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HAEMODIALYSIS-INDUCED ACUTE CHANGES IN T LYMPHOCYTE SUBSETS

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Summary

Leucocyte and lymphocyte counts and T lymphocyte subsets have been studied in 15 haemodialysis patients once on a cuprophan and once on a polyacrylonitrile membrane. On both membranes a progressive decrease in total and suppressor cytotoxic T cells was seen while helper-induced cells remained unaffected. These changes resulted in a significant increase in the T_4/T_8 ratio which was maximal by the end of dialysis but reversible within 24 hours. This phenomenon, seemingly independent of complement activation, must involve mechanisms different from those responsible for dialysis neutropenia.

Introduction

In recent cross-sectional [1] and prospective [2] studies, we have shown that patients on dialysis display characteristic abnormalities of their T lymphocytes: a normal proportion of total T cells and of helper T cells, A low proportion of suppressor-cytotoxic T cells and an increased ratio of helper to suppressor lymphocytes. In addition, their suppressive T cell activity in response to mitogens was markedly impaired and these alterations worsened with time on dialysis. From another point of view, it has been known for almost 20 years that haemodialysis with cuprophan membranes induces a transient, profound and rapidly reversible neutropenia probably mediated by complement activation by the alternate pathway [3,4]. In addition to granulocytopenia, a reduction in the eosinophil count with no or minor changes in monocyte counts has been observed [5,6]. However, little is known about the acute effect of dialysis on lymphocytes [6] which, repeated three times a week over many years, might lead to the abnormalities described above. The present study investigates the behaviour of lymphocytes and particularly of T lymphocyte subsets, identified using monoclonal antibodies, during dialysis using cuprophan and non-cuprophan membranes since haemodialysis leucopenia seems membrane dependent [7].

Materials and methods

Patients

Fifteen patients (6 women, 9 men; mean age 50.3 years) receiving three times weekly haemodialysis for a mean duration of 48.2 months were studied after informed consent. All patients had arteriovenous fistulas, used bicarbonate-containing dialysate, were anticoagulated with heparin; they were free from infection and none was under treatment with drugs known to affect white cell counts or immunity.

Membranes

The study was conducted with cuprophan membranes (Travenol 2308, $1.5m^2$ Gambro GF, $1.8m^2$) and polyacrylonitrile (PAN) membranes (Biospal 2400 S, $1m^2$). Each patient was dialysed once with each membrane in a randomly selected order.

Sampling

Pre-dialysis blood samples were drawn from the cannula at the time of insertion. Samples during the three-hour treatment were drawn from the arterial segment of the extracorporeal circuit at 15, 60 and 180 minutes. Blood samples were analysed on the same day for total and differential white cell counts using a Coulter counter. T lymphocyte subsets were enumerated by an indirect immuno-fluorescence assay using the mouse monoclonal antibodies OKT_3, reacting with all peripheral T cells, OKT_4 identifying only T cells with helper or inducer function and OKT_8 directed against a T cell subset with suppressor-cytotoxic properties [8]. Finally, complement activation was assayed by calculating the ratio of C_{3d} to total C_3 concentration (normal range: 0–5); these complement fractions were measured according to methods previously reported [9].

Analysis of results

All results are expressed as mean±SEM. Student's 't' test for paired data or Wilcoxon test have been used for statistical analysis after logarithmic transformation of the data.

Results

On cuprophan, the mean neutrophil count at 15 minutes fell significantly and was 22.1 per cent of the pre-dialysis value; thereafter, it rose to reach a value at 180 minutes not significantly different from the value at time 0. In contrast, the same patients, when undergoing dialysis on PAN, experienced a fall of only 18.3 per cent which, however, was significant.

