Th1 and Th2 Cytokine mRNA Profiles in Childhood Nephrotic Syndrome: Evidence for Increased IL-13 mRNA Expression in Relapse

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Abstract. Idiopathic nephrotic syndrome of childhood is thought to be associated with T lymphocyte dysfunction often triggered by viral infections, with the production of circulating factor(s) resulting in proteinuria. In view of the conflicting evidence of T cell activation and Th1 or Th2 pattern of cytokine synthesis in this disease, this study examined the mRNA expression of interleukin-2 (IL-2), interferon-γ, IL-4, and IL-13 from CD4+ and CD8+ T cells in steroid-responsive nephrotic patients in relapse and remission. Fifty-five children with steroid-responsive nephrotic syndrome were included in this study, together with 34 normal controls and 24 patient controls with viral infections. RNA was isolated from purified CD4+ or CD8+ cells from peripheral blood and subjected to reverse transcription-PCR. Cytokine mRNA expression was measured semiquantitatively, and a cytokine index was derived from densitometric readings, with cyclophilin as the housekeeping gene. Both cross-sectional and paired data showed an increased CD4+ and CD8+ IL-13 mRNA expression in patients with nephrotic relapse as compared to remission, normal, and patient controls (P < 0.008). This was also associated with increased cytoplasmic IL-13 expression in phorbol myristate acetate/ionomycin-activated CD3+ cells (6.66 ± 3.39%) from patients with nephrotic relapse compared to remission (2.59 ± 1.35%) (P < 0.0001). However, there was no significant difference in CD4+ or CD8+ IL-2, interferon-γ and IL-4 mRNA expression. IL-13 is an important T cell cytokine with anti-inflammatory and immunomodulatory functions on B cells and monocytes. It is conceivable that IL-13 may act on monocytes to produce vascular permeability factor(s) involved in the pathogenesis of proteinuria in patients with relapse nephrotic syndrome.

Our current understanding of the pathogenesis of idiopathic nephrotic syndrome (INS) of childhood is that it is probably the result of a primary immune disturbance. Various studies have attempted to identify lymphocyte subset abnormalities in INS (1), but results have been conflicting. Increased T suppressor cell activity has been described in some children with INS (2–4), which returned to normal after steroid treatment (5). Moreover, an increase in Tγ cells, which are associated with suppressor activity, has been reported in one study (6). On the other hand, a decrease in both Tγ and Tτ cells was described in another study (7).

Despite all this, there is strong evidence that proteinuria, which is the hallmark of this condition, is mediated by cytokines (8). Relapses are often triggered by viral infections, which possibly result in the release of cytokines, causing immunoregulatory imbalances. Evidence for a possible cytokine-mediated role in the pathogenesis of the nephrotic syndrome include clinical response of the nephrotic state to immunomodulating drugs which affect cytokine production, such as steroids and cyclosporin A. Several studies have demonstrated increased in vitro mitogen-stimulated production of interleukin-2 (IL-2) (8,9), IL-4, and interferon-γ (IFN-γ) (10) by lymphocytes from children with steroid-responsive nephrotic syndrome. IL-2 receptor (IL-2R) expression on the surface of T lymphocytes was increased in these patients, rising early in relapse (11–13). Various soluble factors such as soluble immune response suppressor (14) and vascular permeability factor (VPF) (15,16) have also been demonstrated in the serum and supernatants of concanavalin A-stimulated peripheral blood mononuclear cells (PBMC) in children with INS. The PBMC supernatants from these patients were shown to increase proteinuria when injected into rats (17), and also increase the sulfation of isolated glomerular basement membrane (18), a process that leads to alteration in the charge selectivity of the glomerular basement membrane seen in minimal change disease. Recently, increased IL-8 mRNA expression by PBMC and raised IL-8 serum concentrations were found in nephrotic children (19). IL-8 was also shown in vitro to increase sulfation of the glomerular basement membrane.

