

Atopy in childhood idiopathic nephrotic syndrome

Maria Esposito Salsano (maria.espositosalsano@tin.it), Luisa Graziano, Ilaria Luongo, Paola Pilla¹, Mario Giordano², Giuliana Lama

1.Department of Paediatrics, Unit of Paediatric Nephrology, Second University of Naples, Naples, Italy

2.Department of Pathology and Oncology, Second University of Naples, Naples, Italy

3.Unit of Nephrology and Paediatrics Dialysis, Paediatrics Hospital "Giovanni XXIII" Bari, Italy

Keywords

Nephrotic syndrome, T-cell subsets, Th-1/Th-2 cytokines

Correspondence

Dr. Maria Esposito Salsano, Department of Paediatrics, Unit of Paediatric Nephrology, Second University of Naples, Via L. De Crecchio 4, 80138 Naples, Italy.

Tel: +00 39 81 5665457-58 |

Fax: +00 39 81 5665457 |

Email: maria.espositosalsano@tin.it

Received

26 September 2006; revised 22 October 2006;

accepted 29 November 2006

DOI:10.1111/j.1651-2227.2007.00154.x

Abstract

Aim: Aim of the study was to evaluate the immunoallergic pattern and their modulating serum cytokines in children with primary manifestation of nephrotic syndrome, in order to analyse the correlation with disease activity and the outcome of childhood NS.

Materials and methods: We have evaluated 72 children: 58 steroid-sensitive and 14 steroid-resistant; 42 subjects were the healthy controls. In all were measured serum: T cell-subset, cytokines by Th-1, Th-2, total IgE levels and specific IgE antibodies.

Results: Of the 72 children investigated, 35 (48.6%) had either a history of atopy and/or elevated serum IgE; 14 of these children (40%) had clinical sign of an atopic disease (asthma, rhinitis, dermatitis) and 21 (60%) had elevated sIgE. The atopy was more frequent among SS than SRNS patients (52% versus 36%, $p < 0.05$). The CD19 were significantly increased in nephrotic patients compared with controls. IL-4 levels were not different from those in normal control both in SS and SRNS patients, either in relapse than in remission. There was no correlation between the sIgE and IL-4 levels. Therefore, IL-5 and IL-13 levels were significantly higher in SSNS compared to controls, in both pre than posttreatment, and higher in atopic patients. Interestingly, IL-6 and IL-10 levels were significantly increased in SRNS pretreatment compared to posttreatment and controls and, only for IL-10, significantly higher in atopic patients.

Conclusion: In our study, only 40% of atopic children had a positive allergic history and 51.4% of the nephrotic children had normal sIgE levels, both pre and posttreatment, indicating different aetiologies, as immune mechanisms, in the pathogenesis of NS. Therefore, specific IgE antibodies were not related to disease activity, suggesting that IgE production might be co-incident in childhood NS. However, the increased production of IL-5 and IL-13 in atopic SSNS may indicate that these cytokines are involved in the enhanced production of sIgE while IL-4 have a role as controlling cytokine.

INTRODUCTION

Mechanisms underlying the pathophysiology of minimal change nephrotic syndrome (MCNS), the most frequent cause of idiopathic nephrotic syndrome (INS) remain elusive, although recent arguments suggest that immune system function disorders play an important role as already proposed in the past (1). Minimal change nephrotic syndrome is often associated with clinical features of allergy such as bronchial asthma, allergic rhinitis, atopic dermatitis and urticaria.

In the 1951 Fanconi et al. (2) have found hypersensitivity of the skin test in nephrotic children, but they were unable to correlate increased sensitivity with aggravation of the renal disease. The association of pollen sensitivity and seasonal proteinuria was first reported by Hardwicke et al. (3) more than 40 years ago. In the 1971, Gerber et al. (4) investigated the presence of immune complexes (in particular IgE) along the glomerular capillary walls. The meaning of the IgE deposition in glomeruli of patients with NS and the relationship of IgE to other immunoglobulins and complement are not yet known. In asthma the specific localisation of IgE in the bronchial mucosa suggests a pathogenetic role for IgE. Atopy is associated with up 30% of cases of INS (5).