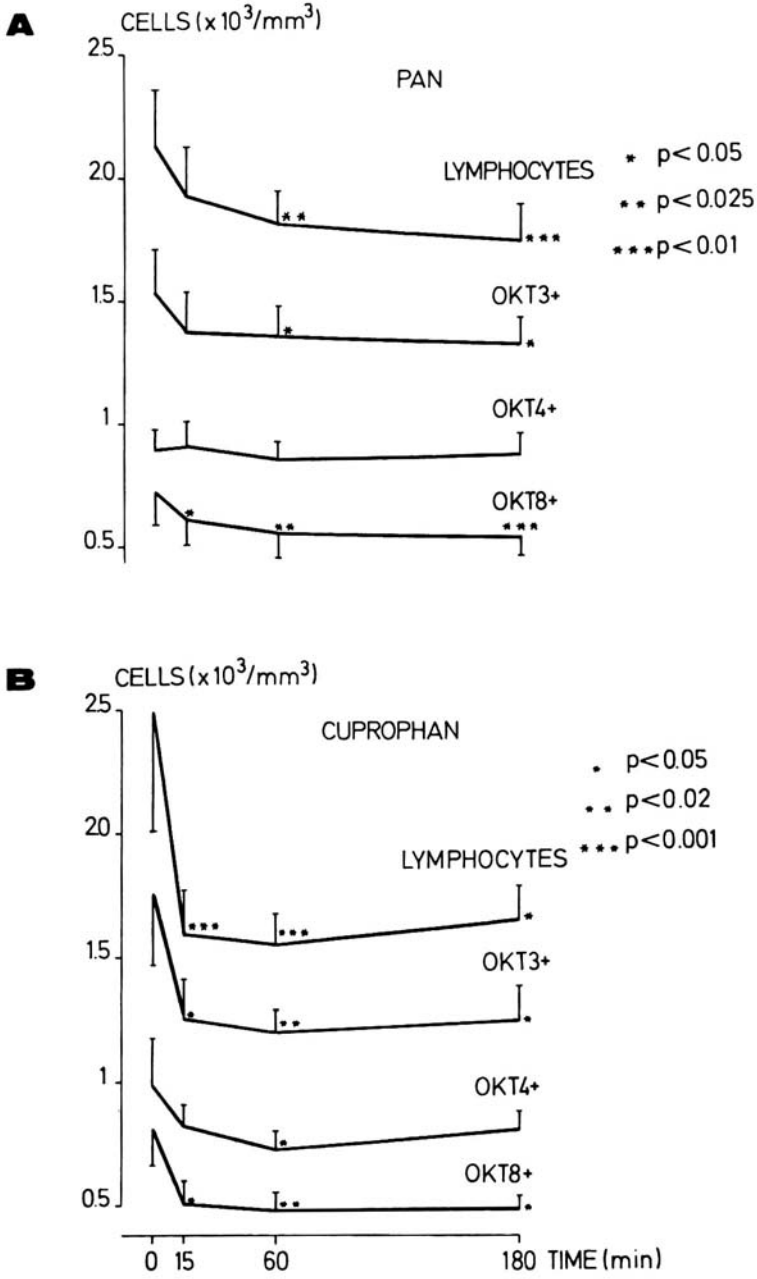

Figure 1. Changes in lymphocyte counts and T lymphocyte subsets during dialysis on a PAN dialyser (A) and on a cuprophan dialyser (B); statistical comparison of absolute cell numbers with pre-dialysis values

T lymphocyte subsets

Dialysis with cuprophan (Figure 1B) induced a rapid, profound and persistent decrease in the total number of lymphocytes, with a similar fall in the number of OKT_3^+ and OKT_8^+ cells, while OKT_4^+ cells decreased only marginally and with some delay (significant fall at 60 minutes; $p<0.05$). The changes in lymphocytes during dialysis with PAN was of the same nature but developed more gradually (Figure 1A): total lymphocytes fell from 2145±220/mm^3 pre-dialysis to 1738±143/mm^3 at the end of the procedure, while OKT_3^+ fell from 1554±196/mm^3 to 1368±118 and OKT_8^+ from 720±124/mm^3 to 522±65 over the same 180 minute period. Meanwhile, the number of OKT_4^+ cells did not change between the beginning and the end of dialysis (910±101/mm^3 and 836±88/mm^3). When the results are expressed as a percentage of the pre-dialysis values to eliminate variations due to differences between patients' white cell counts, as in Figure 2, there was still a significant decrease with both types of membranes