We have previously shown that there is a strong association between frequent relapses and atopy with high IgE levels in
children with steroid-responsive nephrotic syndrome (SRNS) (20). Other studies have subsequently described the association between increased T cell IL-4 mRNA expression and type II IgE receptor expression on B cells, providing a possible link between T lymphocyte activation and atopy (21). However, Kimata and coworkers have shown that the spontaneous production of IgE and IgG4 by PBMC in nephrotic patients was due to T cell IL-13 production, rather than IL-4 production as seen in atopic individuals (22).

In view of the conflicting evidence of T cell activation and Th1 or Th2 pattern of cytokine synthesis in this disease, this study examined the mRNA expression of IL-2, IFN-γ, IL-4, and IL-13 from CD4+ and CD8+ T cells in steroid-responsive nephrotic patients in relapse and remission.

Materials and Methods

Patient Population

Fifty-five children (37 boys and 18 girls) with SRNS were included in a cross-sectional study, and examined in clinical relapse and/or remission. Their ages ranged from 2 to 17 yr (median 8 yr). Paired data for CD4+ cytokine gene expression was obtained in 16 children, i.e., they were studied both in clinical relapse and remission. Likewise, paired data for CD8+ cytokine gene expression was obtained in 10 children with SRNS. Relapse was defined as increased urinary protein excretion (Albustix® ≥2+ for at least 3 consecutive days or >40 mg/m^2 per h) and serum albumin ≤25 g/L. Remission was defined as albumin ≥35 g/L and normal urinary protein excretion (Albustix® trace or negative for at least 3 consecutive days or <5 mg/m^2 per h). Informed consent was obtained from the parents before study.

Heparinized blood was obtained from the study subjects. Children with SRNS were either not on any treatment during the time of blood sampling, or on prednisolone both during relapse and remission, as they were steroid-dependent. None of them was on angiotensin-converting enzyme inhibitors or nonsteroidal anti-inflammatory drugs, and none was previously given cyclosporin or cyclophosphamide, which might affect interpretation of the cytokine gene expression. In addition, 34 normal controls and 24 patient controls (children with SRNS) were either not on any treatment during the time of blood sampling, or on prednisolone both during relapse and remission, as seen in atopic individuals (22).

In a cross-sectional study, and examined in clinical relapse and/or remission, their ages ranged from 2 to 17 yr (median 8 yr). Paired data for CD4+ cytokine gene expression was obtained in 16 children, i.e., they were studied both in clinical relapse and remission. Likewise, paired data for CD8+ cytokine gene expression was obtained in 10 children with SRNS. Relapse was defined as increased urinary protein excretion (Albustix® ≥2+ for at least 3 consecutive days or >40 mg/m^2 per h) and serum albumin ≤25 g/L. Remission was defined as albumin ≥35 g/L and normal urinary protein excretion (Albustix® trace or negative for at least 3 consecutive days or <5 mg/m^2 per h). Informed consent was obtained from the parents before study.

Isolation of CD4+ and CD8+ Lymphocytes

PBMC were isolated using standard Ficoll-Hypaque density gradient centrifugation. The MiniMACS magnetic separation technique (Miltenyi Biotec, Sunnyvale, CA) was used to further purify CD4+ or CD8+ T lymphocytes. CD4+ or CD8+ cells were separated from PBMC using magnetized beads coated with monoclonal antihuman CD4 (Leu™-3a) or CD8 (Leu™-4a) antibodies, respectively. The purity of the positively selected CD4+ or CD8+ T cells was greater than 90% as determined by flow cytometry (FACScan, Becton Dickinson, Sunnyvale, CA).

