Functional and immunological studies in children with chronic renal failure: the effects of uremia and dialysis treatment

Bouts, A.H.M.

Citation for published version (APA):
Chapter 9

Characteristics of peripheral and peritoneal white blood cells in children with chronic renal failure, dialyzed or not.

Antonia HM Bouts\textsuperscript{1,2}, Theo A Out\textsuperscript{2,3}, Cornelis H Schröder\textsuperscript{4}, Leo AH Monnens\textsuperscript{5}, Jeroen Nauta\textsuperscript{6}, Raymond T Krediet\textsuperscript{7}, Jean-Claude Davin\textsuperscript{1}.

\textsuperscript{1} Emma Children's Hospital, \textsuperscript{2} Clinical and Laboratory Immunology Unit, \textsuperscript{3} CLB Sanquin Blood Supply Foundation, Amsterdam, \textsuperscript{4} Wilhelmina Children Hospital, Utrecht, \textsuperscript{5} Department of Pediatrics, St. Radboud University Hospital, Nijmegen, \textsuperscript{6} Sophia Children Hospital, Rotterdam, \textsuperscript{7} Department of Nephrology, Academic Medical Center, Amsterdam, the Netherlands.

ABSTRACT

Objective: To explore further the mechanisms leading to immune deficiency in chronic renal failure and the role of dialysis treatment in these mechanisms.

Patients: We studied 39 children treated with peritoneal dialysis (PD), 23 children treated with hemodialysis (HD), 33 children not yet dialyzed (chronic renal failure [CRF]), and 27 healthy children. Peritoneal cells were also obtained from PD children for analysis.

Methods: White blood cells (WBCs) were isolated from blood and peritoneal dialysis effluent by centrifugation. The number of CD2⁺, CD4⁺ and CD8⁺ T cells, B cells and of natural killer cells were measured by flow cytometry.

Results: The total peripheral blood lymphocyte count was lower in PD children (2.6 x10⁹/L), HD children (2.1 x10⁹/L) and CRF children (2.0 x10⁹/L) compared with healthy children (3.1 x10⁹/L, p<0.05). The B lymphocyte count was also lower in PD children (0.34 x10⁹/L), HD children (0.22 x10⁹/L) and CRF children (0.33 x10⁹/L) children compared with healthy children (0.52 x10⁹/L, p<0.01). Numbers of CD4⁺ T cells were not different, but numbers of CD8⁺ T cells were lower in PD children (0.56 x10⁹/L), HD children (0.63 x10⁹/L) and CRF children (0.53 x10⁹/L) compared with healthy children (0.77 x10⁹/L, p<0.05). The count of natural killer cells was lower in PD children (0.21 x10⁹/L), HD children (0.17 x10⁹/L) and CRF children (0.18 x10⁹/L) compared with healthy children (0.50 x10⁹/L, p<0.0001). The CD4/CD8 ratio of lymphocytes in peritoneal effluent was 0.8 versus 1.9 in peripheral blood (p<0.001). The CD2/CD19 ratio was not different. The cell subsets remained stable during the first year of PD treatment. CD2/CD19 ratio in peritoneal effluent was higher in children with a peritonitis incidence 1 per year.

Conclusion: The reduced number of B lymphocytes, CD8⁺ T cells and natural killer cells found in CRF children, dialyzed or not, may favour the frequent occurrence of infections.
INTRODUCTION

In uremia, the immune system shows signs of activation coexisting with signs of deficiency, such as an increased expression of interleukin-2 (IL-2) receptor on T cells combined with a decreased T-cell proliferative response to mitogens [1]. Lymphopenia may occur in adult patients on dialysis, but their percentages of B cells and T cells are normal [2-5]. Furthermore, most studies report a normal ratio of T-helper (CD4) and T-suppressor (CD8) cells [3,6-8]. Results concerning the percentage or number of natural killer (NK) cells in uremic patients conflict [3,6,7]. Compared with the percentages found in peripheral blood lymphocytes, an increased percentage of peritoneal B lymphocytes and a decreased percentage of peritoneal T cells have been reported in adult PD patients [6,9,10].

