IMPROVED LYMPHOCYTE TRANSFORMATION
IN VITRO OF PATIENTS ON CONTINUOUS
AMBULATORY PERITONEAL DIALYSIS

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Summary

In vitro lymphoblastic transformation and sensitivity to methylprednisolone (MP) was studied in lymphocyte cultures obtained from patients on continuous ambulatory peritoneal dialysis (CAPD), on haemodialysis (HD) and control subjects. The PHA and Con A responses of peripheral blood lymphocytes (PBL) and T cells were identical in CAPD and control cultures, and in both significantly higher than in HD cultures (p<0.05). Both PBL and T cells from CAPD and control cultures were more resistant to the suppressive effects of MP than those from HD cultures. There was no relation between the duration of the dialysis period and the lymphocyte mitogen response in HD patients. Enhanced in vitro cell transformation and resistance to steroids during CAPD treatment may reflect an improved form of dialysis of importance for the general immune defence of the uraemic patient.

Introduction

Previous reports have demonstrated a decreased response of lymphocytes to mitogens in chronic uraemia [1, 2]. Recently a beneficial effect of CAPD has been reported [3, 4]. The present investigation examines in patients on CAPD the lymphocyte response to mitogens and the degree of resistance to the suppressive effect of methylprednisolone (MP) on this response in vitro.

Subjects

In vitro lymphocyte functions were investigated in 11 patients on CAPD (five women and six men, aged 23 to 62 years, mean age 44 years, mean dialysis period 1.7 years), 11 patients on HD (seven women and four men, aged 28 to 66 years, mean age 46 years, mean dialysis period 3.7 years), and 11 normal volunteers (five women and six men, aged 30 to 63 years, mean age 40 years). The effect of age and the duration of the dialysis period on in vitro lymphocyte
transformation were studied in 32 HD patients, and the effect of age alone was finally studied in 74 control subjects.

Materials and methods

Isolation of PBL and T cells

Twenty millilitres of heparinised blood obtained by venepuncture was added to equal amounts of Hank’s balanced salt solution. The PBL were isolated by Lymphoprep® (Nyco, Norway) and washed three times in Hank’s solution. The cells were then resuspended to a concentration of $10^6$ cells/ml in RPMI-1640 (Gipco, Europe), containing 10 per cent fetal calf serum, 500 IU penicillin/ml (Leo, Denmark), and streptomycin 333 µg/ml (Novo, Denmark). The T cells were obtained by the E-rosetting technique. AET- (Sigma 5879, USA) treated sheep red blood cells (SRBC) were added to equal volumes of the above PBL suspension [5]. After centrifugation for five minutes at 160G followed by incubation at 4°C for 60 minutes the sedimented T cell rosettes were resuspended and layered on Lymphoprep® and centrifuged at 500G for 30 minutes. The interface cells were then removed and the sedimented T cell rosettes were isolated and incubated in RPMI-1640 with 15 per cent AB serum for 10–20 minutes at 37°C until lysis of the SRBC. The T cells were washed (Hank’s) three times, counted and resuspended to a concentration of $10^6$ cells/ml suspension in RPMI-1640 with 10 per cent fetal calf serum and antibiotics.

Lymphocyte cultures

Triplicates of PBL, and T cells from controls, CAPD patients, and HD patients were cultured in microtitre plates (Nunc, Denmark) containing $5 \times 10^4$ cells/well. Mitogen stimulated triplicates contained optimal mitogen concentrations, PHA (Difco, Detroit, USA) 20µg/well, or Con A (Pharmacia Fine Chemicals, Sweden) 10µg/well. MP succinate (Urbason®, Hoechst, FRG) was added to the triplicates. The following concentrations were used: 0, 0.05, 0.25, 0.5, 1.0, 2.5µg/ml culture.

The cultures were incubated for 72 hours in humidified atmosphere with five per cent carbon dioxide at 37°C before $^{14}$C-thymidine addition (50 nCi/well, Amersham, UK). The cultures were harvested and counted (Packard, Tri-Carb) 20 hours after $^{14}$C-thymidine addition. The mean number of cpm incorporated in the presence versus absence of steroid was expressed as the % suppression at each concentration. MP resistance of patient and control cultures in terms of the in vitro MP concentration that cause 50 per cent suppression ($ED_{50}$), was calculated from these dose response curves.

Statistics

A non-paired t-test was used to compare group means. Values of $p<0.05$ were considered significant.

Results

Figure 1 shows the PHA (left) and Con A (right) responses in cpm of CAPD, HD and control cultures of PBL. The $^{14}$C-thymidine uptake in cpm of unstimulated
Figure 1. The PHA and Con A responses of peripheral blood lymphocytes (PBL) from controls, CAPD patients, and HD patients. Ordinate: $^{14}$C-thymidine uptake in cpm x $10^3$. Abscissa: concentration of MP in $\mu$g/ml culture. Controls, n = 11. CAPD patients, n = 11. Dotted area: HD patients, n = 11. The SEM values are indicated.

