

RESTORATION OF URAEMIC LYMPHOCYTE RESPONSES IN VITRO BY INTERLEUKIN-2

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Summary

Interleukin-2 (IL-2) has a key role in the activation of lymphocytes, an effect antagonized by steroids. To further clarify this relationship the present study examined in uraemic patients the mitogen responses of lymphocytes to phytohaemagglutinin (PHA) and concanavalin A (Con A). The response of lymphocytes from haemodialysis patients was depressed ($p < 0.01$) at optimal mitogen stimulation, while lymphocytes of continuous ambulatory peritoneal dialysis (CAPD) patients responded normally. At suboptimal mitogen (PHA) stimulation, however; both CAPD and haemodialysis lymphocyte responses were reduced ($p < 0.01$) compared to that of control cultures. Addition of IL-2 to these cultures normalized the CAPD and enhanced the haemodialysis responses. CAPD and haemodialysis lymphocyte cultures were more sensitive to the suppressive effect of methylprednisolone than were control cultures ($p < 0.01$). Addition of IL-2 normalized the uraemic lymphocyte sensitivity to methylprednisolone. It is suggested that IL-2 is a central factor in the impaired uraemic immune response.

Introduction

A decreased response to mitogens and an increased sensitivity to the immunosuppressive effect of steroids on uraemic lymphocytes has previously been demonstrated [1]. Steroids abrogate the release or production of interleukin-2 from activated lymphocytes [2,3]. The present study therefore examined in vitro if the increased uraemic lymphocyte sensitivity to steroids, and the decreased transformation response could be normalized by exogenous IL-2.

Materials and methods

Subjects

The total study was comprised of 32 patients on chronic haemodialysis, 28 patients on continuous ambulatory peritoneal dialysis (CAPD), and 60 healthy

control subjects. Patients and controls were matched with respect to age and sex. The duration of the dialysis periods of the haemodialysis and the CAPD patients were 2.7 ± 2.6 (SD) years, and 1.6 ± 1.2 years respectively. The causes for renal failure were comparable in the two groups of patients.

Preparation of peripheral blood lymphocyte cultures

Peripheral blood lymphocytes were isolated as previously described [1]. Peripheral blood lymphocytes were isolated by lymphoprep (Nyco, Norway), then washed three times in Hanks' balanced salt solution and resuspended to a concentration of 10^6 cells/ml suspension in RPMI-1640, containing 10 per cent fetal calf serum, gentamicin $10 \mu\text{g}/\text{ml}$, and L-glutamine 2mM . Triplicates of peripheral blood lymphocytes were cultured in microtitre plates (Nunc, Denmark) containing 5×10^4 cells/well. Phytohaemagglutinin (PHA; Difco, USA) $8 \mu\text{g}/\text{well}$ (optimal stimulation), $0.4 \mu\text{g}/\text{well}$ (suboptimal stimulation), and concanavalin A (Con A; Pharmacia Fine Chemicals, Sweden) $1 \mu\text{g}/\text{well}$ were used as mitogens.

Methylprednisolone succinate (Urbason[®], Hoechst, FRG) was added to the triplicates ($0.2 \mu\text{g}/\text{ml}$ culture). Part of the cultures were in parallel experiments supplemented with optimal quantities of purified human interleukin-2 (IL-2; Electro-Nucleonics Inc, USA), 20 per cent v/v undiluted both with and without addition of methylprednisolone. All the cultures were incubated for 72 hours in a humidified atmosphere with five per cent carbon dioxide at 37°C before addition of the radioisotope (^{14}C -thymidine, $20 \text{nCi}/\text{well}$, Amersham, UK). The cultures were then harvested and counted (Packard, Tri-Carb, Liquid Scintillation Spectrometer).

Statistical analysis

Student's 't' test was used for comparison of differences between groups.

Results

Interleukin-2 effect on uraemic and normal lymphocyte proliferation

Lymphocyte cultures of control subjects and CAPD patients showed a higher response ($p < 0.01$) to PHA (optimal stimulation, $8 \mu\text{g}/\text{well}$) and Con A stimulation than those of patients on haemodialysis (Figure 1). In order to further elucidate these findings IL-2 was added to control, CAPD, and haemodialysis cultures stimulated with two different concentrations of PHA (Figure 2).

At optimal PHA stimulation ($8 \mu\text{g}/\text{well}$) no additional effect of IL-2 was seen neither in patient nor in control cultures. At suboptimal PHA stimulation ($0.4 \mu\text{g}/\text{well}$) without exogenous IL-2 added the control cultures responded significantly ($p < 0.05$) higher than both CAPD and haemodialysis cultures. Addition of IL-2 to the suboptimally PHA ($0.4 \mu\text{g}/\text{well}$) stimulated cultures restored the low mitogen responses to their respective response levels at optimal ($8 \mu\text{g}/\text{well}$) PHA concentrations. Thus, the CAPD lymphocyte response was

normalized, whereas the haemodialysis lymphocyte response, although clearly enhanced, still was significantly decreased ($p < 0.01$).

