The imbalance in the ratio of Th1 and Th2 helper lymphocytes in uraemia is mediated by an increased apoptosis of Th1 subset

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Abstract

Background. In uraemia there is a reduction in the total number of T lymphocytes and an imbalance in the ratio of Th1/Th2 T-helper (Th) lymphocytes. A higher rate of apoptosis in T lymphocytes has been reported in haemodialysis patients. The aims of the present study were to assess the Th1/Th2 pattern in uraemia and to evaluate whether a relative increase in Th1 apoptosis may explain the Th1/Th2 imbalance observed in uraemic patients.

Methods. Seventeen non-dialysed uraemic patients were evaluated; eight healthy volunteers served as controls. Intracellular interferon-γ (IFN-γ) and interleukin-4 (IL-4) were measured by direct intracellular immunofluorescence and flow cytometry. Apoptosis was determined by flow cytometry using annexin V or TUNEL. Mechanisms of apoptosis were assessed by determination of Fas and Bcl-2 expression.

Results. Cell production of cytokines is significantly higher in uraemic patients than in controls. In addition, in uraemic patients only 5.1±2.1% of the T lymphocytes contained IFN-γ (Th1 cells) while 61.9±14.8% contained IL-4 (Th2 cells) (P<0.0001). The percentage of apoptosis was 29.6±6.3% and 4.7±1.6% in Th1 and Th2 lymphocytes, respectively (P<0.001). Fas expression was higher in Th1 than in Th2 cells and the expression of Bcl-2 was lower in Th1 than in Th2 cells. The apoptosis induced by anti-Fas antibodies was similar in both types of lymphocytes.

Conclusions. In uraemia there is a reduction in the proportion of Th1 lymphocytes due to a higher rate of apoptosis in this subset of lymphocytes. Th1 from uraemic patients show a higher expression of Fas and a lower expression of Bcl-2 than Th2. This makes uraemic Th1 cells more susceptible to apoptosis. The Th1/Th2 imbalance may contribute to alterations in cellular immunity observed in chronic kidney disease patients.

Keywords: apoptosis; Bcl-2; chronic kidney disease; cytokines; Fas; Th1/Th2 lymphocytes

Introduction

Patients with chronic kidney disease (CKD) have an impairment of the immune response. This is illustrated clinically by an increased incidence of bacterial and viral infections, which are the second most frequent cause of death in these subjects. These patients also have a low rate of response to vaccines, a reduction in delayed hypersensitivity and a high incidence of tumours. Although the exact mechanisms of these immunological alterations in uraemia are unknown, several studies have reported an impaired function of neutrophils, a decrease in the number of lymphocytes, a moderate reduction in T CD3+ lymphocytes, a decrease in the proportion of CD4+ to CD8+ and a decrease in the number of B lymphocytes [1,2]. The lymphopenia of patients with advanced CKD may be due to a reduction in cell proliferation [3] and/or an increase in the rate of T- and B-lymphocyte apoptosis [2]. The fact that treatment with haemodialysis reverses some of these abnormalities suggests that uraemia plays a relevant role in the dysfunction of the immune system in CKD patients [3].

Lymphopenia per se does not explain the immune dysfunction described in uraemic patients. The cytokine profile produced by the T lymphocytes determines the immune response; stimulation by antigen exposure causes T-helper lymphocytes to differentiate into two distinct phenotypes, Th1 and Th2. Th1 cells are mainly responsible for phagocyte-mediated host defence and these cells are the principal effectors of cell-mediated immunity and delayed-type hypersensitivity reactions. Th2 lymphocytes are responsible for the immune

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defence not mediated by phagocytes, the recruitment of eosinophils and allergic reactions [4]. Recently, several authors have reported that in uraemic patients there is a change in the profile of Th1/Th2 lymphocytes [5–9], which would explain, at least in part, the immunological abnormalities found in these patients. However, the underlying mechanisms of the Th1/Th2 imbalance in CKD have not been elucidated. One possible explanation for the predominance of Th2 over Th1 in CKD patients could be an increase in the rate of Th1 lymphocyte apoptosis relative to that of Th2.

