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Independence of depressed lectin-dependent cell-mediated cytotoxicity from interleukin 2 production in patients with systemic lupus erythematosus

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SUMMARY

The relationship of lectin-dependent cell-mediated cytotoxicity (LDCC) to interleukin-2 (IL-2) production was studied in healthy subjects and in patients with systemic lupus erythematosus (SLE). Profoundly depressed levels of LDCC were elicited by peripheral blood mononuclear cells (PBMC) from nine patients with active SLE in comparison to LDCC from seven controls, and eleven inactive SLE donors, using ^{3}H-TdR-prelabelled adherent HEP-2 cells as targets in a 24 h assay with 25 μg/ml Con A. In parallel experiments, no individual correlation was found between LDCC activity and IL-2 production for healthy or SLE subjects. Further, no major differences were detected in IL-2 release when the three groups of donors were compared, a tendency observed at the Con A doses (5 and 25 μg/ml) and incubation times (24, 48, and 72 h) used to induce IL-2 production. In additional studies, impaired Con A-induced blastogenesis was noted for PBMC from active SLE patients in comparison to the PBMC from the controls or patients with inactive SLE. While strong individual correlation was obtained between blastogenesis and IL-2 secretion in controls and patients with inactive SLE, no such relationship was found in patients with active SLE. While addition of exogeneous IL-2 to the cytotoxicity assay considerably enhanced LDCC by healthy donors it failed to improve LDCC by patients with active SLE. These data suggest that depressed LDCC and Con A-induced blastogenesis of patients with active SLE may not be related to impaired IL-2 production but rather to an inherent dysfunction of the effector lymphocytes, including their unresponsiveness to IL-2.

Keywords LDCC IL-2 SLE

INTRODUCTION

Human interleukin-2 (IL-2) consists of a polypeptide of 133 amino acids released by stimulated T cells (Robb, 1984). Although IL-2 is considered to be a growth factor required for the clonal expansion of activated T cells (Kern *et al.*, 1981), it may not be restricted to this role. It triggers T cells to produce various lymphokines, such as gamma-interferon and B cell growth factor (Robb, 1984). It also influences helper (Palacios, 1982), and suppressor activities (Palacios & Möller, 1981), enhances natural killing (NK) (Kuribayashi *et al.*, 1981; Miyasaka *et al.*, 1984) and antibody-dependent cell-mediated cytotoxicity (ADCC) (Kimber *et al.*, 1984). Since all of these functions have been documented to be abnormal in systemic lupus erythematosus (SLE), it seemed to be

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interesting to study the role of IL-2 in SLE. Recently, impaired IL-2 production has been reported in SLE, in both active and inactive stages of the disease (Alcocer-Varela & Alarcon-Segovia, 1982; Liner-Israeli *et al.*, 1983), but normal levels of IL-2 secretion were also detected in patients with active SLE, suggesting that defects of IL-2 production might not be as common as previously reported (Sibbitt *et al.*, 1983). On the other hand, both enhanced (Tsokos *et al.*, 1985) and unchanged levels (Sibbitt *et al.*, 1983) of originally depressed NK activity of SLE patients have been reported, following in-vitro treatment with exogeneous IL-2. In the present study we examined the role of IL-2 in lectin-dependent cell-mediated cytotoxicity (LDCC) of healthy subjects, and patients with inactive and active SLE. LDCC activity was evaluated by quantifying the detachment from the monolayer of ^{3}H-TdR-prelabelled adherent HEp-2 cells in the presence of concanavalin A (Con A) (Perl, Gonazlez-Cabello & Gergely, 1983). The effector cells in this system are T lymphocytes (Perl *et al.*, 1983), and include both OKT4$^+$ and OKT8$^+$ T-cells (Perl *et al.*, 1984). This assay proved to be highly sensitive in detecting profoundly depressed levels of LDCC in patients with active SLE (Perl *et al.*, 1982). On the one hand, IL-2 was found to be a strong modulator of NK, and ADCC activities, and on the other hand lectins are potent inducers of IL-2 secretion, so the relationship of IL-2 to LDCC activity, and, especially, to the impaired LDCC of SLE patients seemed to be an intriguing question.

