (Volk *et al.*, 1984; 1986), demonstrating defective IL-2 production capacity of SLE patient derived lymphocytes. It has been claimed that defective monocyte function of SLE patients (decreased HLA-class II antigen expression, increased suppressive activity may be involved in the failure of IL-2 production (Janeway *et al.*, 1984; Shirakawa *et al.*, 1985; Volk & v. Baehr 1984a). We showed that pretreatment of stimulator cells by γ-IFN normalized the defective HLA-class II antigen expression on monocytes derived from SLE patients but failed to restore the defective AMLR proliferation of patients' T cells. Moreover, non-T cells of patients with SLE can induce potent proliferation in allogeneic T cells (data not shown). Furthermore, we have observed that non-T cells from patients with SLE can induce T helper cell activation for B cell maturation in the AMLR. These data indicate that at least a subpopulation of T helper cells derived from SLE patients are intact and are sufficiently activated by non-T stimulator cells in the AMLR.

As reported by others (Hirose *et al.*, 1985; Sakane *et al.*, 1983) and as shown in this report, the enhanced Ig synthesis in the AMLR of SLE patients is accompanied by (i) an increased helper activity of T cells, (ii) an increased sensitivity of B cells to helper cell action, and (iii) a diminished T suppressor cell function.

Recently, the decrease in the capacity of cells derived from SLE patients to produce IL-2 has been attributed to active suppression of IL-2 production by suppressor cells (Linker-Israeli *et al.*, 1985).

Partial correction of the defective proliferative response in AMLR of SLE patients by natural semipurified IL-2 has been reported by others (Takeda *et al.*, 1985). As shown in this report purified human recombinant IL-2 corrected both the proliferative response and the defect in generation of T suppressor cells.

The main alteration in the AMLR-activated immunological processes in SLE patients seems to be located at the level of T4 cells (Takada *et al.*, 1985). T4 cells of these patients failed to produce IL-2 and to develop IL-2 receptors. Exogenous IL-2, therefore, could not induce an AMLR proliferative response by T4 cells derived from SLE patients. In contrast, a failure of T8 cells of SLE patients to proliferate in AMLR appears to be a secondary effect of abnormal function of T4 cells (no IL-2 production). This proliferative defect in T8 cells could be corrected with exogenous IL-2. These observations agree with our results that IL-2 can restore the generation of T suppressor cells that inhibit the enhanced spontaneous Ig secretion observed in such patients.

In summary, it seems that SLE patients have no general defect in the AMLR reactivity but alterations in the response of several subsets and cellular communications. The defect in the cellular circuits between T suppressor inducer and T suppressor effector cells seem to be secondary to the impaired IL-2 production by T4 cells.

Unregulated Ig production in SLE patients has been considered to be a result of inadequate suppression. On the other hand, observations of excessive suppressor cell activity for IL-2 production in SLE patients (Linker-Israeli *et al.*, 1985) may appear paradoxical, but not if one can ultimately distinguish cells that inhibit Ig production from cells that suppress IL-2 production.

Further studies are necessary to show whether treatment *in vivo* with exogenous IL-2 may be indicated in patients with SLE. Experiments are in progress to verify the observations presented in this paper with lymphocytes of other lymphoid organs (lymph nodes, bone marrow) of SLE patients. Furthermore, we have started to investigate the effects of IL-2 and γ-IFN in regulating the secretion of anti-DNA antibodies, autoantibodies with potent pathogenic capacity in SLE disease.

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