Rapid Communication

Effect of renal dialysis therapy modality on T cell cytokine production

Aurelia Zamauskaitė1, Isabel Perez-Cruz1, Mohammed M. Yaqoob3, J. Alejandro Madrigal1,2 and Shara B. A. Cohen1,2

1The Anthony Nolan Bone Marrow Trust, 2The Department of Haematology, The Royal Free Hospital, Pond Street, and 3The Renal Unit, St. Bartholomew’s Hospital, London, UK

Abstract

Introduction. Dialysis has been associated with acute changes in the complement activation status, granulocyte markers, macrophage function, T cell activation and the release of pro-inflammatory cytokines. The most common analysis of cytokine production in patients on dialysis has focused on the changes in monokines (particularly IL-1 and TNFα), however it is becoming clear that T cell cytokines play a major role in the impaired lymphocyte function of dialysis patients.

Methods. To assess the effect of dialysis modality on T cell function we analysed the ability of T cells within peripheral blood mononuclear cell populations (PBMC) to produce cytokines after mitogen (phorbol-12-myristate-13-acetate; PMA and Ionomycin; I) stimulation in patients on peritoneal dialysis (PD) compared to low flux haemodialysis (HD) and normal individuals (controls).

Results. In control PBMC, PMA + I stimulation significantly increased the percentage of CD3+ cells expressing IL-2, IFNγ, TNFα, IL-4 and IL-10, as expected. However, although mitogen stimulation significantly enhanced the percentage of the classical Th1 cytokines (IL-2, IFNγ and TNFα) in the low flux HD PBMC, it had no effect on CD3+ IL-2 or CD3+ TNFα producing cells in the PD group. In contrast, the percentage of T cells producing Th2 cytokines (IL-4 and IL-10) could not be consistently enhanced by mitogen in either dialysis group.

Conclusions. We suggest that PD alters the ability of T cells to produce cytokines, possibly by causing an ‘exhaustion’ of the Th1 cells, thereby preventing cells to produce cytokine on ex vivo stimulation. Furthermore, since T cells from both low flux HD and PD groups could not be induced to produce Th2 cytokines we suggest that uraemia or dialysis per se inhibits T cells from producing Th2 cytokines.

Key words: dialysis; Th1; Th2; intracellular cytokines

Introduction

Various abnormalities of the immune system have been demonstrated in patients on maintenance dialysis. For example, it is well documented that patients on chronic haemodialysis (HD) and peritoneal dialysis (PD) have a tendency to develop infections and have an increased incidence of malignancy, possibly linked to the immune response [1].

These defects in immunity can be categorized into two major groups: firstly, immune defects may be caused by the uraemic state itself. Uraemic patients have clinical evidence of impaired lymphocyte function [2,3]. Furthermore, a number of in vivo and in vitro studies have demonstrated that lymphocyte [4–6], granulocyte [7,8] and monocyte/macrophage [9] function are all altered in uraemic patients. Possible mediators that may induce the immunosuppressive effect include uraemic toxins and parathyroid and steroid hormones [10,11]. Secondly, defects in immunity can occur as a direct consequence of therapy. In dialysed patients, the risk of infections is substantial [12]. Although defects occur in both the humoral and cellular response, the major defects appear to be in cell-mediated immunity [1,13]. Thus, dialysis has been associated with acute changes in the complement activation status [12,14], granulocyte markers [7,15], macrophage function [16], T cell activation [17,18] and the release of various pro-inflammatory cytokines [19]. The most common analysis of cytokine production in patients on dialysis has focused on the changes in monokines (particularly IL-1 and TNFα; 20–24), however it is becoming increasingly clear that T cell cytokines play a major role in the impaired lymphocyte function of dialysis patients [19].

By analysing the cytokine production profile (at both the mRNA and protein levels) of a number of well established murine CD4+ ‘helper’ cell clones, Mosmann and colleagues described two groups of clones. ‘Th1 cells’ secrete IL-2, IFNγ and TNFα but not IL-4 or IL-10 and the other, labelled ‘Th2’ cells, has a reciprocal cytokine secretion profile [25]. These different subsets display functional differences which correlate with their cytokine profile; Th1 type cells
mediate pro-inflammatory reactions and help B cells produce IgG2a, whereas Th2 type cells help B cells in production of antibody IgG1 and IgE [26]. Although these functionally distinct T cell subsets were originally described in the mouse, human T cell clones have subsequently been shown to have a similar, but not identical restricted cytokine profile. Here we have studied the cytokine profile of the T (CD3+) cells within the peripheral blood mononuclear cell (PBMC) population from 45 patients with end stage renal disease on chronic dialysis. To assess the effects of produce cytokines after stimulation in patients who had been on PD compared to low flux HD and normal individuals. This is the first study which analyses the effect of different modes of long term dialysis on T cell cytokine production. We chose to analyse the frequency of T cells producing Th1 and Th2 cytokines using the new technique of intracellular cytokine staining. Our results suggest that the uraemic state inhibits T cells from producing Th2 cytokines, moreover T cells from patients on PD but not low flux HD could not be consistently induced to produce IL-2 or TNFα.