Remarkably little is known about the immune system in children with chronic renal failure, on dialysis or not. No data have been published on the incidence of infections in these patient groups, other than an observation that the peritonitis rate is higher in PD children as compared with adults [11]. Infections are a major cause of morbidity and mortality in patients with chronic renal failure (CRF). The uremic state, the dialysis procedure, and malnutrition all contribute to this situation [11-14]. Hypogammaglobulinemia, or a selective immunoglobulin G (IgG) subclass deficiency, has been demonstrated by several authors in children on PD [15-19]. The mechanism behind this observation is not yet understood.

No studies are available on white blood cell subpopulations in children with chronic renal failure before initiation of dialysis treatment. Two cross-sectional studies have been published on lymphocytes in children treated with PD [20,21]. Hisano et al. [20] described a normal percentage of CD4, CD8 and NK cells in the peripheral blood of children treated with continuous ambulatory peritoneal dialysis (CAPD). Valle et al. [21] reported a CD4/CD8 ratio of peritoneal lymphocytes that was similar to the ratio in peripheral blood.

The aim of the present study was to analyze white blood cell subpopulations and lymphocyte subsets in children with CRF and to note the influence of dialysis treatment on these subpopulations and subsets. The study used a cross-sectional design to determine the lymphocyte subsets and the differentiation between white blood cells in children with CRF who either were not yet dialyzed or who were being treated with PD or HD, and in a control group of healthy children. Peritoneal cells from PD children were also analyzed. In addition, a longitudinal study on the time course of white blood cells in blood and peritoneal effluent was performed in 15 children treated with PD, who were followed for one year starting with the commencement of PD.

PATIENTS AND METHODS

We studied 39 children treated with PD, 23 with HD and 33 children with CRF not yet being dialyzed. The glomerular filtration rate (GFR) in the latter group was estimated by the Schwartz formula [22]:

\[
GFR = 40 \times \frac{\text{height}}{\text{plasma creatinine}}
\]
where height is the patient's height in centimeters and plasma creatinine is expressed in micromoles per liter.

The median GFR was 22 mL/min/1.73 m² (range: 6-50 mL/min/1.73 m²). Among the CRF children 45% (15/33) had moderate renal insufficiency (25-50 mL/min/1.73 m²), 30% (10/33) had severe renal insufficiency (10-25 mL/min/1.73 m²) and 24% (8/33) had pre-terminal renal failure (< 10 mL/min/1.73 m²). Healthy controls consisted of 27 children admitted for minor surgical procedures such as circumcision and inguinal hernia. Table 1 gives the median and range for age, duration of PD or HD treatment, and peritonitis incidence for the dialyzed children. Table 1 also shows the primary renal diseases of these patients. We followed 15 children for 12 months starting from before commencement of PD.

Table 1. Patient characteristics (median and range) and primary renal disease.

<table>
<thead>
<tr>
<th></th>
<th>PD</th>
<th>HD</th>
<th>CRF</th>
<th>HC</th>
</tr>
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<tbody>
<tr>
<td>Number</td>
<td>39</td>
<td>23</td>
<td>33</td>
<td>27</td>
</tr>
<tr>
<td>Age (years)</td>
<td>9.9 (1.7-18.3)</td>
<td>13 (1.7-19.2)</td>
<td>8.8 (0.5-19.9)</td>
<td>8.3 (2.2-17.5)</td>
</tr>
<tr>
<td>Duration of dialysis (years)</td>
<td>1.4 (0.2-8.9)</td>
<td>2.2 (0.4-7.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peritonitis incidence (episodes per year)</td>
<td>0.8 (0-3.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFR (ml/min/1.73 m²)</td>
<td></td>
<td></td>
<td>22 (6-50)</td>
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</table>

Primary renal disease

<table>
<thead>
<tr>
<th></th>
<th>PD</th>
<th>HD</th>
<th>CRF</th>
<th>HC</th>
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</thead>
<tbody>
<tr>
<td>Urologic malformation</td>
<td>11</td>
<td>7</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Glomerulopathy</td>
<td>12</td>
<td>3</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>HUS</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Metabolic disease</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Congenital disease</td>
<td>6</td>
<td>4</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Other diseases</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td></td>
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</tbody>
</table>