Semiquantitative Reverse Transcription-PCR

Total RNA was isolated from CD4+ or CD8+ T lymphocytes using TRizol reagent (Life Technologies, Gaithersburg, MD), which is a modification of the standard guanidinium-phenol-chloroform extraction method (23). cDNA was then synthesized from 200 ng of RNA using 2.5 μM oligo-(dT)20 as primers, 3 mM MgCl2, 1 mM dNTP, 10 nM Tris-Cl buffer, pH 8.3, 50 mM KCl, 20 U RNase inhibitor, and 50 U Moloney murine leukemia virus-reverse transcriptase (Amersham Life Science, Cleveland, OH) (24). The mixture was incubated at 37°C for 1 h, followed by 99°C for 5 min, and then cooled rapidly to 4°C.

The PCR was performed in a final volume of 20 μl, containing 4 μl of cDNA, 2 to 3 mM MgCl2 (depending on the primers used), 10 mM Tris-Cl, 50 mM KCl, 0.2 μM specific cytokine primers, 0.2 mM dNTP, and Taq polymerase 0.5 U (Perkin-Elmer, Roche Molecular Systems, Branchburg, NJ). The primer sequences and the PCR conditions used for the analysis of the mRNA expression of IL-2, IFN-γ, IL-4, and IL-13 are listed in Table 1. A nested PCR was performed for IL-13. The primers were designed such that the resultant product would span several introns of the gene, effectively excluding amplification of contaminating genomic DNA. Cyclophilin, a housekeeping gene, was used as a standard for semiquantitative comparison with the cytokine amplification products. Amplification was performed in a Hybaid TouchDown™ Thermal Cycler (Hybaid, Ted-dington, Middlesex, United Kingdom), using the following conditions: 1 min denaturation at 94°C, followed by 25 to 45 cycles depending on the primers, consisting of 30 s at 94°C for denaturation, 45 s at 60°C for annealing, and a further 30 s at 72°C for elongation. Final extension was completed after 5 min at 72°C. Positive controls were obtained from pokeweed mitogen (PWM)-stimulated PBMC from a normal subject, where RNA was isolated at 24 h after stimulation. For each cytokine, this positive control was used to standardize for the day-to-day variability.

The total PCR product (20 μl) was analyzed by electrophoresis on a 6% polyacrylamide gel, followed by staining with 0.5 μg/ml ethidium bromide. Laser densitometry analysis was performed on a Bio-Rad GS-700 imaging densitometer (Bio-Rad Laboratories, Hercules, CA). Cytokine mRNA expression was measured semiquantitatively, based on a cytokine index derived from the densitometric readings: CI = [X(sample)/Y(sample)] / [X(standard)/Y(standard)], where X is cytokine and Y is cyclophilin, and standard is the PWM-stimulated positive control.

Restriction Endonuclease Analysis

The specificity of the PCR products was analyzed by restriction endonuclease digestion (Sigma Chemical Co., St. Louis, MO). The final PCR products were digested with the relevant restriction endonucleases (Table 2). Cleaved PCR products were visualized by electrophoresis on a 6% polyacrylamide gel, after staining with 0.5 μg/ml ethidium bromide. Specificity of the PCR products was confirmed by the fragment size obtained after restriction endonuclease digestion (Table 2).