**Subjects and methods**

We studied 45 patients with end-stage renal disease on chronic dialysis. Twenty-five patients were on chronic HD and had never been on PD and 20 patients were on PD without having been on HD. Within the HD group there were 16 males and 9 females. Within the PD group there were 14 males and 6 females. The mean age of HD and PD patients was 52.1 and 57.9 years respectively. The patients undergoing HD had 4-h sessions three times a week. These patients were dialysed using only Hemophan membranes (Gambro Dialysators, GmbH and Co, Hechingen, Germany), there was no dialyser reuse.

Seventeen patients in HD group and 12 patients in PD had an initial diagnosis of chronic glomerulonephritis (biopsy proved). Eight patients in HD group and 8 patients in PD had an initial diagnosis of insulin-dependent diabetes mellitus they and developed end-stage renal failure due to diabetic nephropathy (DN). We selected DN patients which had stable and controlled serum glucose levels.

We also studied 10 healthy controls with an age range from 21 to 52, the mean age was 36.2 (4 male, 6 female) without any known disease and not taking medication. This group was department and laboratory staff.

**Patient status**

No patients had active infection, malignancy or an inflammatory state and none were taking immuno-suppressive medication. All patients were HIV-negative and none had Hepatitis B antigen. We selected PD patients which showed no signs of any infection over the previous 3 months. The clinical and biochemical characteristics of the patient groups are described in Table 1. All three groups (HD, PD and control) had white blood cell counts, total serum protein and serum albumen levels within the normal range. Whereas, as expected, the haemoglobin, serum urea and serum creatinine levels were only within the normal range in the control group.

Blood samples

Blood was taken from the HD patients before their dialysis session. Samples from HD, PD and controls were all taken by venipuncture. All blood was collected in heparin and separation of mononuclear cells from peripheral blood was performed by standard techniques (Ficoll gradient). All cells were frozen in liquid nitrogen in a solution of 10% DMSO plus foetal calf’s serum (FCS; Bio Whittaker, Verlyers, Belgium) in RPMI (Bio Whittaker, Wokingham, UK) by standard techniques until use.

**Antibodies**

All antibodies used were fluorescence labelled, either with fluorescence isothiocyanate (FITC) or phycoerythryene (PE). Monoclonal antibodies used to detect surface antigen; anti-CD3-FITC and anti-CD3-PE (T cells) were obtained from Becton Dickinson (Palo Alto, USA). Antibodies used to stain for intracellular cytokines (and their isotype matched controls) were all obtained from Pharmingen (Cambridge Biosciences, Cambridge, UK). The anti-cytokine antibodies were: anti-IL-2-PE (rat IgG2a; clone MQ1-17H12), anti-IL-4-PE (mouse IgG1; clone SD4-8), anti-IL-10-PE (rat IgG2a; clone JES3-19F1), anti-IFN-γ-PE (mouse IgG1; clone 4S.B3), anti-TNF-α-FITC (mouse IgG1; clone MAb11). Isotype matched control antibodies were: clone R35-95 (rat IgG2a, PE conjugated) and clone MOPC-21 (mouse IgG1, FITC or PE conjugated).

Stimulating T cells

Cells were thawed in 12.5% FCS in RPMI (wash buffer) then washed twice in wash buffer. 1×10⁶ cells were then placed in 1 ml RPMI plus 10% human serum (Bio Whittaker) (unstimulated cells) or stimulation mixture (20 ng/ml phorbol-12-myristate-13-acetate; PMA from Sigma, 1 μM Ionomycin; I from Calbiochem, Nottingham, UK, 3 μM Monensin from Calbiochem, and 10% human serum), for 4 h at 37°C in 5% CO₂. Cells were then stained for intracellular cytokines.