MATERIALS AND METHODS

Patients. Twenty female patients satisfying the 1982 revised criteria of the American Rheumatism Association for the classification of SLE (Tan *et al.*, 1982) were examined. Nine patients were in the active stage of disease as defined both clinically and serologically, while eleven patients were inactive at the time their cells were studied. Inactive patients were on no treatment, while active patients were receiving doses of prednisone which varied from 10 to 100 mg per day. Therapy was withheld for 24 h before drawing blood, so it had no significant influence on the parameters examined. As controls, seven age-matched and sex-matched normal volunteers were studied.

Separation of peripheral blood mononuclear cells. Human peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood on Ficoll-Uromiro gradients (Boyum, 1968). PBMC were suspended in TC 199 medium, supplemented with 10% heat-inactivated fetal calf serum (FCS, Phylaxia, Hungary), 25 mM HEPES buffer (Serva, GFR), 2 mM L-glutamine (GIBCO, USA), 100 iu/ml penicillin, and 100 μg/ml gentamicin.

Target cells. Target cells derived from the Cincinnati HEp-2 adherent human epipharynx carcinoma cell line (National Institute for Public Health, Budapest, Hungary) were cultured in Eagle's MEM containing 10% heat-inactivated calf serum, 25 mM HEPES, 2 mM L-glutamine, 300 iu/ml penicillin, 120 μg/ml gentamicin, and 7·5 μg/ml amphotericin B in 100 ml sterile plastic tissue culture flasks (Greiner, GFR). Serial passage of HEp-2 cell monolayers was performed in 3- to 5-day periods. After discarding detached, dead cells with the supernatant medium, HEp-2 target cells for cytotoxicity assay were resuspended from the bottom of culture flasks with 0·5 ml of 0·1% trypsin (Sigma) and 0·05% Versene in TC 199, then washed twice in culture medium. Viability of resuspended target cells was evaluated by trypan blue staining (>99%).

Cytotoxicity assay. Resuspended HEp-target cells (2500 cells in 100 μl culture medium per well) were seeded in flat-bottomed microtitre plates (Greiner), then prelabelled with 0·2 μCi ^{3}H-thymidine (^{3}H-TdR UVVVR, Czechoslovakia) in 20 μl culture medium. To allow cell adhesion, the plates were incubated at 37°C for 24 h in a humidified atmosphere with 5% CO_2. After 24 h, HEp-2 cells grew into monolayers and incorporated sufficient isotope activity (2–2·5 $\times$ 10^4 ct/min) for cytotoxicity studies. This number of target cells per well also allowed the addition of effector cells at a high ratio, up to 50:1. Subsequently, each well was washed twice with 37°C culture medium. The assays were performed in six replicates. To each well 100 μl of an effector cell suspension was added in TC 199 at killer-to-target ratios of 5:1, 25:1, and 50:1. The data shown are from the 50:1 ratio. Similar patterns of results were observed at 5:1 and 25:1 ratios. LDCC cultures also contained Con A (Pharmacia, Sweden) at a final concentration of 25 μg/ml. Target cells incubated in medium alone

or medium plus Con A but without effectors were included in each experiment. No target cell damage due to Con A was observed in the absence of added effector cells. Culture plates were incubated for 24 h at 37°C. Detached target cells (dead according to trypan blue staining) and effector cells were removed by washing twice with 37°C culture medium. Detachment from the monolayer was used as indication of cell damage. The remaining adherent HEp-2 cells were frozen at −20°C. After thawing, the content of each well was aspirated onto filter paper discs by an automated sample harvester (Dynatech, GFR). Isotope determinations were made in a liquid scintillation counter (Isocap 300, Nuclear Chicago, USA). The results were expressed in ct/min using the arithmetic mean of six replicate values. NCMC was calculated taking the medium control as baseline according to the formula:

$$\% \text{ cytotoxicity} = 100 - \frac{\text{ct/min test sample}}{\text{ct/min medium control}} \times 100.$$