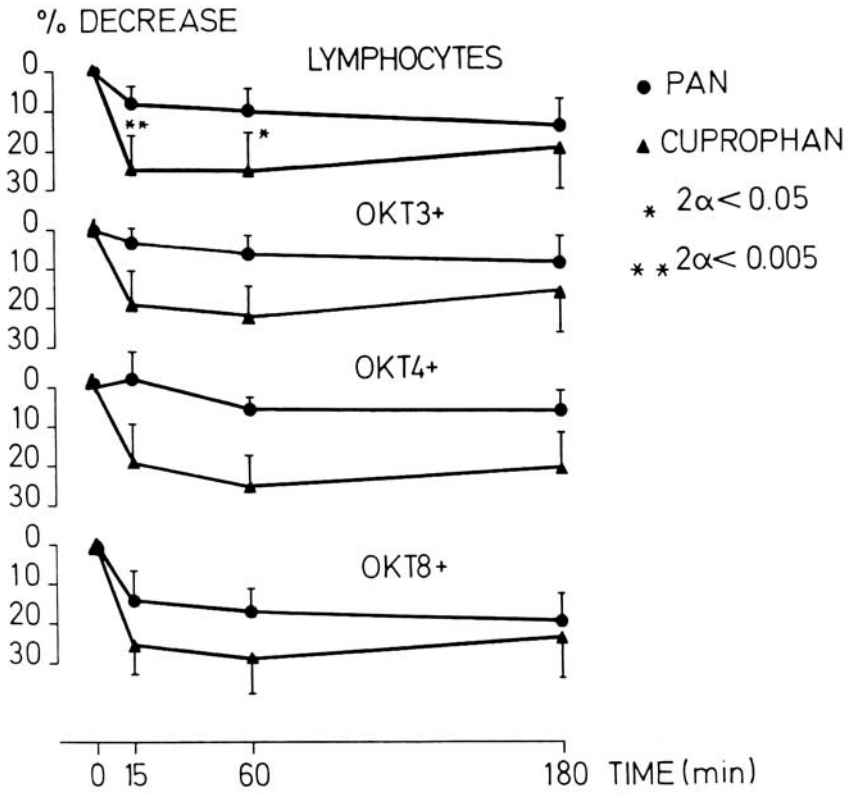

Figure 2. Comparison of lymphocyte counts and T lymphocyte subsets in per cent of pre-dialysis values during dialysis on cuprophan and PAN dialysers

in total lymphocytes, OKT_3^+ and OKT_8^+ cells with no change in OKT_4^+ lymphocytes; once again, the changes seemed more progressive with PAN than with cuprophan and were more pronounced on cuprophan, only with regards to the total number of lymphocytes at 15 and 60 minutes. As a consequence (Figure 3A), the T_4/T_8 ratio significantly and similarly increased during dialysis on both cuprophan and PAN (from 1.53±0.22 and 1.53±0.20 at time 0 to 1.93±0.23