A peripheral white blood cell count and differentiation was performed in all patients on a H3-Technicon Counter (Technicon Instruments, Tarrytown, NY). White blood cells (WBC) were isolated from EDTA-blood by centrifugation (500 g, 10 min) after cell fixation with paraformaldehyde 1% (PFA). Erythrocytes were lysed with ammonium chloride (0.155 mol/L) and potassium-EDTA (0.5 mmol/L) and WBC were subsequently washed with PBAP (phosphate-buffered saline solution supplemented with 0.5% wt/vol bovine serum albumin,
0.01% wt/vol sodium azide and 0.5 mmol/L potassium-EDTA). Peritoneal cells from the peritoneal dialysis effluent of a 4 hour dwell using 1.36% glucose solution (Dianeal®, Baxter BV, Utrecht, The Netherlands) were isolated by centrifugation (500 g, 10 min). This was followed by fixation with 4% PFA. Cytospins were prepared for total cell count and differentiation. WBC and peritoneal cells were incubated with saturating amounts of FITC or PE labeled CD2, CD4, CD8, CD19 and NK monoclonal antibodies (Beckton Dickinson (BD) Immunocytometry Systems, San Jose, California, USA) for 30 minutes on ice in the dark. After incubation the cells were washed with PBAP and again fixated with PFA 1%. Flow cytometry analysis was performed within 12 hours thereafter with the FACSscan (BD). Lymphopenia or subset deficiency was defined as a value <5th percentile of normal values according to age obtained from Coomans-Bitter et al. [23]. Absolute counts of lymphocyte subsets were calculated by the percentage subset - positive lymphocytes obtained from the flow cytometry multiplied by the absolute lymphocyte count.

The study was approved by the Medical Ethical Review Committee of the hospital and written informed consent was obtained from children and/or parents.

**Statistical analysis**

The results are expressed as medians (range). Differences between the groups were tested with Kruskall-Wallis one-way analysis of variance. In case of significance, the Mann-Whitney-U test was performed. Longitudinal data were analyzed with a Friedman trend analysis and differences between two time points with the paired Wilcoxon test. The Fisher exact test was performed to compare frequencies between groups. Two sided p-values < 0.05 were considered significant.

### Table 2. Absolute counts of white blood cells.

<table>
<thead>
<tr>
<th></th>
<th>PD</th>
<th>HD</th>
<th>CRF</th>
<th>HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>39</td>
<td>23</td>
<td>33</td>
<td>27</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>6.9 (3.4-22.2)</td>
<td>7.3 (3.7-18.2)</td>
<td>6.4 (3.5-14.7)</td>
<td>7.7 (4.0-18.0)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>2.6b (1.2-8.2)</td>
<td>2.1b (0.6-5.7)</td>
<td>2.0c (0.9-6.9)</td>
<td>3.1 (1.4-6.5)</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.37b (0.14-0.96)</td>
<td>0.50 (0.14-1.10)</td>
<td>0.36b (0.08-1.25)</td>
<td>0.53 (0.20-1.10)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>3.5 (0.7-13.7)</td>
<td>3.8 (1.8-14.1)</td>
<td>2.8 (1.2-9.4)</td>
<td>3.3 (0.9-13.8)</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.36 (0.03-9.1)</td>
<td>0.27 (0.1-2.1)</td>
<td>0.28 (0.08-1.7)</td>
<td>0.35 (0.06-1.9)</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.03b (0-0.16)</td>
<td>0.01c (0-0.10)</td>
<td>0.03b (0-0.23)</td>
<td>0.06 (0-0.13)</td>
</tr>
</tbody>
</table>

Absolute count expressed as x 10³/L. *p <0.05, *p < 0.01, **p<0.001 when compared with HC.