LDCC was expressed as the percentage increase of cytotoxicity above the NCMC attributable to the presence of Con A:

$$\% \text{ LDCC} = \left(100 - \frac{\text{ct/min test sample with Con A}}{\text{ct/min medium control with Con A}} \times 100\right) - \left(100 - \frac{\text{ct/min test sample without Con A}}{\text{ct/min medium control without Con A}} \times 100\right).$$

Production and quantification of IL-2. $1{\cdot}25 \times 10^6$/ml PBMC were stimulated with 5, or 25 μg/ml Con A for 24, 48, or 72 h at 37°C in a 5% CO_2 atmosphere. The data shown are for stimulation with 25 μg/ml Con A for 24 h, unless indicated otherwise. Control cultures without Con A were included in every experiment. The cells were grown in volumes of 150 μl of 5% FCS-complete RPMI in V-shaped 96-well Greiner trays. After incubation 100 μl of supernatant fractions were harvested, and assayed for IL-2 activity as previously described (Gillis *et al.*, 1978). Samples were evaluated in

Table 1. LDCC, IL-2 production and Con A-induced blastogenesis of PBMC from healthy subjects

Subject	LDCC*	IL-2 production† (u/ml)	Blastogenesis‡ ct/min
1	37·5	7·2	60985
2	31·4	2·5	22664
3	23·7	0·8	4739
4	17·5	1·3	ND
5	62·9	1·5	ND
6	83·7	4·5	21639
7	77·2	4·9	25059
Mean ± s.e.m.	47·7 ± 10·1	3·2 ± 0·9	27017 ± 9224
r §	0·42	—	0·91
P	NS	—	< 0·05

* LDCC was measured by detachment from the monolayer of ^{3}H-TdR-pre-labelled HEp-2 targets in a 24 h assay at 50:1 effector-target cell ratio due to 25 μg/ml Con A

† IL-2 production was evaluated after incubation of PBMC for 24 h with 25 μg/ml Con A.

‡ Data show ^{3}H-TdR-incorporation of PBMC after incubation for 72 h in the presence of 25 μg/ml Con A.

§ *r* values refer to the correlation of LDCC, and blastogenesis, respectively, with IL-2 production.

¶ *P* values regard the significance of correlation.

ND = not determined.

NS = not significant.

triplicate in microtitre plates. Supernatants fractions were diluted 1:2, 1:4, 1:8, and 1:16, and 50 μl was added to each well. To these, 50 μl/well of 5×10^3 CTLL cells (an IL-2 dependent murine line of cytotoxic T lymphocytes) were pipetted, and the rays were incubated for 24 h at 37°C in 5% CO_2, pulsed with 1 μCi ^{3}H-TdR for 6 h, and harvested as described above. In every assay the incorporation of ^{3}H-TdR by CTLL cells in response to the tested samples was compared to their response to a standard IL-2 preparation. Highly purified standard reference IL-2 ($13{\cdot}1 \times 10^6$ reference units/mg, Lot: ISDP-841) was kindly provided by Dr G.B. Thurman (BRMP, Frederick, MD). Results are expressed as units of IL-2/ml (u/ml). In every experiment at least one healthy subject was tested along with SLE patients. Con A in itself did not stimulate CTLL cells.

Con A-induced blastogenesis. Peripheral blood mononuclear cells (2×10^5) suspended in TC 199, supplemented with 10% heat-inactivated calf serum, 25 mM HEPES, 2 mM L-glutamine, 100 iu/ml penicillin, 100 μg/ml gentamicin, and 7·5 μg/ml amphotericin B were placed in each microplate well using four parallel samples. Con A was added in an optimal concentration of 25 μg/ml. Control cultures without Con A were included in each experiment. The plates were incubated at 37°C in a humidified atmosphere with 5% CO_2 for 72 h. The cultures were pulsed with 0·4 μCi ^{3}H-TdR 8 h before termination. The cells were harvested as described above.

Statistics. Data are given as mean ± s.e.m. of *n* experiments. Statistical analysis was performed using Student's *t*-test.