Several authors have reported allergic symptoms in patients with steroid-sensitive nephrotic syndrome (SSNS) (6,7). Higher serum IgE levels in nephrotic children have been related to poor outcome with frequent relapses (8,9) or poor responses to steroid therapy (10). However, it is uncertain whether increased levels of IgE in childhood INS are pathogenetic or coincident (11). Some factors are known to modulate IgE synthesis and investigation of the cytokine-regulating network of serum IgE is necessary to clarify the relationship between IgE production and INS.

More recently, the activity of T-helper 1 (Th1) and T-helper 2 (Th2) cells was assessed. Th1 cells produce IL-2, interferon- γ (IFN- γ); these cells are predominantly associated with delayed-type, cell mediated hypersensitivity reactions. In contrast, Th2 cells produce mainly IL-4, IL-10 and IL-13 and are primarily involved in the regulation of humoral immune responses and atopic reactions (12). The IFN- γ , typical Th1 cytokine, has a crucial role in the initiation of T-cell activation and particularly activates CD8⁺ suppressor/cytotoxic lymphocytes as well as CD16⁺ NK cells (13). IL-4 and IL-13 plays a key role in the regulation of B cell; while IFN- γ has an inhibitor role on production of IgE (14). IL-10, which is expected to inhibit the production

of other cytokines, might increase the activity of vascular permeability factor known to be stimulated in INS (15). Therefore, a significantly higher expression of CD23, the type II IgE receptor has been observed, on B cells from active MCNS patients, than in normal controls and the up-regulation of CD23 correlates with greater IL-4 activity (16). More recently, it has been investigated the association of gene polymorphism of IL-4, its receptor (IL-4R) with MCNS, demonstrating a significant difference between the MCNS group and controls in genotypic distribution of IL-4 promoter gene polymorphism, but not in the IL-4R (17). However, it is not clear whether the increased IL-4 activity is primarily responsible for the elevated serum IgE level observed in NS patients. Other cytokines that synergize or inhibit the action of IL-4 would be play a modulator role in the IgE response in these patients. It is known that even IL-13, have the same role than IL-4, on IgE synthesis by B cells (14) and both target the Cepsilon gene for switch recombination (18). We examined the immunoallergic pattern and their modulating serum cytokines in children with primary manifestation of NS, in order to analyse the correlation with disease activity and the outcome of childhood NS. Also, to determine whether there were any potential regulatory effect of corticosteroid on cytokine levels, children were investigated pre and posttreatment and, therefore, stratified in SSNS and SRNS.

MATERIALS AND METHODS

We have studied 72 children with primary NS, 52 boys and 20 girls, with mean age at the diagnosis of 4.6 ± 3.2 (range 3–10 years). These children, collected between 1997 and 2004, were selected for the study because all they were had primary manifestation of INS and were studied sequentially prior to initiation of treatment. Eleven patients were referred at our Department that served as a tertiary referral center, from another hospital. The diagnostic criteria for INS were based on the International Study of Kidney Diseases in Children (19). The initial treatment for INS, was prednisone at the dosage of 60 mg/1.73 m²/day as single dose for 4 weeks, followed by 40 mg/1.73 m²/day, given on alternate days for the next 4 weeks. Remission patients were considered those with protein-free urine for 3 alternate days and with restored normal albuminemia levels and total absence of edema. SSNS were defined the patients being free of proteinuria within 4 weeks of prednisone therapy and SRNS the patients after 4 weeks of ineffective therapy and three pulses of methylprednisolone (1 g/m²/day) (19). The history and physical examinations were obtained for each patient to evidence an infections or allergic illness immediately preceding or accompanying the diagnosis of NS. *Atopy* is defined by atopic history associated with positive skin test or specific IgE. Particular attention was paid to the time of year, associated symptoms, treatment, activities, diet and domicile at the time of onset of the NS. A questionnaire about allergy in the child and family was completed separately by the two of us. The information was recorded separately, but at the end of each session this information was compared

