CONTINUOUS AMBULATORY PERITONEAL DIALYSIS AND CELLULAR IMMUNITY

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Summary

E+ cells were studied in 16 patients on continuous ambulatory peritoneal dialysis (CAPD) to evaluate the impairment of cell-mediated immunity. E-rosette forming cells (E-RFC) were below the normal range at the beginning of treatment in 10/16 patients, after which their number increased and reached normal levels in the majority of patients in three to six months.

In this phase of therapy, the same result was obtained with OKT11 monoclonal antibody, while OKT4+/OKT8+ ratio was in the normal range.

Normal human lymphocytes, pre-incubated with uraemic peritoneal fluid, showed a significant reduction of E-RFC.

Maximum inhibition was observed with the less than 500 daltons fraction of peritoneal fluid. Extraction with chloroform almost completely abolished inhibitory activity, suggesting that the toxic substance(s) has the characteristic of a polar lipid.

Immunodeficiency in CAPD patients seems therefore partly restored by the removal through the peritoneum of inhibitors capable of blocking sheep-cell receptors.

Introduction

It is well established that uraemia impairs cell-mediated immunity and that repeated haemodialysis treatment does not reverse this deficiency [1–3]. Numerous hypotheses have been put forward to explain this observation [4,5] and some reports have suggested that inhibitory factors retained in the serum of uraemic patients may cause cellular immunodeficiency [6–8]. More recently lymphocyte function appeared to be significantly less impaired in patients treated with continuous ambulatory peritoneal dialysis (CAPD).

This was observed by studying lymphoblastic transformation [9], delayed hypersensitivity skin reactions [10] and surface markers for lymphocyte sub-populations [11].
The hypothesis that inhibitor(s) capable of blocking sheep-cell receptors is selectively removed through the peritoneum, was therefore tested in normal human lymphocytes by enumeration of T-cell subpopulations after incubation with peritoneal fluid.

Spontaneous sheep red-cell rosette formations and monoclonal anti-human T-lymphocyte antibodies were employed. The range of the inhibitory activity was determined by separation of peritoneal fluid on Amicon ultrafilters.

Patients and methods

E-rosette forming cells (E-RFC) were determined in 54 uraemic patients (25 male, 29 females; 15–69 years, with an average age of 51): 22 on haemodialysis (HD), 19 on intermittent peritoneal dialysis (IPD), 16 on CAPD. The same test was performed on normal human lymphocytes after the incubation (1 ml vol/vol – 37°C for two hours) with peritoneal dialysis fluid from patients on CAPD.

Normal human lymphocytes were obtained from healthy untransfused donors.

As control fluid we used ascitic fluid from patients with alcoholic cirrhosis and normal renal function. Peritoneal fluid was pre-treated with ultrafiltration through PM30, UM10, UM05 Diaflo ultrafilters (Amicon, Lexington, Ma) in an Amicon’s stirred cell.

Three fractions with about 30,000–10,000, 10,000–500, and less than 500 daltons molecular weight (MW) were obtained.

The solubility in chloroform was tested by mixing one part of sample to one part of chloroform (Chloroform FU, C. Erba, Milano). The chloroform extracted samples were lyophilised to completely remove the solvent and resuspended in a volume of sterile water equal to the original fluid.

Lymphocyte suspensions for rosette formation were obtained by Ficoll-Isopaque centrifugation [12]. E-RFC were determined by the method of Wybran [13]. Tests were performed in duplicate. A rosette was considered only when five or more RBC were found attached to a lymphoid cell. The values were estimated as percentage of E-RFC among uraemic lymphocytes (normal range 62 ± 5) and as a percentage of inhibition for normal lymphocytes.

The reactivity of lymphocyte cell suspensions with OKT3, OKT4, OKT8 and OKT11 monoclonal antibodies (Ortho Pharmaceutical Co) was determined by indirect immunofluorescence staining using fluorescein isothiocyanate-conjugated goat anti-mouse IgG (G/M FITC; Meloy Laboratories). Two hundred cells were counted.

The F test was used for statistical analysis.