Cytosplasmic Cytokine Assay by Flow Cytometry

We have attempted to measure the serum IL-13 levels using the enzyme immunoassay (EIA) kit (Quantikine R&D System, San Jose, CA). The EIA method, however, was not sensitive enough to detect any circulating IL-13 in the serum of nephrotic patients, despite a good standard curve. Therefore, to corroborate our reverse transcription (RT)-PCR results, cytosplasmic expression of IL-13 was measured using FastImmune™ intracellular staining system (Becton Dickinson Immunocytometry Systems, San Jose, CA). Briefly, PBMC collected in sodium heparin were activated with phorbol myristate acetate (PMA) at 25 ng/ml and ionomycin at 1 μg/ml for 4 h, in the presence of Brefeldin-A (Boehringer Mannheim) at 10 mg/ml to inhibit intra-
controls showed more than 90% positivity for CD69. Experiments, both the intracellular staining controls and activation similarly activated T cells (activation control) (25). In all of the staining control) was also compared with non-Brefeldin A-treated, plus ionomycin-activated Brefeldin A-treated T cells (intracellular isotype control). Cytoplasmic CD69 staining on PMA isotype control, US is the unstimulated sample, and UIC is the unstimulated isotype control. Peridinin chlorophyll protein-conjugated monoclonal antibody to IL-13 was added. The cells were subsequently lysed with FACS™ Lysing Solution (Becton Dickinson). After washing, FITC-conjugated intracellular monoclonal antibody to CD3 was added. The red blood cells were subsequently lysed with FACS™ Lysing Solution (Becton Dickinson). Results were analyzed using 2-color dot-plot gating on CD3+ cells using CELLQUEST software. The percentage of CD3+ cells positive for cytoplasmic IL-13 was calculated based on the formula: (AS − AIC) / AS, where AS is the activated sample, AIC is the activated isotype control, US is the unstimulated sample, and UIC is the unstimulated isotype control. Cytoplasmic CD69 staining on PMA plus ionomycin-activated Brefeldin A-treated T cells (intracellular staining control) was also compared with non-Brefeldin A-treated, similarly activated T cells (activation control) (25). In all of the experiments, both the intracellular staining controls and activation controls showed more than 90% positivity for CD69.

Statistical Analyses
Statistical analysis was performed using Statistical Package for the Social Sciences for Windows. The cytokine indices (CI) were expressed as mean values ± SD. The difference between the CI of the nephrotic patients in relapse and remission was compared with both the normal controls and patient controls using the Mann–Whitney rank sum test. Paired data for the nephrotic patients in relapse and remission were compared using the Wilcoxon signed-rank test.

Results

Standardization of PCR
Figure 1 shows the PCR amplification products of the Th1 and Th2 cytokine gene expression in CD4+ cells. The negative control consisted of appropriate buffer added to the RT-PCR reagent mix starting from the reverse transcription stage. Cyclophilin was used as the “housekeeping” gene to standardize the amount of cDNA obtained after RT-PCR. The number of amplification cycles chosen for cyclophilin was 30, as this had been shown to lie on the linear portion of a standard curve, before saturation of the PCR product (Figure 2). Similarly, the number of amplification cycles for IL-2, IFN-γ, IL-4, and IL-13 was determined in preliminary experiments, such that they had not reached saturation in terms of band density of the PCR product.

Cross-Sectional Data
The CI for mRNA expression in CD4+ and CD8+ cells are shown in Table 3. Because the amount of RNA isolated was variable depending on the number of CD4+ or CD8+ cells isolated from each patient, it was not possible to study all of the cytokines from a single sample in some of the patients, accounting for the variability in patient numbers for the cytokines studied. Both CD4+ and CD8+ cells from patients with SRNS in relapse had increased IL-13 mRNA expression compared with normal controls, patient controls with viral infections, and nephrotic children in remission (P < 0.008 and P < 0.005, respectively). There was no significant difference in IL-2, IFN-γ, and IL-4 mRNA expression comparing CD4+ and CD8+ cells for nephrotic patients in relapse and remission with normal and patient controls. The patient controls were children who were seen for viral infections, a common trigger for relapses of nephrotic syndrome.

Effect of Steroids
The patients were stratified into those who were steroid-dependent, and therefore on steroid treatment, and those who

Table 1. Primers and experimental conditions for PCR

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Primer Sequence</th>
<th>Product (bp)</th>
<th>Amplification Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophilin</td>
<td>5'-GTGACTTCACACGCCATAATGGC-3'</td>
<td>365</td>
<td>30</td>
</tr>
<tr>
<td>IL-2</td>
<td>5'-AGTGACAGATCCTCTTCTGGAATGG-3'</td>
<td>458</td>
<td>45</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>5'-GTCACTTCCATGCTAGCTGAGC-3'</td>
<td>332</td>
<td>35</td>
</tr>
<tr>
<td>IL-4</td>
<td>5'-CTTCCACGTCCTCTGCTTGG-3'</td>
<td>345</td>
<td>40</td>
</tr>
<tr>
<td>IL-13</td>
<td>5'-ATTGCTCTCTACTTGGGTCCTG-3' (outer)</td>
<td>170</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 2. Restriction endonuclease digestion of specific PCR products