to provide an agreed allergy status sheet for each patient. We did this to avoid bias in the collection and interpretation of possible allergy but in practice there was agreement between us on nearly all the children. The following allergic illnesses were sought by direct questioning: asthma (recurrent wheezing), eczema (infantile or flexural), allergic rhinitis (hay fever), urticaria recurring at least twice, or recurring twice in response to a known provoking agent; therefore, questions were asked also about food allergy. A history of the same disorders was sought in the child's 1st-degree relatives. Before the study, none of the children was on non-steroidal anti-inflammatory drugs and none was previously given cytotoxic immunosuppressive therapy, which might affect interpretation of the lymphocyte cytoplasmic cytokine expression. Moreover, none of the children was given antihistamines at the time of study. Normal controls were taken from 42 healthy patients (32 boys and 10 girls) with mean age of 4.1 ± 3.7 years, selected from the outpatient clinic; they had no evidence of atopy, infection, systemic or renal disease from their history and physical examinations. Moreover, 15 children (10 boys and five girls) age-matched with nephrotic patients, with history of atopy and/or positivity for skin prick tests were the allergic controls.

Informed consent was obtained from each child and/or parents before the patients entered the study and this study was approved by Ethics Committee of University Hospital.

Methods

The blood sample were collected from all children at the initial manifestation of INS, prior to steroid therapy (pretreatment) and 4 weeks postinitial treatment (during steroid therapy, posttreatment), in order to obtain paired determinations. In all patients we investigated, at the onset, T cell-subsets and the serum levels of Th-1 (IL-2, IFN- γ), Th-2 (IL-4, IL-5, IL-6, IL-10 and IL-13) cytokines and total serum IgE (sIgE). Also, in children with positive history of atopy, either a skin-prick tests and a radioallergosorbent test (RAST) for specific sIgE were available for diagnosis.

The membrane phenotype of peripheral blood lymphocytes was determined using a panel of monoclonal antibodies directed against the total T lymphocyte population (CD3), T helper lymphocytes (CD4), T cytotoxic lymphocytes (CD8), B lymphocytes (CD19), Natural Killer cells (NK, CD16), CD25⁺. The samples were analysed using a flow cytometer.

IL-2, IFN- γ ; IL-4, IL-5, IL-6, IL-10 and IL-13. Serum cytokines concentrations were assessed by ELISA using a murine monoclonal antibody specific. A standard curve was prepared from six standard dilutions for IL-2, IL-4, IL-10 and IFN- γ and serum sample concentration determined for each cytokines. When the concentration of cytokine was below the detectable level, a value of zero was assigned for the statistical management of data.

Skin prick tests were performed on the ventral surface of the forearm using disposable No. 25 gauge hypodermic needles. All patients were tested against solutions of house-dust, house-dust mite (*Dermatophagoides pteronyssinus*), grass pollen mix, mould mix, egg, fish and cereal. If the child had regular contact with a pet at home or at school, skin tests

were performed with the relevant extract-for example cat fur or dog hair. Any child with suspicious history for any food or allergen was tested against that allergen if available.

Total serum IgE levels were measured by Pharmacia CAP System (Pharmacia and Upjohn Diagnostics AB, Uppsala, Sweden). The test procedure followed the direction of the kit manual.

Specific IgE antibodies directed against mites, milk, eggs were measured by Allergen ImmunoCAP using the *Pharmacia CAP System*. In this test the cut-off IgE level was 0.35 kU/L and values >0.70 kU/L represented a positive result.

Serum creatinine levels were measured by the Jaffe method and creatinine clearance (C_{Cr}) was calculated by the formula of Schwartz et al. (20). Urinary excretion of protein was assessed by a 24-h collection. The measurement of blood pressure was made by Dinamap.

Statistical analysis

All the data were expressed as mean $\pm$ standard deviations and analysed using paired/unpaired, two-tailed Student's *t*-test. Spearman's coefficient (*r*) was used to correlate serum cytokine levels and total IgE. Comparison and correlation were considered significant when *p*-values below 0.05 were considered significant.