Results

In 26/41 patients on RDT, the percentage of E-RFC was reduced. E-RFC were below the normal range in 59 per cent of HD patients (X: 48 ± 12) and in 68 per cent of IPD patients (X: 52 ± 17). These values were independent of the duration of dialysis. The same behaviour was observed in 62 per cent of CAPD patients (X: 51 ± 20) at the beginning of treatment; subsequently the number of
E-RFC increased and reached normal in 72 per cent of patients after three to six months of CAPD therapy. The mean percentage of E-RFC in the patients on therapy from six to 24 months was 69.8 ± 6.3. About the same value was obtained with OKT11 monoclonal anti-human T-cells, which reacts with E⁺-cells. The ratio helper/suppressor T-cells (OKT⁺4/OKT⁺8) was in the normal range (X̄: 1.8 ± 0.5).

<table>
<thead>
<tr>
<th>Peritoneal fluid (n=13)</th>
<th>Untreated</th>
<th>&gt;30,000</th>
<th>30,000–10,000</th>
<th>10,000–500</th>
<th>&lt;500</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>28.46</td>
<td>28.77</td>
<td>33.15</td>
<td>39.07</td>
<td>59.07</td>
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<tr>
<td>SD</td>
<td>16.36</td>
<td>10.89</td>
<td>7.08</td>
<td>15.78</td>
<td>11.49</td>
</tr>
<tr>
<td>F</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>30.47*</td>
</tr>
</tbody>
</table>

* p<0.005

The results of experiments which tested the inhibitory effect of peritoneal fluid on E-RFC of normal human lymphocytes are presented in Table I.

The uraemic untreated samples induced a mean reduction of E-RFC among normal lymphocytes of 28 per cent. The maximum inhibition (59%; F 30.47, p<0.005) was observed with the less than 500 daltons fraction.

A dose-effect relationship was observed with the dilution of this last fraction.

No effect was observed with the incubation of normal lymphocytes with untreated ascitic fluid, while an inhibition of about 13 per cent was observed with the other fractions. This inhibitory effect was not obtained by incubating normal lymphocytes with urea, creatinine or glucose. The extraction with chloroform of the fraction with less than 500 daltons reduced the E-RFC inhibition (Figure 1) from 59 to 20 per cent (p<0.01).

The number of E-RFC observed at the beginning of CAPD treatment shared an indirect relationship (Figure 2) with the inhibitory activity of the less than 500 daltons peritoneal fluid fraction.

This relation was almost completely lost after six to 24 months of therapy when E-RFC were within the normal range.

The mean inhibitory activity of peritoneal fluid on E-RFC among normal lymphocytes retested on this occasion, was less pronounced than previously (33.2 ± 21.6). The inhibition appeared reduced to only 12 per cent (± 10.7) when E⁺-cells were counted by OKT11 monoclonal antibodies, a value comparable with the one observed in controls.

An intermediate blocking effect (19.2 ± 10.1) was reported for helper T-cells (OKT⁺4). In this case excessive chloroform extraction reduced the inhibitory activity to less than 13 per cent (12.5 ± 5.4%).

Besides the discrepancies in the enumeration of E⁺-cells with the two techniques, we noted a low density staining of T-cells when incubated with peritoneal fluid fractions. The staining was weaker for OKT11 antibodies.
Figure 1. Percentage of E-RFC inhibition among normal human lymphocytes by CAPD-peritoneal fluid fractions

Figure 2. Relationship between E-RFC of CAPD patients after different periods of therapy and the inhibitory activity of less than 500 daltons peritoneal fluid fraction among human normal lymphocytes
Discussion

Substantial changes in immune response are associated with renal failure. Prolonged skin graft survival [14,15], impaired delayed hypersensitivity [16,17] and acquired susceptibility to infection [18] have all been reported. Overall lymphocyte function has been assessed in attempting to define immunological defects associated with uraemia, but with quite variable results. Controversial data have been reported for lymphocyte mitogen responsiveness [19–25] and relative and absolute number of lymphocyte subpopulations [3,23,26–32].

Our results, in agreement with other authors [26–29], confirm that in haemodialysis patients the average number of E-RFC is significantly lower than in normal subjects.

The same behaviour was observed in CAPD patients at the beginning of treatment, after which the number of T-cells increased very rapidly in the first months of therapy and remained subsequently stable around normal values in the majority of patients. This result was reported to be associated with an improvement of delayed hypersensitivity skin tests [10]. In contrast to the artificial membrane, peritoneum appears therefore able to remove substance(s) which inhibits several T-lymphocyte functions.