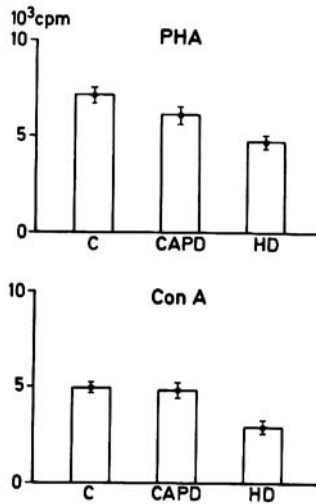


Figure 1. The ¹⁴C-thymidine uptake (cpm) of optimal (8 μg/well) PHA (n=30), and Con A (n=20) stimulated PBL cultures from haemodialysis (HD) and CAPD patients, and control subjects (C). Mean ± SEM

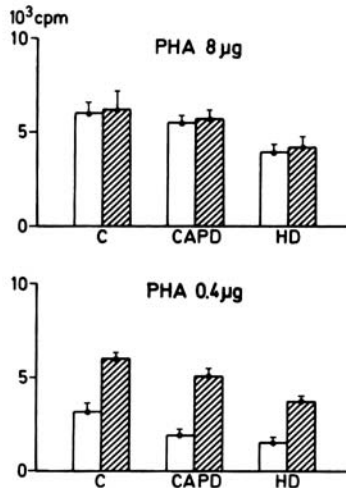


Figure 2. The effect of addition of IL-2 (hatched columns) to PHA stimulated PBL cultures (blank columns) from haemodialysis (HD) patients (n=15), CAPD patients (n=15), and control subjects (n=15) at various PHA concentrations (8 and 0.4 μg PHA/well). Ordinate: Mitogen response in cpm

Combined effect of interleukin-2 and steroid on uraemic and normal lymphocyte proliferation

At optimal PHA stimulation (Figure 3, top) CAPD and haemodialysis lymphocyte cultures were significantly more sensitive ($p < 0.05$) to methylprednisolone ($0.3 \mu\text{g}/\text{ml}$ culture) than control lymphocyte cultures, which were almost as resistant to the inhibition by methylprednisolone as the cultures supplemented with IL-2 (hatched columns). In contrast, in cultures supplemented with IL-2 was this difference between patients and control subjects absent ($p > 0.05$). At suboptimal mitogen stimulation were control and patient lymphocyte cultures equally sensitive to methylprednisolone. However, addition of IL-2 rendered control as well as patients lymphocyte cultures unresponsive to the effect of steroid.

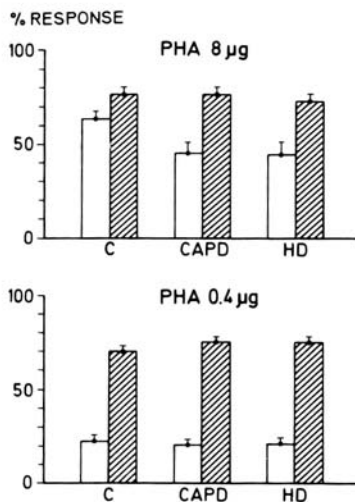


Figure 3. The combined effect of addition of methylprednisolone ($0.3 \mu\text{g}/\text{well}$) to PBL cultures (blank and hatched columns) and addition of IL-2 (hatched columns) to PHA stimulated PBL cultures from haemodialysis (HD) patients ($n=15$), CAPD patients ($n=15$), and control subjects ($n=15$) at various PHA concentrations (8 and $0.4 \mu\text{g}$ PHA/well). Ordinate: per cent response of maximal mitogen response (without methylprednisolone) of cultures with methylprednisolone ($0.3 \mu\text{g}/\text{ml}$)

Discussion

Lymphocyte cultures of haemodialysis patients have lower responses to optimal mitogen stimulation than those of control cultures. In contrast, CAPD lymphocyte cultures respond normally (Figure 1). Previous studies have shown no relation between the time on chronic haemodialysis and responsiveness to mitogen stimulation [4]. The two groups of patients were comparable in all other clinical aspects. Studies of the cellular subsets (helper and suppressor/cytotoxic T cells) in peripheral blood lymphocytes have not demonstrated any

significant differences between uraemic patients (haemodialysis) and healthy control subjects which could explain the impaired immune response of haemodialysis patients [5]. In vitro normalization of cellular immunity has not previously been demonstrated in CAPD patients.

Interleukin-2 has a keyrole in the activation of lymphocytes [6,7]. Therefore we examined in uraemic lymphocytes the influence of IL-2 on mitogen induced T cell proliferation. At suboptimal PHA stimulation (0.4 μ g/well) CAPD lymphocyte proliferation was lower than that of control cultures. Addition of exogenous IL-2 not only restored the response of the control cultures to the level of optimal PHA stimulation, but restored the response of CAPD cultures as well (Figure 2). Contamination of IL-2 with mitogen can be excluded since the stimulatory effect of IL-2 alone was below 20 per cent of mitogen stimulated cultures (data not shown). The additive effect at suboptimal PHA stimulation (0.4 μ g/well) between IL-2 and mitogen activation was still deficient in lymphocytes of haemodialysis patients.

The inhibitory effect of steroids on lymphocyte proliferation is mediated through an abrogation of the IL-2 production from IL-2 producing T cells [2,3]. Steroids are thus potent antagonists to the IL-2 mediated cell proliferation. IL-2 was therefore added to PHA stimulated haemodialysis, CAPD, and control cultures (Figure 3). At optimal mitogen stimulation (PHA 8 μ g/well) control lymphocyte cultures without exogenous IL-2 were almost as resistant to the suppressive effect of methylprednisolone as control cultures supplemented with exogenous IL-2, while lymphocytes of uraemic patients were highly sensitive to methylprednisolone. Addition of IL-2 rendered the uraemic cultures resistant to steroids. At suboptimal stimulation (PHA 0.4 μ g/well) control as well as uraemic lymphocyte cultures were inhibited by steroids, but all cultures could be normalized after addition of IL-2 to the methylprednisolone containing cultures.

Thus the decreased uraemic lymphocyte response in vitro to mitogen stimulation may partly be due to a reduced capability to produce IL-2.

Acknowledgements

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