Intracellular cytokine staining

Aliquots of 10⁵ cells/tube were washed twice in staining buffer (Dulbecco’s PBS from Gibco BRL Paisley, UK; 1% heat-inactivated FCS, 0.1% (w/v) sodium azide from BDH Laboratory Supplies, Poole, UK; pH 7.4–7.6). The pellet was resuspended in 30 μl staining buffer plus anti-CD3-FITC or anti-CD3-PE. Cells were incubated for 20 min at 4°C, washed, fixed in Cell FIX (Becton Dickinson, San Jose, USA) and left for 20 min at 4°C in the dark. To permeabilize the cells they were washed twice in permeabilization buffer (staining buffer plus 0.1% saponin; Sigma, Poole, Dorset, UK). Cells were then spun down and resuspended in a previously determined optimal concentration of an anti-cytokine or control conjugated antibody. After incubation at 4°C for 30 min in the dark cells were washed with permeabilization buffer. Cells were analysed within 24 h by a FACScan flow cytometer after acquiring 10000 cells (Becton Dickinson). Data was evaluated by Consort 30 software (Becton Dickinson) and is expressed as the percentage of double stained (anti-cytokine and anti-CD3) positive cells in the total population. Samples were analysed by either Student’s t-test or one tailed paired t-test. We assumed significance at P<0.05.
increased the percentage of T cells expressing all the However, the cells through chronic
in vivo
P
Y
+ cells before and after PMA
n
Serum albumin (g/l) 23 0.2 7.0 1.2
Serum creatinine (µmol/l) 842.3 ± 94.8 714.0 ± 46.4 115.2 ± 30.0 < 120
Total serum protein (g/l) 70.4 ± 3.5 70.3 ± 2.0 81.5 ± 2.5 65.0–85.0
Serum albumin (g/l) 36.5 ± 2.8 35.8 ± 2.8 40.7 ± 2.6 35.0–50.0
Recombinant human erythropoietin treatment

White blood cells counts, haemoglobin levels, serum urea, serum creatinine, and serum albumin were all measured in the routine hospital lab (St. Bartholomew’s Hospital, London). Samples were taken for clinical and biochemical analysis at the same time that we took blood for cytokine analysis. Some patients were treated with recombinant human erythropoietin (‘Eprex’; Cilag and ‘Recormon’; Boehringer Mannheim) at an average dose of 25 units/kg.

Table 1. Clinical and biochemical characteristics of subjects (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>HD group (n=25)</th>
<th>PD group (n=20)</th>
<th>Control (n=10)</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells (× 10/l)</td>
<td>8.5 ± 1.5</td>
<td>8.5 ± 0.9</td>
<td>5.7 ± 0.7</td>
<td>4.0–9.0</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>9.9 ± 1.1</td>
<td>11.3 ± 1.3</td>
<td>13.5 ± 1.3</td>
<td>12.0–18.0</td>
</tr>
<tr>
<td>Serum urea (mmol/l)</td>
<td>25.2 ± 3.2</td>
<td>20.8 ± 2.2</td>
<td>6.0 ± 0.6</td>
<td>5.0–8.0</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>842.3 ± 94.8</td>
<td>714.0 ± 46.4</td>
<td>115.2 ± 30.0</td>
<td>&lt; 120</td>
</tr>
<tr>
<td>Total serum protein (g/l)</td>
<td>70.4 ± 3.5</td>
<td>70.3 ± 2.0</td>
<td>81.5 ± 2.5</td>
<td>65.0–85.0</td>
</tr>
<tr>
<td>Serum albumin (g/l)</td>
<td>36.5 ± 2.8</td>
<td>35.8 ± 2.8</td>
<td>40.7 ± 2.6</td>
<td>35.0–50.0</td>
</tr>
</tbody>
</table>

White blood cells counts, haemoglobin levels, serum urea, serum creatinine, and serum albumin were all measured in the routine hospital lab (St. Bartholomew’s Hospital, London). Samples were taken for clinical and biochemical analysis at the same time that we took blood for cytokine analysis. Some patients were treated with recombinant human erythropoietin (‘Eprex’; Cilag and ‘Recormon’; Boehringer Mannheim) at an average dose of 25 units/kg.

*P<0.05 compared to control values.

Results and discussion

To delineate the T cell response we set up a system to analyse the frequency of cytokine producing T cells within the PBMC of our dialysis patients. A total of 10000 events were acquired and analyses was performed on a live gate selected by forward and side scatter characteristics (Figure 1a).