The E-RFC inhibitory activity of peritoneal fluid on normal lymphocytes can be explained by dialysis through the peritoneum of toxic factors which block lymphocyte receptors for sheep-cells. This leads to the belief that plasmatic factors constitute essential causative elements of the reported uraemic immuno-deficiency. Several toxic substances, which are retained by insufficient elimination or defective catabolism or produced in excessive amounts due to the metabolic abnormalities of renal failure, are present in uraemic serum.

'Middle molecules' have a very considerable influence on cellular proliferation, in particular in the response to phytomitogens and, to an even greater extent, allogenic responses [8,33].

This toxic role has been attributed to the 'middle molecules' of 500–2,000 daltons [6], but more recently toxic substances of less than 500 daltons have been considered [34].

Considering the nominal 'cut-off' of Amicon membranes, in our experiments, the maximum inhibitory activity on E-RFC was observed in the peritoneal fluid fraction smaller than 500 daltons.

This substance(s) appears to be almost totally removed by chloroform extraction. This material showed therefore the characteristic of a polar lipid substance, similar to that reported for the erythopoietic inhibitory factor [35].

The reason why no definite relationships exist between E-RFC and peritoneal fluid inhibitory activity, specifically when the number of E-RFC returns to normal may suggest that other factors are involved.

In fact it is questionable that only the retention of toxic molecules is responsible for immunodeficiency. The accumulation of toxic substances has been found to be linearly related to the urea generation rate [36] and it is well documented that CAPD patients show a positive nitrogen balance [37].

In view of the weaker reactivity with monoclonal antibodies of human normal lymphocytes incubated with peritoneal fluid, it is possible that different staining
intensity defines functionally distinct T-cell subsets, as previous work suggested [38]. The weak fluorescence pattern observed with OKT11 antibody could mean a partial block of E-receptor T-cells, capable of impairing the E-RFC test.

In conclusion, the decrease in cell-mediated immunity of uraemic patients appears partly restored during CAPD. From these results this effect appears dependent on the removal of toxic or inhibitory serum factor(s) in the range of less than 500 daltons. Further studies must be performed in order to obtain a better definition of the chemical structure of this fraction.

References

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Open Discussion

RINGOIR (Chairman) You suggest that you have improved removal of the inhibitor of the immunity by haemodialysis. Was this haemodialysis by Cuprophan or by other permeable membranes?

GIANGRANDE All our haemodialysis patients were treated with $1m^2$ Cuprophan membranes for four to five hours three times weekly.

CANTAROVICH (Buenos Aires) I would like to ask a question and make a comment please. I would like to know if you measure immune complexes in your dialysate?

GIANGRANDE No, I have not tried this.

CANTAROVICH The comment is that we think that CAPD carries the possibility for modification of immunological status in immunological diseases. We treated three patients with lupus erythematosus on previous long-term corticosteroid therapy and in end-stage renal failure. They improved with peritoneal dialysis. After one year of treatment they were clinically free of symptoms with negative serology. When two of them returned to haemodialysis they became symptomatic.

GIANGRANDE There is a very recent report from Dr Nolph's group which described a clearance of immune complexes through the peritoneum in CAPD patients.

LA GRECA (Vicenza) Do you have any information concerning E-rosette formation during infective episodes?

GIANGRANDE I have no data here to show you. There are some reports describing a reduction in the number of E-rosettes during acute episodes of infection, mainly peritonitis. This agrees with my comments on the number of factors in this situation; changes in the clearance of molecules, metabolic changes, mainly in nitrogen balance. In this respect it is interesting to remember Dr Bergstrom's correlation between the increasing amounts of toxic substances and infective episodes.

RINGOIR Are you continuing studies to further identify the factor?

GIANGRANDE At present I have not enough chromatographic data to reply. As far as the heat stability is concerned I can say that all our fluid samples were pre-treated at $56^\circ C$ for 30 minutes.
BRIGGS (Glasgow) I am very interested in your information because we have been comparing haemodialysis and CAPD patients as far as CMI is concerned by DNCB testing. By this technique we could not demonstrate any difference in the CMI between these two groups. One other point of evidence is that we ourselves and I think others have not observed any difference in transplant outcome between these two groups which is further evidence against any important clinical degree of difference in CMI.

GIANGRANDE I agree with you that my data must be confirmed by functional studies.

RINGOIR Was there any difference between the incidence of clinical infections in your CAPD and your haemodialysed patients?

GIANGRANDE I think this is a very difficult comparison, because the causative factors are quite different.