RESULTS

In accordance with previous studies (Perl *et al.*, 1984) ^{3}H-TdR-prelabelled HEp-2 target cells were resistant to natural cell-mediated cytotoxicity (data not shown), while significant lectin-dependent cell-mediated cytotoxicity (LDCC) was elicited by PBMC from healthy subjects (47·7 ± 10·1%,

Table 2. LDCC, IL-2 production, and Con A-induced blastogenesis of PBMC from patients with inactive SLE

Subject	LDCC* (%)	IL-2 production† (U/ml)	Blastogenesis‡ (ct/min)
1	76·0	3·2	46966
2	44·9	2·0	4034
3	18·6	1·6	24700
4	43·9	1·1	6676
5	71·7	1·7	23643
6	14·1	1·2	1556
7	6·3	1·3	7742
8	40·1	1·2	1257
9	83·1	1·9	15716
10	41·5	2·4	ND
11	12·5	2·2	7983
Mean ± s.e.m.	41·2 ± 8·1	1·8 ± 0·2	14027 ± 4525
r §	0·44	—	0·76
P	NS	—	< 0·02

* LDCC was evaluated at 50:1 effector-target ratio against HEp-2 cells in a 24 h assay with 25 μg/ml Con A.

† IL-2 production was measured after incubation of PBMC for 24 h with 25 μg/ml Con A.

‡ Data show ^{3}H-TdR- incorporation of PBMC after incubation for 72 h in the presence of 25 μg/ml Con A.

§ *r* values refer to the correlation of LDCC, and blastogenesis, respectively, with IL-2 production.

¶ *P* values regard the significance of correlation.

ND = not determined.

NS = not significant.

Table 3. LDCC, IL-2 production and Con A-induced blastogenesis of PBMC from patients with active SLE

Subject	LDCC (%)	IL-2 production (u/ml)	Blastogenesis (ct/min)
1	16·0	1·8	2457
2	−78·4	4·6	1042
3	−31·0	1·6	14516
4	−41·7	0·3	ND
5	−42·0	0·2	ND
6	−9·1	0·3	ND
7	−39·9	1·6	4443
8	10·0	0·6	941
9	−25·3	2·9	ND
Mean ± s.e.m.	−26·8 ± 9·7	1·54 ± 0·5	4680 ± 2539
r	−0·48	—	−0·22
P	NS	—	NS

* LDCC was measured by detachment from the monolayer of ^{3}H-TdR-prelabelled HEp-2 targets in a 24 h assay at 50:1 effector-target ratio with 25 μg/ml Con A. Negative cytotoxicity values represent diminished detachment of HEp-2 cells from the monolayer in comparison to control cultures containing target cells without effectors.

† IL-2 production was assessed after incubation of PBMC for 24 h with 25 μg/ml Con A.

‡ Data show ^{3}H-TdR- incorporation of PBMC after incubation for 72 h in the presence of 25 μg/ml Con A.

§ *r* values refer to the correlation of LDCC and blastogenesis, respectively, with IL-2 production.

¶ *P* values regard the significance of correlation.

ND = not determined.

NS = not significant.

$P < 0{\cdot}01$; Table 1). Similar to control donors, patients with inactive SLE showed strong LDCC activity against HEp-2 cells ($P < 0{\cdot}001$) while active SLE patients showed none. Negative LDCC values by seven of nine patients with active SLE represent decreased detachment from the monolayer (increased survival) of HEp-2 targets in the presence of PBMC from SLE patients in comparison to control cultures containing target cells without effectors.

To evaluate whether or not IL-2 production may play a role in the depressed LDCC of patients with active SLE, IL-2 production was examined in parallel experiments. As shown in Tables 1, 2, and 3 no individual correlation was found between LDCC and IL-2 secretion for either control or SLE donors. Contrary to the impaired LDCC activity considerable levels of IL-2 production were detected in patients with SLE, similar to those in patients with inactive SLE and in healthy controls.

Con A-induced blastogenesis was also simultaneously investigated in 20 of the donors. Contrary to the strong individual correlation between blastogenesis and IL-2 production in control and inactive SLE persons (Tables 1 and 2), the independence of depressed blastogenesis ($P < 0{\cdot}05$, as compared to that of controls) from IL-2 secretion was found in patients with active SLE (Table 3).