RESULTS

Clinical and demographic data of patients are reported in Table S1. Of the 72 nephrotic children, 52 were boys and 20 girls (72.2% versus 27.8%, $p < 0.05$) and the male to female ratio was 3:1. The mean age of all patients at the diagnosis that correspond to age of the study, was 4.6 ± 3.2 years and there was no difference between boys and girls; 58 children (43M/15F) were SS and 14 children (9M/5F) SR (80.6% versus 19.4%, $p < 0.05$). Serum creatinine levels averaged 0.67 ± 0.16 mg/dL (range 0.33–0.93 mg/dL) and estimated C_{Cr} averaged 116 ± 16 mL/min (range 83.3–142.5 mL/min) and blood pressure was in normal range for sex and age.

Serum IgE levels

Of the 72 children investigated, 35/72 had atopy and, then, there was no difference in the proportion of atopic and nonatopic patients (48.6% versus 51.4%, $p < 0.5$). However, 14/35 of the atopic patients (40%) had either clinical sign of an atopic disease (asthma, rhinitis and dermatitis) than elevated sIgE, while 21/35 (60%) had elevated sIgE and/or skin prick test and specific IgE positivity, but no symptoms of allergy. Also, there was a significantly higher prevalence of atopy in SS than SRNS (52% versus 36%, $p < 0.05$) as well as an higher prevalence of atopy in boys than girls (77% versus 23%, $p < 0.01$) and in SS than SRNS boys (64.8% versus 28.6%, $p < 0.01$) (Table S1). At the diagnosis of NS, sIgE levels were elevated (506 ± 427 kU/L) in all nephrotic children compared with normal (38 ± 18 kU/L) and atopic controls (112 ± 60 kU/L $p < 0.05$). Then, 37 nephrotic children had elevated IgE levels without atopy. Also, sIgE were significantly higher in atopic than nonatopic children ($460 \pm$

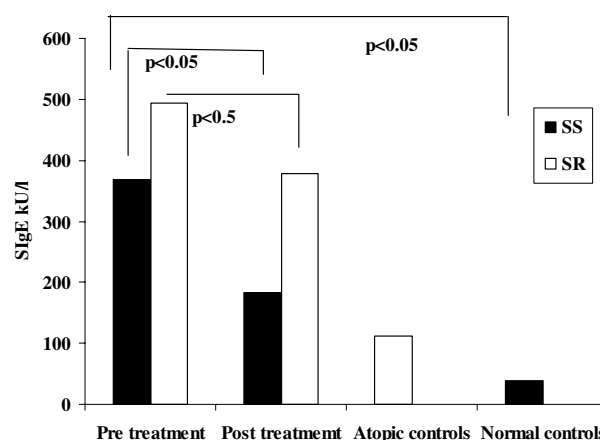


Figure 1 Serum IgE levels in patients with SS and SRNS compared to allergic and normal controls.

327 kU/L versus 69 ± 16 kU/L, $p < 0.05$) but no difference were found in patients with and without symptoms of allergy. However, there was no significant difference between sIgE levels in SS and SR pretreatment. In contrast, children with SRNS posttreatment had a significantly higher sIgE levels than SSNS in remission and posttreatment (Fig. 1). Serum specific IgE was positive in all the 35 atopic patients; the most common specific IgE antibodies were directed against dust mite (29%) and grass pollen mix (33%); asthma or allergic rhinitis were the more frequent (65%) symptoms, and, however, in all of the patients there was correspondence between skin prick test and specific IgE antibodies ($r = 0.80$).

Lymphocyte subpopulations

The lymphocyte subpopulations in patients and in control group are given in Table S2. There were no significant differences in the proportions of CD3 cells in SS and SR patients as compared to controls. In SS children, the proportion of CD4 cells increased significantly pretreatment as compared to posttreatment and to controls ($p < 0.005$, respectively). In SRNS, also, were showed reduced CD4 cells as compared to controls ($p < 0.05$). No increase in expression of CD8 was noted in SS. However, in SR CD8 count was increased only in pretreatment group as compared to controls ($p < 0.005$). The $CD4^+/CD8^+$ ratio was reduced to the same degree in SS and SRNS. No significant difference in NK cells was found pretreatment as compared to posttreatment and controls in SS; while, in SR the proportion of NK cells was significantly higher in group pretreatment, with heavy proteinuria, compared to controls (Table S2). Then, in SRNS patients we found a decreased ratio of helper/inducer cells ($CD4^+$) versus suppressor/cytotoxic cells ($CD8^+$) and an increased proportion of natural killer cells ($CD16^+$) in patients with marked proteinuria versus controls, as a sign of an elevated cytotoxic potential.