**RESULTS**

**Cross-sectional analysis in peripheral blood**

The medians (ranges) of white blood cells are listed in Table 2. Peripheral blood lymphocytes and subset numbers are listed in Table 3. The analysis of peritoneal cells in
Peritoneal dialysis effluent is given in Table 4. No significant differences for age (p=0.07) and leukocyte counts (p=0.22) were found among the groups. Lymphopenia was found in only one PD child, one HD and 2 CRF children and one healthy child. The numbers of total lymphocytes in PD (p=0.02), HD (p=0.002) and CRF (p=0.0005) children were lower as compared with HC. The number of neutrophils was not different among the groups. Monocyte count was lower in PD (p=0.007) and CRF (p=0.02) children compared to HC whereas no significant difference was found between the PD, HD and CRF groups. The number of basophils was higher in HC (p<0.01) compared to PD, HD and CRF children.

Table 3. Median (range) of absolute numbers of lymphocytes and subsets in peripheral white blood cells.

<table>
<thead>
<tr>
<th></th>
<th>PD</th>
<th>HD</th>
<th>CRF</th>
<th>HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>39</td>
<td>23</td>
<td>33</td>
<td>27</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>2.6(^{a}) (0.14-8.2)</td>
<td>2.1(^{b}) (0.6-5.7)</td>
<td>2.0(^{c}) (0.9-6.9)</td>
<td>3.1 (1.4-6.5)</td>
</tr>
<tr>
<td>CD2</td>
<td>1.9(^{a}) (0.93-3.62)</td>
<td>1.69(^{b}) (0.36-3.36)</td>
<td>1.57(^{c}) (0.66-4.36)</td>
<td>2.32 (1.08-5.09)</td>
</tr>
<tr>
<td>CD4</td>
<td>1.22 (0.56-2.97)</td>
<td>0.91 (0.18-1.82)</td>
<td>0.88(^{c}) (0.33-2.66)</td>
<td>1.01 (0.51-2.09)</td>
</tr>
<tr>
<td>CD8</td>
<td>0.6(^{a}) (0.23-1.14)</td>
<td>0.63(^{b}) (0.1-1.91)</td>
<td>0.53(^{c}) (0.24-1.62)</td>
<td>0.77 (0.34-2.15)</td>
</tr>
<tr>
<td>NK</td>
<td>0.21(^{a}) (0.05-0.78)</td>
<td>0.17(^{b}) (0.05-0.77)</td>
<td>0.18(^{c}) (0.01-1.21)</td>
<td>0.5 (0.12-1.37)</td>
</tr>
<tr>
<td>CD19</td>
<td>0.34(^{a}) (0.03-1.63)</td>
<td>0.22(^{b}) (0.04-1.82)</td>
<td>0.33(^{c}) (0.06-1.48)</td>
<td>0.52 (0.25-1.79)</td>
</tr>
<tr>
<td>CD4/CD8</td>
<td>1.9(^{a}) (0.91-5.9)</td>
<td>1.5(^{b}) (0.54-3.39)</td>
<td>1.6(^{c}) (0.6-2.61)</td>
<td>1.3 (0.66-2.39)</td>
</tr>
</tbody>
</table>

PD, peritoneal dialysis; HD, hemodialysis; CRF, chronic renal failure; HC, healthy controls. Absolute numbers expressed as x 10\(^{9}\)L. \(^{a}\) p<0.05, \(^{b}\) p<0.01, \(^{c}\) p<0.001 when compared with HC. PD vs CRF: p=0.02, \(^{a}\)PD vs CRF: p=0.03, \(^{b}\)PD vs HC: p=0.04, \(^{c}\)PD vs HD p=0.02.