We then set quadrants around our negative control (unstained PBMCs from a normal individual; Figure 1b) and used these quadrants for all further analyses. Further negative controls were the PBMCs stained with FITC or PE conjugated antibodies, isotype matched to the anti-cytokine antibodies plus staining with the corresponding anti-CD3 PE or FITC conjugate. Our positive controls were the PBMC derived from normal individuals, stimulated with PMA + I and stained with anti-cytokine FITC or PE antibody plus the corresponding anti-CD3 PE or FITC conjugated antibody. Figure 1b shows an example of PBMC obtained from a normal individual stained as described above. The percentage of CD3+ cells within this PBMC population was approximately 73% and the percentage of PBMC doubled stained with anti-CD3 and anti-cytokine isotype matched control antibody was always less than 5%. The percentage of cytokine producing T cells was greater than 10% with the exception of IL-10. This cytokine was difficult to detect in the normal individuals PBMC T cells, possibly due to the late kinetics of its detection [27], nevertheless a 4 h stimulation of PMA + I did enhance the detection of this cytokine significantly (P<0.05; Figure 3) and so we continued to analyse the frequency of T cells producing this cytokine.

In general a T cell will not produce cytokines unless stimulated, therefore to analyse the frequency of cells with the potential to produce certain cytokines T cells are generally stimulated with a mitogen in vitro [28,29]. Here we analysed the frequency of cytokine producing cells before and after PMA + I stimulation in PBMCs derived from our three groups of individuals. In our control group of individuals PMA + I significantly increased the percentage of T cells expressing all the cytokines analysed (IL-2, IFNγ, TNFα, IL-4 and IL-10) as expected. However, we did not see this for the low flux HD and PD groups (Figure 2). We found that mitogen stimulation significantly enhanced the percentage of the classical Th1 cytokines (IL-2, IFNγ and TNFα) in the low flux HD PBMCs, but had no consistent effect on CD3+ IL-2 or CD3+ TNFα producing cells in the PD group.

There are many differences between PD and low flux HD modalities, these differences range from differences in the dialysate [30,31] to biocompatibility issues [32,33]. However there are also differences in the patients well-being and an individual on PD is more likely to have multiple infections [31]. Although our PD group was selected on the basis that they showed no clinical infection for a minimum of 3 months it is likely that they had sub-clinical infections. It therefore seems likely that individuals on PD will have chronically stimulated T cells, due to multiple infections and ex vivo are exhausted. This exhaustion of T cells has previously been described, for example in diabetes T cells ex vivo make little IL-2 [34] and has recently been attributed to pre-translational regulation [35].

Thus, we saw a trend, which was significant, which suggested that Th1 cells in the PBMC of patients on PD could not always be stimulated to produce cytokines. This was not true for IFNγ and we have two explanations for this. Firstly, because CD4 expression detect in the normal individuals PBMC T cells, possibly due to the late kinetics of its detection [27], nevertheless a 4 h stimulation of PMA + I did enhance the detection of this cytokine significantly (P<0.05; Figure 3) and so we continued to analyse the frequency of T cells producing this cytokine.

In general a T cell will not produce cytokines unless stimulated, therefore to analyse the frequency of cells with the potential to produce certain cytokines T cells are generally stimulated with a mitogen in vitro [28,29]. Here we analysed the frequency of cytokine producing cells before and after PMA + I stimulation in PBMCs derived from our three groups of individuals. In our control group of individuals PMA + I significantly increased the percentage of T cells expressing all the cytokines analysed (IL-2, IFNγ, TNFα, IL-4 and IL-10) as expected. However, we did not see this for the low flux HD and PD groups (Figure 2). We found that mitogen stimulation significantly enhanced the percentage of the classical Th1 cytokines (IL-2, IFNγ and TNFα) in the low flux HD PBMCs, but had no consistent effect on CD3+ IL-2 or CD3+ TNFα producing cells in the PD group.

There are many differences between PD and low flux HD modalities, these differences range from differences in the dialysate [30,31] to biocompatibility issues [32,33]. However there are also differences in the patients well-being and an individual on PD is more likely to have multiple infections [31]. Although our PD group was selected on the basis that they showed no clinical infection for a minimum of 3 months it is likely that they had sub-clinical infections. It therefore seems likely that individuals on PD will have chronically stimulated T cells, due to multiple infections and ex vivo are exhausted. This exhaustion of T cells has previously been described, for example in diabetes T cells ex vivo make little IL-2 [34] and has recently been attributed to pre-translational regulation [35].