Recent reports show that in CKD there is an increased rate of apoptosis of polymorphonuclear and mononuclear cells, including T lymphocytes [1,2,10–13]. Apoptosis is regulated by an intracellular signalling system and Fas and Bcl-2 are important mediators of cell apoptosis [14–16]. There is recent evidence of increased Fas expression in T lymphocytes from uraemic patients [10,11]. Fernández-Fresno et al. [2] have reported that the B-cell lymphopenia observed in uraemia is related to an increased apoptosis due to a reduction in Bcl-2 expression. Finally, since in CKD patients there is a reduction in T-lymphocyte proliferation [3], potential differences in the rate of proliferation between Th1 and Th2 lymphocytes may explain an imbalance of Th1/Th2 in CKD patients.

The aims of the present study were (i) to assess the Th1/Th2 cell profile in advanced CKD patients; (ii) to evaluate the influence of proliferation and apoptosis in the final count of Th1 and Th2 lymphocytes; and (iii) to investigate the mechanisms that regulate T-helper-lymphocyte apoptosis in uraemia. In order to avoid the influence of the dialysis membrane and dialysate, the present study was performed in patients with advanced CKD just before the initiation of chronic dialysis therapy.

**Subjects and methods**

**Patients**

Seventeen patients (six females and 11 males) with advanced CKD were evaluated. The mean age was 60.5 ± 9.8 years (range: 24–70 years). Creatinine clearance, measured by urinary excretion of creatinine, was 14.7 ± 4.7 ml/min (range: 9.1–20.4 ml/min). None of the patients included had diabetes mellitus, malignancy, infection or autoimmune disease. Hepatitis B and C viruses and human immunodeficiency virus were negative in all cases. Patients were not on steroids, immunosuppressive medications, non-steroidal anti-inflammatory drugs, inhibitors of the renin–angiotensin system or calcitriol. Since parathyroid hormone (PTH) has been described to have an effect on peripheral mononuclear blood cell function, all patients included had PTH levels <400 pg/ml. Fourteen patients were receiving treatment with erythropoietin. The causes of CKD were obstructive uropathy (n = 1), nephritisis (n = 2), nephrosclerosis (n = 3), polycystic kidney disease (n = 5), chronic interstitial nephritis (n = 2), chronic glomerulonephritis (n = 1) and unknown (n = 3). The control group included eight healthy individuals (three females and five males) with normal renal function and an average age of 57.6 ± 11.5 years (range: 26–68 years). Additional information relative to patients and controls is presented in Tables 1 and 2.

**Table 1.** Baseline clinical and laboratory characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Healthy subjects</th>
<th>CKD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Age (years) [range]</td>
<td>57.6 ± 11.5 [26–68]</td>
<td>60.5 ± 9.8 [24–70]</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>5/3</td>
<td>11/6</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>98.2 ± 3.6</td>
<td>14.7 ± 4.7</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>14.4 ± 1.3</td>
<td>11.8 ± 2.2</td>
</tr>
</tbody>
</table>

Values are given as means ± SD.

**Table 2.** Clinical characteristics of CKD patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroke history [n (%)]</td>
<td>1/17 (6%)</td>
</tr>
<tr>
<td>Ischaemic cardiopathy history [n (%)]</td>
<td>3/17 (18%)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>152 ± 18</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>82 ± 8</td>
</tr>
<tr>
<td>Number of antihypertensive drugs/patient</td>
<td>1.8 ± 1.1</td>
</tr>
<tr>
<td>Diuretics [n (%)]</td>
<td>11/17 (65%)</td>
</tr>
<tr>
<td>Serum urea (mg/dl)</td>
<td>166 ± 58</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>7.3 ± 2.1</td>
</tr>
<tr>
<td>Serum calcium (mg/dl)</td>
<td>9.1 ± 1.8</td>
</tr>
<tr>
<td>Serum phosphate (mg/dl)</td>
<td>5.2 ± 0.9</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)</td>
<td>10.2 ± 3.1</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.4 ± 3.8</td>
</tr>
</tbody>
</table>