A deficiency of B-cells (CD19\(^{+}\) lymphocytes) was observed in 4 PD, 3 HD and 5 CRF children compared to no HC child (ns) (Figure 1). The median B-lymphocyte counts were lower in PD (p=0.004), HD (p<0.0001) and CRF (p=0.0007) children than in HC. HD children had lower B-lymphocytes counts than PD children (p=0.04). A CD4\(^{+}\) T-cell deficiency was observed in 0 PD, 1 HD and 1 CRF child compared to no HC child (ns). The median count of CD4\(^{+}\) T-cells was lower in CRF compared to PD children. A CD8\(^{+}\) T-cell deficiency was observed in 2 PD, 1 HD and 1 CRF child compared to 0 HC child (ns). The median CD8\(^{+}\) T cell count was lower in PD (p=0.0008), HD (p=0.02) and CRF (p=0.0003) children compared to HC. The CD4/CD8 ratio was higher in PD (p=0.0002) HD (p=0.05) and CRF (p=0.004) children compared to HC (Table 3). An NK cell deficiency was found in 6 PD (p=0.03), 2 HD (ns) and 5 CRF (ns) children compared to no HC child (Figure 2). Furthermore, we found lower numbers of NK cells in the PD, HD and CRF group compared to HC children (p<0.0001).
Figure 1. The absolute number of CD19+ B cells in peripheral blood of children with chronic renal failure treated with peritoneal dialysis (PD), hemodialysis (HD) or not dialyzed (CRF) and healthy controls (HC). (---) 5th and 95th percentile of normal values according to age.

Figure 2. The absolute number of NK cells in peripheral blood of children with chronic renal failure treated with peritoneal dialysis (PD), hemodialysis (HD) or not dialyzed (CRF) and healthy controls (HC). (---) 5th and 95th percentile of normal values according to age.
Table 4. Peritoneal cells in dialysis effluent (Median and range).

<table>
<thead>
<tr>
<th></th>
<th>Percentage</th>
<th>Absolute number per exchange</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell number</td>
<td></td>
<td>6.0x10^5 (0.6 - 68 x10^5)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>5.8 (0-19)</td>
<td>0.3x10^5 (0.03 - 8.8 x10^5)</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>4 (0-68)</td>
<td>0.21x10^5 (0.0 - 23 x10^5)</td>
</tr>
<tr>
<td>Macrophages</td>
<td>65 (17-92)</td>
<td>3.7x10^5 (0.17 - 45 x10^5)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>13 (5-42)</td>
<td>1.0 x10^5 (0.52 - 8.4 x10^5)</td>
</tr>
<tr>
<td>Mesothelial cells</td>
<td>6 (0.9-19.7)</td>
<td>0.24x10^5 (0.03 - 2.8 x10^5)</td>
</tr>
<tr>
<td>CD4/CD8</td>
<td>0.8 (0.13-5.6)</td>
<td>1.00 x10^5 (0.52 - 8.4 x10^5)</td>
</tr>
<tr>
<td>CD2/CD19</td>
<td>4.9 (0.6-29)</td>
<td>0.24x10^5 (0.03 - 2.8 x10^5)</td>
</tr>
</tbody>
</table>

Study in PD children

The number of peritoneal cells in the dialysis effluent from 31 PD patients was 6 x 10^5 per exchange (range 0.6 - 68 x 10^5). The median peritoneal cell population consisted of 6% neutrophils, 4% eosinophils, 65% macrophages, 13% lymphocytes and 6% mesothelial cells (Table 4). The peritoneal CD4/CD8 ratio was 0.8 compared to 1.9 in peripheral blood (p<0.001). The CD2/CD19 ratio of peritoneal lymphocytes was 4.6 compared to 4.7 in peripheral blood (ns) (Figure 3).

Figure 3. The CD2/CD19 and CD4/CD8 ratio of peripheral blood lymphocytes (PBL) in PD children compared to peritoneal lymphocytes (PL).

Peritonitis: No differences were found in the white blood cell differentiation and lymphocyte subsets of children with a peritonitis incidence of one or more episode(s) per year compared to less than one. In the peritoneal dialysis effluent the lymphocyte CD2/CD19 ratio was higher in the group with a higher peritonitis incidence (Figure 4). The CD4/CD8 ratio was not different between the two groups.
Figure 4. The CD4/CD8 and CD2/CD19 ratio of peritoneal lymphocytes (PL) in children with a high peritonitis incidence (PI ≥ 1 episode per year) and a low peritonitis incidence (PI < 1 episode per year).