Thus, we saw a trend, which was significant, which suggested that Th1 cells in the PBMC of patients on PD could not always be stimulated to produce cytokines. This was not true for IFNγ and we have two explanations for this. Firstly, because CD4 expression is down modulated by PMA [36] we had to restrict our analysis to CD3+ cells. We have previously shown that CD8+ cells produce very high levels of IFNγ [37], we therefore cannot overlook the possibility that CD8+ cells are contributing to the IFNγ detected and we are not looking at the T helper population. Thus we may have masked the Th1 IFNγ response by not analysing only CD4+ cells.

However, a more plausible explanation may be that the Th1 cells in the PBMC from patients on PD have been over-stimulated in vivo. This is manifested in vitro by our observation that we cannot always further stimulate the cells to produce IL-2 or TNFα. However, the cells through chronic in vivo stimulation,
Fig. 1. Setting up the system of analysis. Peripheral blood mononuclear cells (PBMC) from normal individuals was either placed in 1 ml RPMI plus 10% human serum (unstimulated cells) or stimulation mixture (10 μg/ml phorbol-12-myristate-13 acetate; PMA, 1 μM Ionomycin; 1 and 3 μM Monensin, and 10% Human serum, for 4 h 37 °C in 5% CO2. Cells were then stained extracellularly with anti-CD3 and intracellularly for cytokine or with a control antibody, as described in the materials and methods section. Cells were analysed within 24 h by a FACScan flow cytometer after acquiring 10 000 cells. Data was evaluated by Consort 30 software (Becton Dickinson). A total of 10 000 events were acquired and analyses was performed on a live gate selected by forward and size scatter characteristics (A). We then set quadrants around our negative controls and used these quadrants for all further analyses (B). Results are expressed as a percentage of cells stained in the total population.
Cytokine production by T cell from dialysis patients

are spontaneously producing IFN\(_\gamma\) when removed from the patient. This hypothesis is supported by our observation that the mean percentage of CD3\(^+\) cells producing IFN\(_\gamma\) spontaneously in the PD group (Nil stimulation) (14.5 ± 13.0) was significantly higher by student’s t-test, \((P < 0.005)\) than the control group (0.6 ± 0.5). In addition the mean percentage of IFN\(_\gamma\) producing T cells after mitogen stimulation, was significantly higher in the PD group (22.8 ± 13.5) compared to the control (7.1 ± 3.0; \(P < 0.005\)).

When we analysed the percentage of T cells producing the ‘classical’ Th2 cytokines (IL-4 and IL-10) we found a different pattern of response. Neither the percentage of T cells producing IL-4 nor IL-10 could be consistently enhanced by mitogen stimulation in either of the dialysis groups, although the control PBMC had an increase in frequency of IL-4 \((P < 0.05)\) and IL-10 \((P < 0.05)\) with PMA+I stimulation (Figure 3). After cells had been removed from the patient, the mononuclear cells were separated out from the blood, washed several times and then stimulated in vitro, thus any factor acting on the T cells must have a long term effect. Since T cells from both the low flux

Fig. 2. Th1 cytokine production by cells from patients in different dialysis groups before and after stimulation. Peripheral blood mononuclear cells (PBMC) from individuals on haemodialysis (HD), peritoneal dialysis (PD) or the control group were either placed in 1 ml RPMI plus 10% human serum (Nil) or stimulation mixture (10 \(\mu\)g/ml phorbol-12-myristate-13-acetate, 1 \(\mu\)M Ionomycin; and 3 \(\mu\)M Monensin, and 10% human serum; PMA+I), for 4 h at 37°C in 5% CO\(_2\). Cells were then stained extracellularly with anti-CD3 and intracellularly for cytokine, as described in the materials and methods section. Data was analysed as described for Figure 1. Cells were analysed for their ability to produce the Th1 type cytokines (IFN\(_\gamma\), IL-2 and TNF\(_\alpha\)). The figures represent the changes in the percentage of CD3\(^+\) cells producing cytokines in different dialysis groups and the control. The changes in the percentage of CD3\(^+\) P values were determined by paired t-test, comparing unstimulated to PMA+I stimulated cells. Results were assumed significant if \(P < 0.05\).

Fig. 3. Th2 cytokine production by cells from patients in different dialysis groups before and after stimulation. PBMC were stimulated and analysed as described for Figure 3. Cells were analysed for their ability to produce the Th2 type cytokines (IL-4 and IL-10).
HD and PD group of individuals could not be induced to produce the Th2 cytokines we can assume that uraemia or dialysis per se is effecting the T cells in a chronic manner.