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>PCR Product (bp)</th>
<th>Restriction Endonuclease</th>
<th>Fragment Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophilin</td>
<td>365</td>
<td>HaeIII</td>
<td>228, 137</td>
</tr>
<tr>
<td>IL-2</td>
<td>458</td>
<td>HaeIII</td>
<td>208, 250</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>332</td>
<td>TaqI</td>
<td>286, 46</td>
</tr>
<tr>
<td>IL-4</td>
<td>345</td>
<td>AluI</td>
<td>250, 95</td>
</tr>
<tr>
<td>IL-13</td>
<td>170</td>
<td>AluI</td>
<td>131, 27, 12</td>
</tr>
</tbody>
</table>
were not on steroids during the time of blood sampling. We found that IL-13 mRNA expression in CD4+ cells was significantly increased in the nonsteroid group that had relapse nephrotic syndrome (CI = 0.99 ± 0.29, n = 17), as compared with those in remission (CI = 0.57 ± 0.18, n = 10), normal and patient controls (P < 0.0005) (Figure 3). Additionally, the CD4+ IL-13 mRNA expression for the nonsteroid group was significantly higher than the steroid-treated group in relapse

Figure 1. Amplification products of Th1 (interleukin-2 [IL-2] and interferon-γ [IFN-γ]) and Th2 (IL-4 and IL-13) cytokine gene expression in CD4+ cells after reverse transcription (RT)-PCR. Lanes 1 and 2, normal controls; lanes 3 and 4, patient controls (with nonspecific viral infections); lanes 6 and 7, 8 and 9, 10 and 11, respectively; paired data from patients with nephrotic syndrome in relapse and remission; lane 12, negative control for RT-PCR (buffer). Cyclophilin was used as the “housekeeping” gene to standardize the amount of cDNA.

Figure 2. Standardization curve for RT-PCR. The number of amplification cycles chosen for each cytokine was based on the linear portion of the standard curve for the band density of the respective cytokines after pokeweed mitogen stimulation of PBMC, before saturation of the PCR product.
CI than their respective groups in remission (n = 23), patients with nephrotic relapse (n = 17), nephrotic controls (n = 30), and nephrotic remission (n = 22). There was no significant difference in cytokine index between nephrotic relapse and normal controls (n = 23). However, as shown in Figure 5, CD4+ cytokine gene expression both during relapse nephrotic syndrome and remission. Of these, seven were steroid-dependent and were on prednisolone during the time of blood sampling. Matched-pair analysis for CD8+ IL-13 mRNA expression from the nephrotic children in relapse (n = 17) was significantly higher compared with remission (n = 22) (P < 0.0005). Ninety percent of these children had values at least 1 SD higher than the normal controls. There was no significant difference in CD8+ IL-2, IFN-γ, and IL-4 mRNA expression in the nephrotic children when in relapse compared with remission.

### Intracellular IL-13 Expression

To determine whether the increased IL-13 mRNA expression seen in both CD4+ and CD8+ T cells was associated with protein synthesis, the level of cytoplasmic IL-13 in CD3+ cells was measured using flow cytometry after PMA/ionomycin stimulation. The percentage of CD3+ cells positive for cytoplasmic IL-13 was significantly higher in 12 children with relapse nephrotic syndrome (6.66 ± 3.39%) compared to 18 children in remission (2.59 ± 1.35%) (P < 0.0001) and 12 normal controls (2.44 ± 1.76) (P < 0.002).