Values are means ± SD unless otherwise stated.
Intracellular cytokine staining

To determine the Th1 and Th2 phenotype, the intracellular expression of cytokines was examined at 24 h of culture. After 20 h of culture, 10 μl brefeldin A (BFA) (2 μg/ml BFA; Sigma Chemical Co., Poole, UK) was added over 4 h to inhibit the cytokine secretion by lymphocytes. To assure that T lymphocytes were T-helper cells, after 24 h of culture cells (10^6/ml) were washed, fixed and stained with surface anti-lymphocyte T-helper monoclonal antibodies (anti-CD4) conjugated with peridinin chlorophyll protein (PerCP; Becton Dickinson, San José, CA, USA). Immediately after, cells were washed and permeabilized using FACS permeabilizing solution (Becton Dickinson, San José, CA, USA). Before analysis, 100 μl of cells were incubated with the permeabilizing solution. After vortexing, cells were incubated for 5 min at room temperature in the dark. Then, cells were washed (5 min at 1500 r.p.m. Heraeus Labofuge 400R) in wash buffer [phosphate-buffered saline (PBS) + 0.1% NaN₃ + 10% autologous serum]. In all experiments, the appropriate conjugated intracellular antibody or the corresponding isotype negative antibodies were used as intra-experimental controls. Mouse monoclonal antibodies (mAbs) against human cytokines were used to stain intracellular cytokines. Antibodies anti-IL-4, anti-IL-10, anti-IFN-γ and anti-IL-2 were conjugated with fluorescein isothiocyanate (FITC). After 30 min of incubation, cells were washed and resuspended in 0.5 ml of 1% formaldehyde and stored at 4°C until flow cytometric analysis. Samples were analysed on a FACSCalibur System (Becton Dickinson, Mountain View, CA, USA).

Apoptosis of lymphocytes CD4+

Apoptosis of lymphocytes was measured after 48 h of culture using annexin V staining. One of the cell membrane changes during the early and intermediate stages of cell apoptosis is the translocation of phosphatidylserine from the inside to the outside of the cell membrane. Annexin binds only to cells in which phosphatidylserine has been translocated to the outside membrane. To evaluate apoptosis, cells were washed in PBS and their density was adjusted to 5 × 10^5/ml. They were then resuspended in binding buffer (10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM CaCl2; filtered through a 0.2 μm filter) and 5 μl annexin V/FITC (Bender MedSystems, Vienna, Austria) was added to 195 μl cell suspension. After 10 min incubation in the dark, cells were washed and resuspended in binding buffer and 10 μl propidium iodide stock solution (20 μg/ml). The degree of apoptosis was assessed by flow cytometry. Live cells were considered those that were negative for both dyes, dead cells were positive for both fluorochromes, while apoptotic cells were positive only for annexin V/FITC and negative for propidium iodide. To assure that T lymphocytes are T-helper cells, after annexin labelling cells were washed, fixed and stained with surface anti-CD4 mAbs, conjugated with PerCP.

Apoptosis of Th1 and Th2 cells

Additional experiments were performed to evaluate the effects of apoptosis on the Th1/Th2 profile. Apoptosis and cytokine expression were measured simultaneously in all cell samples, after 24 h culture. In this experiment, apoptosis was measured by the TUNEL method, which allows cell permeabilization, which is required for intracellular cytokines measurement. After washing, cells were fixed, permeabilized and labelled with Phycoerytrin (PE)-conjugated anticytokine antibodies and TUNEL–FITC. In all cases, the appropriate PE and TUNEL-labelled controls were used. After staining, cells were analysed in a FACScan flow cytometer, acquiring 10000 events. To determine the phenotype (Th1 or Th2) of apoptotic lymphocytes, single-cell measurement of intracellular cytokines by flow cytometry was performed as described above. Apoptosis-associated DNA strand breaks were evaluated using a kit based on the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labelling (TUNEL) method (Boehringer Mannheim, Indianapolis, IN, USA). After washing, cells were decanted and resuspended in 50 μl TUNEL reaction mixture (5 μl TUNEL enzyme containing TdT, mixed with 45 μl TUNEL label containing fluorescein-dUTP and dNTP nucleotides) or in 50 μl TUNEL label as negative control. After 60 min of incubation at 37°C in a humid atmosphere, cells were washed three times in wash buffer (PBS + 0.1% NaN₃ + 10% autologous serum) and analysed by flow cytometry.