The B lymphocyte count (CD19) was not different among all the groups of patients studied but was significantly higher in nephrotic patients than controls. However, there was no significant difference in the percentage of lymphocyte count among atopic and nonatopic nephrotic children.

Th-1/ Th-2 cytokines

Serum levels of Th-1/ Th-2 cytokines in SS and in SR patients are reported in Table S3. In Tables S4 and S5 those levels from atopic nonatopic patients, both SS and SR, are compared. Serum IL-2 levels were not detectable in SS and SRNS, as atopic than nonatopic patients. Conversely, IFN- γ levels were higher, but not significantly, in SSNS patients pretreatment compared to those posttreatment or controls and there was no variation between atopic and nonatopic patients (Table S4). No difference in IFN- γ levels was found in SRNS (Table S5). IL-4 levels were not different between SS and SRNS, either pre than posttreatment and compared to controls. All the same, there was no difference between atopic and nonatopic patients (Tables S4 and S5). In SSNS, serum IL-5 levels were significantly higher pre than posttreatment ($p < 0.01$) and higher than controls ($p < 0.05$); while, in SRNS, these cytokine levels were different only in respect of normal controls ($p < 0.05$) (Table S3). Intra-group comparison, between atopic and nonatopic patients, showed significantly higher values of IL-5 in SS ($p < 0.01$) (Table S4) and no significant difference in SR (Table S5). In SRNS, serum IL-6 levels pretreatment were significantly higher compared to those posttreatment ($p < 0.01$) or controls ($p < 0.05$) and, also, higher compared with SSNS (Table S3). However, there was no difference between atopic and nonatopic patients, as SS than SRNS (Tables S4 and S5). Similarly, IL-10 levels were significantly higher in SRNS pretreatment compared to those posttreatment ($p < 0.01$) and controls ($p < 0.05$) (Table S3), and, also, there was a statistical difference between atopic and nonatopic patients (Table S5). In contrast, there was no significant variation in IL-10 levels between the SS patients and controls and no difference between atopic and nonatopic patients (Table S4). In SSNS, serum IL-13 levels were significantly higher pretreatment compared to those posttreatment and controls ($p < 0.05$) and significantly higher in atopic than nonatopic patients. Conversely, in SRNS serum IL-13 levels were significantly different only in respect of controls (Table S5). However, there was no correlation between sIgE levels and Th-1/Th-2 cytokines, in SS atopic and nonatopic ($r = 0.18$) and in SR atopic and nonatopic patients ($r = 0.2$).

DISCUSSION

Many previous studies have focused on the relationship between NS and atopy in different populations (5,8,9). Increased IgE levels have been noted in several cohorts with NS, regardless of atopic history (11). It is still uncertain whether increased levels of IgE in childhood NS are pathogenetic or coincident. A strong Th2 profile (characterized by increased levels of IL-4, IL-5 and IL-13) was found in the children developing atopy and is due to increased production of Th2 cytokines, possibly caused by impaired allergen-induced IFN- γ production. There is evidence for enhanced activity of IL-4 in stimulated peripheral mononuclear cells of relapsing NS patients than during remission or in controls (12). This findings are in contrast with a subsequent study reporting that serum levels of IL-4 were reduced both