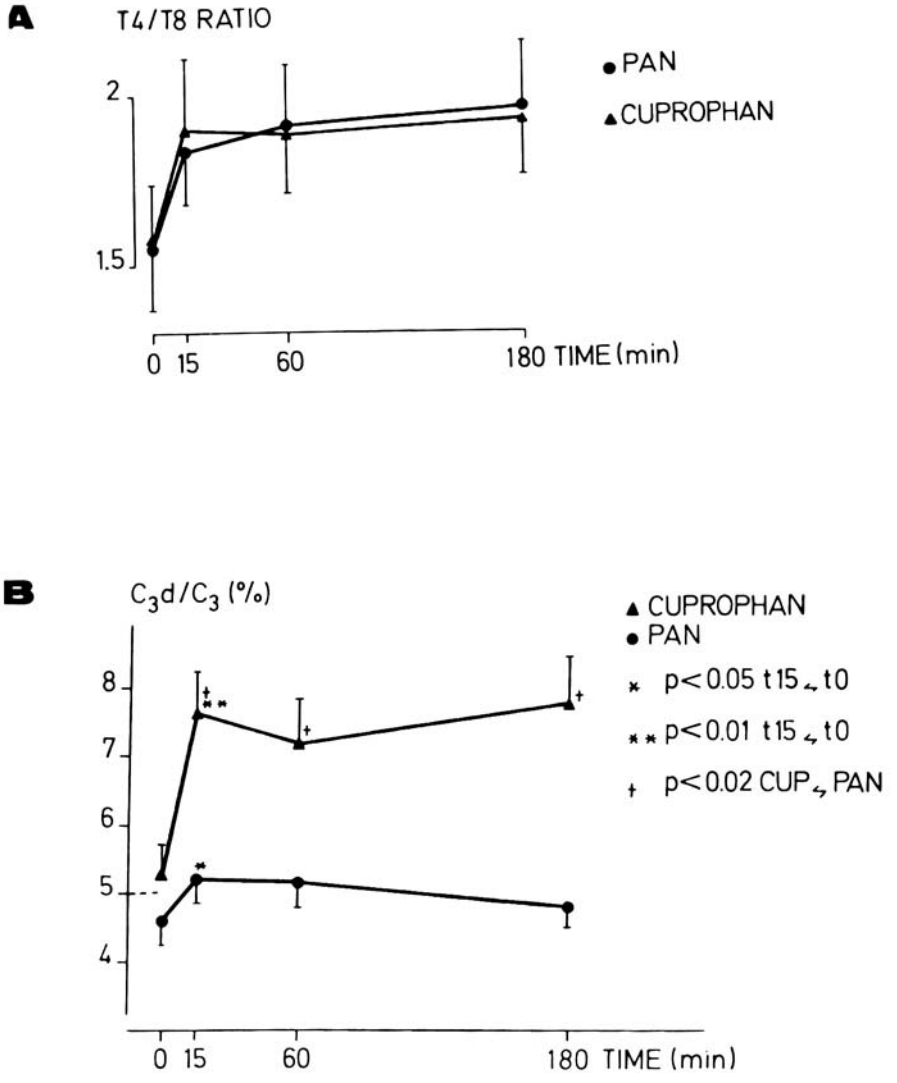

Figure 3. Changes in T_4/T_8 ratio (A) and of C_3d/C_3 ratio (B) during dialysis with cuprophan and PAN membranes

and 1.95±0.20 at 180 minutes, respectively). When measured 24 hours after the end of a dialysis session in four patients, the T_4/T_8 ratio came back to its pre-dialysis value.

Complement activation

As shown in Figure 3B, dialysis with a PAN dialyser activated complement only minimally and transiently (4.5±20.3% at 0 minute to 5.2±0.3% at 15 minutes; $p<0.05$). In contrast, the interaction of blood with cuprophan produced a significant increase in C_{3d}/C_3 ratio which was sustained from the beginning to the end of dialysis (5.5±0.3% at 0 minute to 7.6±0.8% at 15 minutes; $p<0.01$). The differences in complement activation between the two membranes used were significant throughout the whole procedure.

Discussion

The present study demonstrates that in addition to granulocytopenia, haemodialysis also affects lymphocytes. Although more progressively with PAN than with cuprophan, dialysis with both types of membranes induce lymphocytopenia and selective modifications of T lymphocyte subsets resulting in an increased T_4/T_8 ratio. When compared to granulocytopenia [3,4], this phenomenon has several characteristics: first, although it occurs early in the course of dialysis as does neutropenia, it lasts much longer (at least 3 hours) but is also reversible, albeit within 24 hours; second, as complement activation is unimpressive during dialysis with PAN as shown in the present and previous studies [10], it cannot be held as the major factor responsible for the changes in T lymphocyte subsets which are similar during dialyses with both PAN and cuprophan where, in contrast, marked complement activation occurs. Thus other hypothetical mechanisms must be advocated: liberation by the extracorporeal circuitry of some known (interleukins) or yet unsuspected factor(s) having an activity towards lymphocytes; trapping of T cells and preferentially OKT_8^+ lymphocytes, in lymphoid organs such as lymph nodes or the spleen explaining the longer delay for reversibility; change of antigenic characteristics leading to a change in the cells recognition by monoclonal antibodies; sequestration of lymphocytes in the dialyser.

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