Induction of apoptosis in Th1/Th2 purified cells

Lymphocytes from uraemic patients were treated with anti-Fas mAb (anti-human CD95; Becton Dickinson, San José, CA, USA) for 6 h in order to induce apoptosis. Thereafter, lymphocytes were cultured in the presence of BFA to preserve intracellular cytokine content (Th1 and Th2 cells). Th1 and Th2 subpopulation cells were then sorted (FACSVantage; Becton Dickinson, Mountain View, CA, USA) using anti-IL-4–PE or anti-IFN-γ–PE. In Th1 and Th2 isolated subsets, CD95 and Bcl-2 expression as well as apoptosis were determined. CD95 and Bcl-2 were labelled with FITC and apoptosis was measured by annexin V binding.

Determination of Fas and Bcl-2

To identify mechanisms that regulate cell apoptosis, the expression of Fas and Bcl-2 were measured in T lymphocytes from 11 patients and seven control subjects. Fas and Bcl-2 expression were measured immediately after obtaining the T-cell pellets and before cell culture. To determine the expression of Fas/APO-1 (CD95) molecules on T helper lymphocytes, cells were stained with PerCP-mAb anti-CD4 and with anti-human CD95–PE (Becton Dickinson, San José, CA, USA). Bcl-2 is a proto-oncogen that offers cells a selective survival advantage by blocking apoptosis. For Bcl-2 staining, cells were fixed and permeabilized after incubation with mAbs for surface markers. This was followed by staining with anti-human Bcl-2–FITC or with an anti-isotype as negative intraexperimental control (Becton Dickinson, San José, CA, USA).

Proliferation assay

To determine whether cell proliferation occurred in specific subsets of cells (Th1 or Th2), cell proliferation was measured by flow cytometry in 11 CKD patients using a kit containing anti-PE–proliferating cell nuclear antigen (PCNA) antibody (BD Pharmingen, San Diego, CA, USA). After 48 h of culture, cells (10^5/ml) were then washed, fixed,
permeabilized and stained as described above. After permeabilization, an anti-PCNA antibody or the corresponding isotype control was added; simultaneously, anti-IL-4 or anti-IFN-γ conjugated with FITC was also added. After incubation, cells were washed and resuspended in 0.5 ml 1% formaldehyde and stored at 4°C for flow cytometry analysis.

**Statistical analyses**

Results are expressed as means±SD. Means from non-parametric data were compared using the two-sided Mann–Whitney U-test or Wilcoxon signed ranks test for unpaired or paired comparisons, respectively. Significance of the differences in age, peripheral blood neutrophils and lymphocytes between groups was evaluated by the Student’s t-test for unpaired data. Statistical significance was considered when the P-value was <0.05.

**Results**

**Leukocyte and differential cell count**

Leukocyte count was not significantly different in CKD patients as compared with healthy subjects (Table 3). The number of lymphocytes, polymorphonuclear leukocytes and monocytes were similar in patients and controls.

**Effects of uraemia on the Th1/Th2 lymphocyte profile**

The percentage of lymphocytes producing cytokines was significantly higher in uraemic patients than in healthy subjects (Figure 1). This suggests that in uraemia there is spontaneous activation of T lymphocytes. Following 24 h in culture, the intracellular content of IFN-γ and IL-2 were significantly greater in CKD patients than in controls (5.1 ± 2.1% vs 1.37 ± 1.2%, P < 0.001 and 7.4 ± 3.2% vs 2.1 ± 0.3%, P < 0.001, respectively). Furthermore, the percentage of T lymphocytes containing IL-4 and IL-10 was also higher in CKD patients than in controls (61.9 ± 14.8% vs 11.1 ± 2.9%, P < 0.001 and 32.1 ± 15.4% vs 2.1 ± 1.1%, P < 0.001, respectively). In addition, the Th1/Th2 ratio measured as the ratio IFN-γ/IL-4 was 0.13 in healthy subjects and 0.08 in uraemics. When the ratio was calculated as the ratio IFN-γ/IL-10, the difference was even more remarkable: 0.66 in healthy subjects and 0.16 in uraemic patients.