### Discussion

Immune responses are strongly influenced by the type of cytokines produced by T cells after antigenic stimulation. In general, IL-2 and IFN-γ are thought to be produced by CD4+ Th1 cells, whereas IL-4, IL-10, and IL-13 are Th2 cytokines.

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**Table 3.** Th1 and Th2 cytokine gene expression in CD4+ and CD8+ cells from children with steroid-responsive nephrotic syndrome (cross-sectional data)\(^a\)

<table>
<thead>
<tr>
<th>Group</th>
<th>IL-2</th>
<th>IFN-γ</th>
<th>IL-4</th>
<th>IL-13</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>normal controls</td>
<td>0.59 ± 0.13</td>
<td>0.59 ± 0.16</td>
<td>0.59 ± 0.16</td>
<td>0.62 ± 0.16</td>
</tr>
<tr>
<td>(n = 23)</td>
<td>(n = 23)</td>
<td>(n = 23)</td>
<td>(n = 23)</td>
<td>(n = 23)</td>
</tr>
<tr>
<td>patient controls</td>
<td>0.63 ± 0.10</td>
<td>0.55 ± 0.15</td>
<td>0.60 ± 0.17</td>
<td>0.55 ± 0.15</td>
</tr>
<tr>
<td>(n = 17)</td>
<td>(n = 17)</td>
<td>(n = 17)</td>
<td>(n = 17)</td>
<td>(n = 17)</td>
</tr>
<tr>
<td>nephrotic relapse</td>
<td>0.55 ± 0.13</td>
<td>0.61 ± 0.21</td>
<td>0.58 ± 0.16</td>
<td>0.93 ± 0.28(^b)</td>
</tr>
<tr>
<td>(n = 30)</td>
<td>(n = 31)</td>
<td>(n = 26)</td>
<td>(n = 39)</td>
<td></td>
</tr>
<tr>
<td>nephrotic remission</td>
<td>0.62 ± 0.20</td>
<td>0.62 ± 0.20</td>
<td>0.58 ± 0.17</td>
<td>0.59 ± 0.17</td>
</tr>
<tr>
<td>(n = 22)</td>
<td>(n = 23)</td>
<td>(n = 18)</td>
<td>(n = 23)</td>
<td></td>
</tr>
<tr>
<td>CD8+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>normal controls</td>
<td>0.57 ± 0.08</td>
<td>0.57 ± 0.10</td>
<td>0.61 ± 0.10</td>
<td>0.66 ± 0.13</td>
</tr>
<tr>
<td>(n = 23)</td>
<td>(n = 23)</td>
<td>(n = 23)</td>
<td>(n = 23)</td>
<td></td>
</tr>
<tr>
<td>patient controls</td>
<td>0.59 ± 0.08</td>
<td>0.60 ± 0.23</td>
<td>0.64 ± 0.14</td>
<td>0.66 ± 0.13</td>
</tr>
<tr>
<td>(n = 17)</td>
<td>(n = 17)</td>
<td>(n = 17)</td>
<td>(n = 17)</td>
<td></td>
</tr>
<tr>
<td>nephrotic relapse</td>
<td>0.54 ± 0.09</td>
<td>0.57 ± 0.14</td>
<td>0.72 ± 0.24</td>
<td>0.91 ± 0.15(^c)</td>
</tr>
<tr>
<td>(n = 15)</td>
<td>(n = 18)</td>
<td>(n = 16)</td>
<td>(n = 22)</td>
<td></td>
</tr>
<tr>
<td>nephrotic remission</td>
<td>0.54 ± 0.09</td>
<td>0.54 ± 0.17</td>
<td>0.70 ± 0.24</td>
<td>0.63 ± 0.11</td>
</tr>
<tr>
<td>(n = 18)</td>
<td>(n = 21)</td>
<td>(n = 19)</td>
<td>(n = 25)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) The cytokine index is expressed as mean ± SD.

\(^b\) P < 0.008, comparing nephrotic relapse with normal controls, patient controls, and nephrotic remission.