In further studies we investigated IL-2 production after incubation of PBMC for 24, 48, and 72 h with 5, and 25 μg/ml Con A respectively. Table 4 demonstrates that no major differences were observed in IL-2 secretion between control and active SLE subjects at any of the Con A doses or incubation times examined.

The effect of exogeneous IL-2 on LDCC activity was assessed by addition of IL-2, up to a final concentration of 1 u/ml to the cytotoxicity assay. While LDCC by healthy donors was markedly

Table 4. Time- and Con A dose-dependence of IL-2 production of PBMC from control and active SLE subjects

	24 h	48 h	72 h
Controls			
5 μg/ml Con A	3·2±1·4*	1·2±0·1	1·0±0·2
25 μg/ml Con A	4·9±1·8	2·2±0·4	2·2±0·5
SLE patients			
5 μg/ml Con A	1·7±0·1	1·0±0·3	0·9±0·1
25 μg/ml Con A	2·5±0·5	1·9±0·5	1·8±0·1

* Data show mean±s.e.m. of IL-2 production in u/ml by PBMC from four control and four SLE donors, as examined in parallel experiments.

enhanced (+27·0±4·0%, $n=4$, $P<0·01$), that of patients with active SLE was further reduced by 1 u/ml exogeneous IL-2 (−22·5±13·0%, $n=8$); although the latter difference was not statistically significant.

DISCUSSION

In the present study the impaired LDCC activity in patients with active SLE (Perl *et al.*, 1982) has been confirmed. Since treatment with Con A of PBMC is known to induce IL-2 secretion, the possible role of IL-2 in LDCC is a pivotal question to be determined. These data have revealed the lack of correlation between LDCC and IL-2 production both in seven healthy subjects and in 20 patients with SLE. This suggests that IL-2 may not have a major role in the LDCC reaction, and supports the early observations of Kirchner & Blaese (1973) that LDCC activity is independent of lymphokine secretion. LDCC is thought to be mediated by an immature/prekiller T cell subpopulation having insufficient density or affinity of antigen receptors to serve as a directly cytotoxic cell (Schubert & Lucas, 1981). The role of lectin in LDCC is two-fold: bridging the effector cell to its target, and activation of the killer cell (Green, Ballas & Henney, 1978). In recent experiments (Perl, Gonzalez-Cabello & Gergely, 1984) we found no difference in binding of the effector cells to targets in LDCC between control and active SLE persons, suggesting a post-binding defect in the LDCC lytic machinery of SLE patients. The stimulation by exogeneous IL-2 of LDCC by control subjects, but not by patients with active SLE, refers to an inherent dysfunction, including irresponsiveness to exogeneous IL-2 at the level of LDCC effector cells. The defect of LDCC activity in SLE affects both $OKT4^+$ and $OKT8^+$ T cell subsets (Perl *et al.*, 1984), pointing out that neither of these subsets has a predominant role in depressed LDCC. This fact also supports the independence of LDCC from IL-2 production, as T cells with helper (OKT4) phenotype appeared to be the major source of IL-2 (Robb, 1984).

On the other hand, a consistent correlation of Con A-induced blastogenesis with IL-2 production was found in both the control and inactive SLE groups. By contrast, IL-2 release did not correlate with depressed blastogenesis in patients with active SLE. Exogeneous IL-2 up to 1 u/ml also failed to normalize Con A-induced blastogenesis in active SLE patients (data not presented). These findings suggest that depressed Con A-induced blastogenesis, similar to depressed LDCC of patients with active SLE, may not be related to impaired IL-2 secretion. These observations are consistent with the findings of Sibbitt *et al.* (1983) who demonstrated the independence of decreased NK activity of SLE patients from both endogenous and exogenous IL-2. Thus, impaired IL-2 production may not be a primary defect in impaired cytotoxic and proliferative responses of lymphocytes from patients with active SLE, as recently reported (Alcocer-Varela & Alarcon-Segovia, 1982). In conclusion, the present data suggest that impaired LDCC activity and Con A-induced blastogenesis of patients with active SLE may not be related to decreased IL-2 production

but rather to an inherent defect of the effector lymphocytes, including their unresponsiveness to IL-2.

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