Longitudinal: No alteration of the peripheral blood lymphocyte number was found during the first year of PD treatment. This was also found for the various subsets (Figure 5, only CD2/CD19 and CD4/CD8 ratios shown). The contribution of lymphocytes to the peritoneal cell population in the dialysis effluent showed no significant change during the first year of dialysis treatment, although a trend of a slight increase with the duration of PD treatment was observed (data not shown). The CD2/CD19 and CD4/CD8 ratios of peritoneal lymphocytes did not change significantly during the first year of PD treatment (Figure 5).

Figure 5. Follow-up of the CD4/CD8 and CD2/CD19 ratio of peripheral blood lymphocytes (PBL) and peritoneal lymphocytes (PL) during the first year of PD treatment.
DISCUSSION

Peripheral blood lymphocyte counts were lower in children with chronic renal failure, treated with dialysis or not, than in healthy children, although an absolute lymphopenia did not occur more frequently than in the control group. Low numbers were especially found for CD8\(^+\) T-cells, NK cells and B-lymphocytes. Also the monocyte number was lower in the patient groups with exception of HD children.

The definition of lymphopenia in the literature is not always the same. Some define a count of less than \(1.5 \times 10^9/\text{L}\) as lymphopenia [24] and others when a significantly lower level is present, compared to healthy controls [3,9,20,25,26]. The 5th percentile, used as lower limit, in healthy children older than 5 years is \(1 \times 10^9/\text{L}\) [23]. No excess of lymphopenia was found in our population of children with CRF, dialyzed or not. However, non-dialyzed children with CRF had the lowest total lymphocyte counts. Hisano et al. compared CAPD children with HC and found equal numbers of lymphocytes in the two groups [20]. We did not find a significant difference in lymphocyte count between the HD and PD children. Other studies on this in children are not available, but our results are in accordance with those in adult patients [12,17,27].

The B-cell count was lower in PD, HD and CRF children whereas no significant differences were found in the prevalence of B-cell deficiencies among all groups. Furthermore HD children had a lower B-cell count than PD children. In adult CAPD patients mostly normal B-cell numbers have been found [7,9,28,29]. However the results were expressed as proportions of the total lymphocyte count in stead of absolute numbers. One study in HD patients demonstrated a reduction of the percentage B-lymphocytes [3]. Braun et al. described a decrease of the proportion B-lymphocytes during the hemodialysis session that normalized thereafter [30]. This suggests that the alterations caused by the hemodialysis procedure do not persist in the interdialytic interval. The HD children in our study demonstrated a reduced proportion of B-cells already before the hemodialysis session had started. We do not know whether the reduction of peripheral blood B-cells in our patients resulted in decreased Ig production. Previous results from our group have demonstrated that the median serum IgG level was lowest in PD and CRF children [19] whereas in the present study the lowest B-lymphocyte count was found in HD children. In healthy children the number of B-lymphocytes decreases with advancing age whereas the serum Ig levels increase. No difference exists between the spontaneous IgG secretion by lymphocytes from children or adults [31]. Consequently no positive relationship is likely between total B-cell number and serum Ig level nor Ig production in a physiological steady state. It is not known, whether B-cell subsets in children with CRF, reflecting their maturity, might give more information about the capacity of Ig production.

The total number of T-cells was lower in HD and CRF patients whereas for PD children this was not significant \((p=0.1)\). CRF children had the lowest count. An absolute T-cell deficiency did not occur more frequently in uremic children. The T-helper (CD4\(^+\)) cell counts in PD, HD and CRF children were not different compared to HC whereas the cytotoxic T-cell counts (CD8\(^+\)) were reduced. This resulted in a higher CD4/CD8 ratio in...
uremic children which is different from the results obtained by Hisano et al. who described a normal CD4/CD8 ratio in CAPD children [20]. A normal ratio has been described in the majority of studies performed in adult PD and HD patients [3,4,8,25,26,32,33]. Some showed a decreased ratio [34-36]. Only Collart et al. found an increased CD4/CD8 ratio in adult PD and HD patients which is in accordance with our results in children [37]. T-helper cells are necessary for the cell-mediated immune response and for B-cells to differentiate into antibody producing cells. Cytotoxic T-cells are important in the host defense against viral infections. This might implicate that children with chronic renal failure would be more susceptible for such infections. A high susceptibility to influenza virus or a more severe clinical course has been reported in adult uremic patients [32,38,39]. PD children had a higher CD4 count than CRF children. It is speculative whether the PD procedure itself would be responsible for this.