\(^c\) P < 0.005, comparing nephrotic relapse with normal controls, patient controls, and nephrotic remission.

(CI = 0.76 ± 0.13, n = 14) (P < 0.007). However, in the steroid-treated nephrotic patients, there was no significant difference between those in relapse and those in remission (CI = 0.60 ± 0.17, n = 13).

With regard to CD8+ IL-13 mRNA expression (Figure 4), both the patients who were not on steroids (CI = 0.91 ± 0.08, n = 9) and the steroid-treated patients (CI = 0.87 ± 0.10, n = 11) had significantly higher levels during a nephrotic relapse than their respective groups in remission (nonsteroid group CI = 0.64 ± 0.10, n = 10 and steroid-treated group CI = 0.61 ± 0.08, n = 13) (P < 0.0003).

### Paired Data

Paired data for CD4+ cytokine gene expression in both relapse and remission were available for 16 children. Eight children were not on steroids during the time of blood sampling, and the other eight were children with steroid-dependent nephrotic syndrome, and hence were on prednisolone treatment, yet they relapsed. Matched-pair analysis confirmed the cross-sectional data in that there was no significant difference in IL-2, IFN-γ, and IL-4 mRNA expression in the CD4+ cells of nephrotic patients either in relapse or in remission (Figure 1). However, as shown in Figure 5, CD4+ IL-13 mRNA expression was significantly increased in the nephrotic patients when in relapse (CI = 0.97 ± 0.37) as compared to remission (CI = 0.61 ± 0.18) (P < 0.005). Sixty-nine percent of these children had values at least 1 SD higher in relapse than in normal controls.

Similarly, 10 children had paired data for CD8+ cytokine gene expression both during relapse nephrotic syndrome and remission. Of these, seven were steroid-dependent and were on prednisolone during the time of blood sampling. Matched-pair analysis for CD8+ IL-13 mRNA expression from the nephrotic children in relapse (CI = 0.93 ± 0.20) was significantly higher compared with remission (CI = 0.64 ± 0.23) (P < 0.005). Ninety percent of these children had values at least 1 SD higher than the normal controls. There was no significant difference in CD8+ IL-2, IFN-γ, and IL-4 mRNA expression in the nephrotic children when in relapse compared with remission.
However, studies on activated CD4+ Th0, Th1, Th2, and CD8+ cells have shown that IL-10 can be produced by Th1 clones, and IL-13 is also produced by both CD4+ and CD8+ clones. Hence, Th1 and Th2 phenotypes in humans are largely defined on the basis of IFN-γ:IL-4 ratios (27).

Our studies have clearly shown that both CD4+ and CD8+ IL-13 gene expression was increased in children with relapse nephrotic syndrome, and this tended to decrease after clinical remission. Although not all nephrotic children in relapse had elevated IL-13 mRNA expression compared with normal con-

Figure 3. Cytokine index for IL-13 mRNA expression in CD4+ cells. Comparison of the effect of steroid treatment at the time of blood sampling in nephrotic patients during relapse and remission. *P < 0.04, comparison between steroid-treated nephrotic patients in relapse and patient controls. @P < 0.0005, comparison between nephrotic patients without steroids during time of blood sampling, in relapse and remission, and normal and patient controls.

Figure 4. Cytokine index for IL-13 mRNA expression in CD8+ cells. Comparison of the effect of steroid treatment at the time of blood sampling in nephrotic patients during relapse and remission. *P < 0.004, comparison between steroid-treated nephrotic patients in relapse and remission, and normal and patient controls. @P < 0.003, comparison between nephrotic patients without steroids during time of blood sampling, in relapse and remission, and normal and patient controls.
controls, analysis of paired data showed that most had significantly higher IL-13 mRNA expression when compared with the remission state (Figure 5). This was true for both CD4+ and CD8+ cells isolated from the nephrotic children. The increased IL-13 gene expression was also associated with increased intracytoplasmic IL-13 expression in PMA/ionomycin-activated CD3+ cells. Analyses of the effect of steroids on IL-13 gene expression showed that only CD4+ IL-13 gene expression was affected by treatment with prednisolone, as these patients had a significantly lower cytokine index (CI) during the relapse phase, compared with those who were not on steroid therapy (Figure 3). On the other hand, CD8+ IL-13 gene expression was not affected by steroid treatment, as shown by the elevated levels in steroid-dependent patients at the time of relapse, despite being on prednisolone (Figure 4). None of these patients had received other immunosuppressive drugs before this study, excluding their effect on cytokine production.