We are aware there are a large number of factors which could influence the immune response other than dialysis treatment. For example, nutritional status, dialysis adequacy, erythropoietin therapy and ferritin levels may all play a role. Although we have tried to take these factors into account in selecting our population (for example: the nutritional status was adequate, verified by a normal range of serum albumen and protein levels, Table 1: all samples were taken from patients who had dialysed efficiently, the erythropoietin treatment was on average 25 U/kg and the ferritin levels were all within the normal range, 23–862 pmol/l), we cannot overlook the possibility than we may have been looking at the effects of unknown factors. Nevertheless our differences between the two dialysis groups seems clear cut. In addition, the mean control group age and sex of the control group is different from the dialysis groups. However, these parameters were similar between dialysis groups, therefore they do not effect our conclusions regarding differences between types of dialysis.

In this paper we talk of the ‘classical’ Th1 and Th2 cytokines. There is currently suggestion that in the human the Th1/Th2 paradigm is not as strict as in the mouse. Indeed we have shown that human Th1 type cells (defined by a high IFNγ and low IL-4 production) can produce IL-10 [27] (which is defined as a Th2 cytokine in the mouse). However, the cytokines grouped into Th1 and Th2 have similar function i.e. Th1 type cytokines (IL-2, IFNγ, TNFα) have pro-inflammatory mechanisms, whereas Th2 type cytokines (IL-4 and IL-10) will help B cells function. In this paper we are therefore defining a cell according to the function of the cytokine it produces.

Since patients can have renal failure for a multitude of reasons we carefully selected our patient groups further by selecting from individuals who had DN or insulin dependant diabetes mellitus. We saw no clear cut effect of dialysis modality on individual patient groups regarding the ability of the T cells within the PBMC to be stimulated to produce cytokines. However, there was a trend for patients on PD with insulin dependant diabetes mellitus to spontaneously produce the Th2 cytokines, although this was not significant (data not shown).

This is the first study which analyses the effect of different modes of long term dialysis on T cell cytokine production. We chose to analyse the frequency of T cells producing Th1 and Th2 cytokines using the new technique of intracellular cytokine staining. The data presented in this study suggests that, in patients with end stage renal disease and on low flux HD, the ability of the T cells to produce Th1 type cytokines is preserved. However, patients on PD failed to produce IL-2 and TNFα consistently on stimulation and there appeared to be spontaneous production of IFNγ. However, in both low flux HD and PD patients, Th2 cytokine production was impaired, suggesting that the uraemic state inhibits production of the Th2 cytokines. The results of the study may explain the cellular mechanism of increased susceptibility to multiple infections, particularly in PD.

In addition to playing different roles in protection, T cell responses can also be responsible for different types of immunopathological reactions in humans. Th1 dominated responses are involved in the pathogenesis of Type 1 diabetes mellitus [38], acute kidney allograph rejection [39] and unexplained recurrent abortions. In contrast, Th2 type responses are predominant in conditions associated with immunological tolerance such as pregnancy [40] and transplant tolerance [39]. Moreover, allergen-specific Th2 responses are responsible for ectopic disorders [41] in genetically susceptible individuals. Further work is needed to identify the significance of these differential effects of dialysis modalities on Th1/Th2 paradigm, particularly in terms of successful future renal transplantation. We have preliminary data (unpublished) which suggests that patients on PD have less incidence of acute cellular rejection following transplantation as compared to dialysis patients. It is possible to speculate that the mechanism of this observation could be because of preserved Th1 responses responsible for graft rejection in HD patients; however, the Th1 response are impaired in patients on PD.

We feel that, in patients with end stage renal disease, the understanding of the mechanisms involved in the differential activation and function of T cells will help us learn more about the side effects of treatment and enable us to learn how to manipulate the immune system for a better prognosis.

Acknowledgements. A. Zamauskaite, I. Perez-Cruz, J. A. Madrigal and S. B. A. Cohen are supported by The Anthony Nolan Bone Marrow Trust. We would like to thank Novartis for sponsoring this work.

References
8. Abrutyn E, Solomon N. Granulocyte function in patients with
26. Street NE, Mossmann TR. Functional diversity of T lymphocytes due to secretion of different cytokine problems. FASEB J 1991; 5: 171–177
41. Mossmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today 1996; 17: 138–146

Received in revised form: 2.10.98