We also found reduced counts of NK cells in PD, HD and CRF children compared to their healthy controls. The prevalence of a NK deficiency in the PD group (20%) was significantly higher compared to HC. This was not found for the HD and CRF groups. NK cells are important in the immune response against viral infections and possibly against malignancies [40]. Furthermore NK cells can kill antibody-coated bacteria [40]. An increased incidence of malignancies in adult PD or HD patients before renal transplantation has been described [41,42]. In children this has only been demonstrated after renal transplantation [43]. For all these lymphocyte subsets it is not clear whether a lower but not deficient count results in a clinical defective immune response. The children with a deficiency of one or more lymphocyte subsets did not have a higher peritonitis incidence. However, the number of PD children with a lymphocyte subset deficiency in our study was low.

The total peritoneal cell count in the dialysis effluent from 31 PD children was lower compared to that reported by Betjes et al. in adult patients, probably because of the longer dwell time used in the latter study [44]. The mean total cell count of normal human peritoneal cells is 2.5 x 10^6 [45]. It consists mostly of lymphocytes (mean 50%) and to a lesser degree of macrophages (mean 32%) than we found in PD children [45]. The lower number of monocytes in PD children might be explained by a higher migration to the peritoneal cavity. It is speculative whether the uremic state influences the maturation and release of cells from the bone marrow reflecting the lower monocytes counts in CRF children. The CD4/CD8 ratio of peritoneal lymphocytes was lower compared to that in peripheral blood lymphocytes. This is in agreement with results from others obtained in adult patients [29,45-48]. On the contrary Valle et al. showed a similar CD4/CD8 ratio of peritoneal and blood lymphocytes in CAPD children [21]. The CD4/CD8 ratio of peritoneal lymphocytes in adult CAPD patients was higher compared to that in healthy controls undergoing laparoscopy suggesting an activated peritoneal T-cell population in CAPD patients [45,46]. The CD2/CD19 ratio (T/B-cells) in PDE was not different from blood. This is in contrast to the situation in adults where a decrease of peritoneal T cells combined with an increase of peritoneal B-cells has been described [7,9,48].
Children with a peritonitis incidence of one or more episodes per year had a higher peritoneal T/B lymphocyte ratio whereas no significant differences were found in WBC differentiation and the separate lymphocyte subsets both in blood and the PDE. Most authors did not find a relationship between peritonitis and lymphocyte subsets [9,49]. However, Giacchino et al. described a reduced peritoneal IgA+ B-lymphocytes number in patients with a high peritonitis incidence [29].

During the first year of PD treatment we did not find a significant change of absolute numbers of peripheral blood and peritoneal lymphocytes and subsets. However, the percentage of peritoneal lymphocytes tended to increase probably because of a decrease in the total cell number. This is in agreement with findings of others [6,9,46,50]. Alternatively, significant changes could possibly not be established because of the relative low number of longitudinal patients.

We conclude that a reduced number of total lymphocytes, B-cells, cytotoxic T-cells, NK cells and monocytes in children with chronic renal failure before and after starting dialysis treatment is present. An absolute lymphocyte deficiency was observed in only a few patients. The PD children had a higher CD4+ T-cell count and HD children a lower CD19+ B-cell count for which the dialysis procedure might be responsible. Although we could not find a direct relationship between peritonitis incidence and lower lymphocyte subsets the disturbances in the balance of immune cells may contribute to the increased susceptibility to viral or bacterial infections and malignancies in these children.

ACKNOWLEDGMENTS

This study was supported by the Dutch Kidney Foundation (grant C95.1464). We thank the Department of Pediatric Surgery for providing blood samples from healthy children as a control.

REFERENCES