Conversely, the expression of Th1 cytokine genes, i.e., IL-2 and IFN-γ, was not increased, unlike the findings in previous studies, which looked at cytokine production and cytokine receptor expression on lymphocytes from nephrotic patients (8–10,12,28). In many of these studies, PBMC production of cytokines or cytokine expression was studied under different in vitro conditions, such as after stimulation with various mitogens, which may account for the discrepancy in the experimental findings. Additionally, the population of nephrotic children tends to be heterogeneous, with some in relapse or remission. As viral infections tend to trigger a relapse in many of these children, it is important to control for this factor when studying the cytokine responses. Hence, we included a group of children with febrile viral upper respiratory tract infections as a patient control group in this study. We did not demonstrate any significant increase in CD4+ or CD8+ cytokine gene expression in this group of children compared with the normal controls.

The close relationship between atopy and INS suggests a common immune pathway (20,29). Stimuli such as allergens may activate common immune mechanisms, which subsequently result in proteinuria in children with INS. There is clear evidence that the balance between Th1 and Th2 cytokines is intimately linked to disease pathogenesis. In allergic diseases, the balance is tipped in favor of Th2 cytokines (30,31). Cho and coworkers have previously shown that increased production of IL-4 by PBMC from patients with minimal change nephrotic syndrome was associated with increased expression of type II IgE receptor expression on B cells and high IgE production (21). We were unable to demonstrate any increase in IL-4 gene expression from CD4+ cells of nephrotic children in relapse. However, the increased IL-13 gene expression from both CD4+ and CD8+ cells in children in relapse was consistent with Kimata’s study, in which IL-13 was shown to be important for the spontaneous production of IgE and IgG4 by PBMC in nephrotic patients (22). This was in contrast to atopic individuals in whom IL-4 was the controlling cytokine. Moreover, in the nephrotic patients, IL-13 binding was increased not only on B cells, but also on monocytes and natural killer cells.
Although IL-13 shares many of its biologic activities with the Th2 cytokine IL-4, including induction of B cell class switch to IgE, it is also an important modulator of monocyte function (32–35). IL-13 has important anti-inflammatory and immunoregulatory activities as it inhibits IL-12 production, which is a known inducer of the Th1 cytokines IL-2 and IFN-γ (36). It inhibits production of tumor necrosis factor-α, IL-1α, IL-1β, IL-6, IL-8, and IL-10 by activated monocytes (33,37). There is convincing evidence that a monocyte factor is the mediator for increased glomerular permeability and proteinuria in minimal change disease (38). IL-10, which regulates cytokine production by activated monocytes in vitro, has also been demonstrated to inhibit VPF release by PBMC in patients with minimal change disease (39). Matsumoto and coworkers subsequently demonstrated that IL-13 was able to act synergistically with IL-10 to inhibit concanavalin A-stimulated release of VPF from monocytes of children with INS (40). Hence, it is conceivable that IL-13 is an important regulatory cytokine produced by both CD4+ and CD8+ cells from patients with nephrotic relapse. In fact, many earlier studies have shown a tendency toward increased T suppressor cell activity or increased CD8+ cells in patients with relapse nephrotic syndrome (2–4,13,28). Thus, the immunoregulatory actions of IL-13 on monocytes from patients with relapse of nephrotic syndrome is currently under investigation in our laboratory.

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