**Effects of uraemia on T-lymphocyte apoptosis**

In CKD patients the percentage of apoptotic T lymphocytes was significantly higher than controls (24.05 ± 3.7% vs 2.25 ± 1.2%; P < 0.001; Figure 2). The expression of Fas was higher in the T lymphocytes of CKD patients than in the control group; however, the difference was not statistically significant (27.5 ± 5.5% vs 22.6 ± 4.9%; Figure 3). Bcl-2 expression was significantly lower in T lymphocytes from CKD

**Table 3.** Leukocyte and differential cell count in CKD patients and healthy subjects

<table>
<thead>
<tr>
<th>Test</th>
<th>Healthy subjects</th>
<th>CKD patients</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes (cells/mm³)</td>
<td>7356 ± 1220</td>
<td>7121 ± 1098</td>
<td>0.9</td>
</tr>
<tr>
<td>Lymphocytes (cells/mm³)</td>
<td>2398 ± 429</td>
<td>2237 ± 443</td>
<td>0.7</td>
</tr>
<tr>
<td>Monocytes (cells/mm³)</td>
<td>432 ± 44</td>
<td>406 ± 39</td>
<td>0.9</td>
</tr>
<tr>
<td>Polymorphonuclear cells (cells/mm³)</td>
<td>4526 ± 870</td>
<td>4478 ± 910</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Values are given as means±SD.
patients than from controls (7.9 ± 2.2% vs 36.6 ± 8.4%; \( P < 0.001 \); Figure 4).

Apoptosis and proliferation of Th1 and Th2 lymphocytes

To investigate the effect of apoptosis as a cause of predominance of Th2 phenotype over Th1 in uraemia, we performed the TUNEL technique simultaneously with the intracellular staining of cytokines IFN-\( \gamma \) (Th1) or IL-4 (Th2). The results are expressed as the mean percentage of apoptosis in T lymphocytes containing IFN-\( \gamma \) (Th1) or IL-4 (Th2). \( * P < 0.001 \).

Expression of Fas and Bcl-2 in Th1 and Th2 cells

To identify mechanisms of increased Th1-lymphocyte apoptosis in uraemia, the expression of Fas and Bcl-2 were separately measured in both Th1 and Th2 cells. The results are presented in Table 4. Fas expression was significantly higher in Th1 than in Th2 lymphocytes (41.6 ± 5.6% vs 18.2 ± 3.7%; \( P < 0.001 \)). Furthermore, the expression of Bcl-2 was significantly lower in Th1 than in Th2 cells (8.5 ± 2.1% vs 34.8 ± 4.3%; \( P < 0.001 \)).

Discussion

The present study shows that in CKD patients there is a decrease in the proportion of lymphocytes Th1 in relation to Th2. It is also shown that Th1 lymphocytes from CKD patients are highly susceptible to undergo apoptosis and this is associated with a low expression of the anti-apoptotic Bcl-2 gene. Consequently, the Th2 immune response prevails over the Th1 response in CKD patients. These findings are caused by uraemia, independently of exposure to haemodialysis membrane.

Our results show that in uraemic patients a high percentage of lymphocytes are activated, as indicated by the high percentage of lymphocytes that spontaneously express both Th1 and Th2 cytokines (Figure 1).
Other studies in CKD patients have reported an exacerbated immune activation, including increased expression of lymphocytes activation markers such as CD69 antigen [10]. We have measured the intracellular cytokine profile of lymphocytes to assess Th1/Th2 functional properties of lymphocytes. Measurement of lymphocytes containing cytokines has been used previously in studies of viral infections or autoimmune diseases and has been demonstrated to be an efficient method to detect Th1/Th2 differences [17, 18]. In our uraemic patients, the proportion of Th2 lymphocytes (containing IL-4 and IL-10) is higher than that of Th1 lymphocytes (IFN-γ and IL-2). Our results are in agreement with previous reports showing that CKD patients mainly exhibit a Th2 response [7–9]; however, other authors have found a predominant Th1 response in CKD patients [5, 6]. It is possible that the non-uniform results from different reports are due to the fact that many of these studies have been performed in CKD patients undergoing haemodialysis therapy and, in some cases, using haemodialysis membranes with low biocompatibility. To avoid the influence of dialysis membrane and dialysate, we have performed our study in patients with advanced CKD who had not yet started dialysis therapy. Thus, the increase in Th1 and also Th2, found in uraemics as compared with controls, is likely the result of lymphocyte activation induced by uraemia, which was also previously suggested by others [5, 6].