Figure 2. The PHA and Con A responses of T lymphocytes from controls, CAPD patients, and HD patients. Ordinate: $^{14}$C-thymidine uptake in cpm x $10^3$. Abscissa: concentration of MP in $\mu$g/ml culture. Controls, n = 11. CAPD patients, n = 11. Dotted area: HD patients, n = 11. The SEM values are indicated.
cultures (not shown) was 3–10 per cent of mitogen stimulated cultures. The mitogen responses of CAPD and control cultures without MP (left bars) were similar, and significantly higher than that of the HD cultures (p<0.05). This difference was found throughout the concentration range of MP. Steroid resistance in terms of ED₅₀ values were similar in CAPD and control cultures (PHA: 0.38µg/ml, 0.35µg/ml and Con A: 0.28µg/ml, 0.23µg/ml, respectively), but higher than in HD cultures (PHA: 0.07µg/ml and Con A: 0.1µg/ml).

Figure 2 shows the T lymphocyte PHA (left) and Con A (right) responses. The CAPD T cell responses were higher than the HD T cell responses and the difference was significant in the Con A stimulated T cell cultures without MP as well as in the MP suppressed cultures (p<0.05). The T cell resistance to steroid was higher in the CAPD and control cultures than in the HD cultures. The ED₅₀ values were; PHA: 0.11µg/ml, 0.13µg/ml, and 0.04µg/ml and Con A: 0.02µg/ml, 0.05µg/ml, and 0.005µg/ml, respectively.

The relation between age and PBL response to PHA stimulation was studied in HD patients and normal controls. There was no relationship between age and PHA response in the control group (r = -0.19, p>0.05, n = 74). In the HD group, however, there was a significant trend of lower PHA responses with increasing age (r = -0.40, p<0.05, n = 32).

The relationship between the duration on HD and PHA transformation response is shown in Figure 3. The PHA responses are not significantly lower after several years on HD (r = -0.28, p>0.05, n = 32).

![Figure 3. The relation between the duration of the period on haemodialysis and the PHA response of peripheral blood lymphocytes (PBL) from 32 patients. Ordinate: ¹⁴C-thymidine uptake in cpm x 10⁵. Abscissa: duration of haemodialysis period in years](image-url)
Discussion

In chronic uraemia the in vivo cell mediated immune response is depressed and in vitro mitogen transformation decreased [1, 2, 6]. However, the mechanism by which uraemia impairs the T cell function remains obscure, although several factors, e.g. serum factors, have been suggested [7]. More recently a significantly increased E-rosette formation has been observed during CAPD treatment [4]. Serum factors blocking sheep cell receptors have been suggested to be selectively removed through the peritoneum during CAPD treatment [8]. The OKT4/OKT8 ratio has been reported to be within the normal range in CAPD patients [8]. In vivo evidence of enhanced delayed hypersensitivity skin reactions has been reported [4].

In the present study PHA and Con A responses of both PBL and T cells from CAPD patients, HD patients and control subjects were compared. In general both PBL and T cells from CAPD patients responded like the controls with significantly improved responses compared to the PBL and T cell responses of the HD patients. One reason could be patient selection i.e. ‘best risk patient’ would be on CAPD. In this study all patients were clinically well. The HD group was slightly older than the CAPD group but the present findings and previous reports [9, 10] have demonstrated mitogen responses to be almost unaffected by age, except at age extremes [9]. As shown in Figure 3 the slightly longer period of dialysis of the HD group compared to the CAPD group was not likely to explain the normal mitogen response of the CAPD patients, who being uraemic would be expected to respond subnormally to mitogens and antigens in vitro. The higher in vitro resistance to MP of both CAPD patients and controls supports the findings of an enhanced in vitro lymphocyte function during CAPD and may suggest an improved dialysis during CAPD treatment.

Acknowledgments

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References

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Open Discussion

GOLDSMITH (Chairman) May I congratulate you on your very nice paper. In view of what we know about biological (circadian) rhythms in lymphocytes, were all the samples taken at the same time of the day in the different patient groups?

LANGHOFF All the samples were taken at the same time of the day from both the controls, the haemodialysis group, and the CAPD group.

BINSWANGER (Chairman) Would you like to speculate on the clinical importance of your findings in respect to transplantation of different patient groups?

LANGHOFF Theoretically this question may be of some importance, but I do think that in the transplanted group this difference might disappear because of the heavy immunosuppression.

SCHREIBER (Cleveland, USA) If one considers there exists a multipotent stem cell population, did you attempt to correlate your results of T-cell suppression with erythropoiesis. Were the haematocrits in the patients which had good T-cell responses increased in comparison to those patients who were on haemodialysis or CAPD, thus looking for a common underlying stimulatory factor for multi-stem cell development?

LANGHOFF We did not find a relation between improved erythropoiesis and an improved lymphocyte transformation in vitro.

SCHREIBER Were you able to reverse this response by any changes in regard to crossing patients over from CAPD to haemodialysis or using some addition of dialysate or serum to inhibit these responses?

LANGHOFF We have not had the opportunity yet of following patients who are changed from CAPD to haemodialysis treatment.

SCHREIBER So you don’t really know what regulates this response?

LANGHOFF The whole explanation is probably not a toxic plasma factor since uraemic plasma from patients on haemodialysis does not seem to change a normal lymphocyte response to a uraemic lymphocyte response.