In addition to lymphocyte activation we and others have observed that in CKD there is an increase in mononuclear cell apoptosis [2, 10–13]. However, these studies were not designed to evaluate a possible different rate of apoptosis in Th1 vs Th2 lymphocytes. In the present study, we have hypothesized that an increased rate of apoptosis in Th1 cells may explain the shift in Th1/Th2 observed in our CKD patients. We have observed that total lymphocyte apoptosis was largely increased in CKD patients as compared with controls after 48 h of culture; according to our results, this increase in total lymphocyte apoptosis is related to a decrease in Bcl-2 expression (Figure 4). Additional experiments by TUNEL demonstrated that, in our CKD patients, the rate of apoptosis was greater in Th1 than in Th2.

Furthermore, in Th1 lymphocytes from CKD patients the expression of Fas was significantly higher and the expression of Bcl-2 was significantly lower than in Th2 cells. This suggests that the increase in Th1 apoptosis is mediated by the Fas/Fas-ligand pathway as well as a decrease in Bcl-2 expression. To investigate whether uraemia may cause an alteration of the Fas apoptotic signal pathway in Th1 and Th2 cells, lymphocytes from CKD patients were cultured with anti-Fas antibodies. We observed that the percentage of apoptosis in the cells that expressed Fas was similar in the Th1 and Th2 cells. This indicates that both types of cells are equally susceptible to undergo apoptosis and the high rate of apoptosis of Th1 cells may be explained by the increased percentage of cells expressing Fas. In healthy subjects IFN-γ is required for activation-induced death of T lymphocytes and Th1 cells are more sensitive to this apoptotic pathway than Th2 [19]. The apoptosis induced by IFN-γ is not related to an increase in expression of Fas, so the action of IFN-γ may be ‘down-stream’ to formation of the death-inducing signal complex, possibly through caspase expression. Although IFN-γ may play an important role in apoptosis in uraemia, in our study, Th1 cells clearly display an increased expression of Fas and a decreased expression of Bcl-2 as compared with Th2 cells, mechanisms independent of IFN-γ action.

Although the increase in Th1 apoptosis explains the predominance of Th2 in CKD patients, it is important to evaluate the rate of Th2 proliferation in relation to that of Th1 cells. For this purpose, the proliferation rate was measured by PCNA staining. The results showed that Th1 cells from CKD patients exhibit a lower proliferation rate than controls; however, the proliferation of Th2 is similar in CKD and controls. Our patients do not have lymphopenia. We showed that apoptosis of Th1 in uraemics is 21% greater and proliferation is 2% smaller than in controls. Considering that the percentage of Th1-positive cells in CD4 lymphocytes is low, the final percentage of apoptosis may not represent a sufficient number of lymphocytes to produce lymphopenia. In fact, we have observed that the difference in the average number of lymphocytes between controls and uraemic patients is 7% (which does not reach statistical significance). The mechanisms underlying these abnormalities are not known, but there are data in the literature providing evidence for a role of uraemic toxins (polyamines, aminoguanidine and glucose-modified proteins) in polymorphonuclear cell apoptosis [20]. Thus, it is possible that some uraemic toxins may act directly on Th1 lymphocytes, inducing activation and also inhibition of cell proliferation. Once they have been activated, Th1 die by apoptosis rather than proliferating, as usually occurs.

In summary, our results suggest the following.

(a) Uraemia causes an increase in T-lymphocyte apoptosis, an effect that is related to a decrease in the expression of Bcl-2;

(b) In uraemia there is a predominance of Th2 over Th1 lymphocytes (due to an increase in Th1 apoptosis and also a decrease in Th1 proliferation rate);

(c) The predominance of anti-inflammatory Th2 lymphocytes and the deficit in Th1 cells may be, in part, responsible for the alteration in cellular immunity observed in uraemic patients.

In conclusion, our results seem to indicate that apoptosis of Th1 lymphocytes induced by uraemia may participate in the imbalance of the immune response characteristic of uraemic patients. Further studies addressed to identify the role of the different uraemic toxins in the activation and apoptosis of Th1 cells would be most interesting. This might contribute to improve the immune response of uraemic patients.
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Conflict of interest statement. None declared.

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