in relapse and in remission of SS as well in SR patients compared to controls, but increased in patients with short- and long-term remission (13). Growing evidence shows that INS is the results of a primary T-cell dysfunction and leads to secondary anatomical and functional glomerular changes. Several studies have shown increased *in vitro* production of IL-2, IL-4 and IFN- γ by lymphocytes and high plasma and urine concentration of the soluble IL-2 receptor in patients during a relapse. In addition, patients with SSNS often display a defect in delayed-type hypersensitivity response, suggesting an abnormal Th1-dependent cellular immunity (13). Kemper et al. (21) reported parallel abnormalities of both CD23-mediated B as well as CD-25 mediated T-cell activation and suggested that INS is not solely a disorder of T-cell dysfunction. However, the molecular link between the immune system and the kidney disease is still lacking. In the present study, we observed no difference in age between SS and SR, but a significantly higher prevalence of atopy in SS compared with SR NS subjects. Therefore, the atopy would be an indicator for SS. It is worth noting a decrease of CD23 levels in SSNS in remission following steroid therapy (15). The sIgE levels, pretreatment, in both SS and SR children studied, were significantly higher than in normal controls. Patients with SR tend to have higher sIgE after steroid treatment, while patients with SS tend to have lower IgE after treatment. This characteristic feature mean that steroid response in childhood's NS is associated with decrease of Th2 reaction and that in patients with SRNS an excessive Th1 response may limit the Th2 immune response (22).

Also, there was no correlation between the elevated sIgE levels and a history of atopy in this group of patients. Th2 cytokines are primarily involved in the regulation of humoral immune responses and atopic reactions. The IL-4 and IL-13 cytokines are produced by stimulated Th-2 lymphocytes and the activation of Th-2 is known to play a key role in atopy. These data confirm the observation that the increased IgE levels during nephrotic relapses probably reflect some humoral immune dysregulation, but do not indicate a direct role in the pathogenesis of NS (23). Studies have shown that both IL-4 and IL-13 enhance the production of sIgE in nonatopic controls, while IFN- γ inhibits IgE secretion (17, 24), and, therefore, patients in relapses have increased CD4⁺ and CD8⁺ IL-13 mRNA expression as compared to patients in remission or to controls (13). We have previously shown that, in supernatant of peripheral blood mononuclear cells (PMBC), levels of IL-4, which are related to IgE productions and CD19 cells, were increased in relapse of SS as well during heavy proteinuria in SR patients (25). We found that serum IL-4 levels were not significantly different between NS patients and normal controls and, also, not different between SSNS and SRNS, both atopic than nonatopic patients. The spontaneous production of IFN- γ , IL-4 and IL-10, without stimulation, can explain the difference with our previous findings. Therefore, the last data, confirm the results of Tain et al. (26), but seem to be at variance with previous reports referring that serum IL-4 levels decreased during relapse but increased in nephrotic patients with long-term remission (12). All the same, Cheung et al. (27) were

unable to demonstrate any increase in the percentage of CD3+ cells producing IFN- γ and IL-4 in nephrotic children in relapse and in those in remission or in nonatopic controls. However, a recent study showed that MCNS relapses are associated with low levels of IL-4, a target gene of *c-maf* (transcription factor involved in Th polarization), whereas no significant changes in IL-10 and IFN- γ expression between relapse and remission were observed (28). One reason for the unchanged serum IL-4 may be that IL-13 is more important than IL-4 in modulating IgE production in NS (29). Indeed Tain (30) suggests that IL-13 might play a protective role in SSNS. Furthermore, the decreased allergen-induced IL-10 levels observed in the atopic children may result in a lack of down-regulation of the inflammatory process (31). In the past years efforts have been made to identify this pathogenetic cytokine as well as to understand the mechanism(s) for the increased release of this factor. Using different methodologies, investigators have focused on the production of cytokines in patients with INS during relapse and remission. This has resulted in a plethora of data without definitive conclusions. The pathogenetic cytokine has not been identified, and it is questionable whether there is a Th2 dominance in INS (32). The review of the available data illustrates the difficulties encountered when one is studying the cytokine secretory pattern in patients with INS. Differences in patient population, type of cells studies, sample preservation, and methodology used to measure cytokines are some of the factors that could account for the disparity of observed results (33). Our data give evidence that there was no difference between atopic and nonatopic nephrotic patients and that there was a significantly higher prevalence of atopy in SS than SRNS subjects. However, only 40% of atopic children had a positive allergic history and 51.4% of the nephrotic children had normal sIgE levels, both pre and posttreatment, indicating different aetiologies, as immune mechanisms, in the pathogenesis of NS. Therefore, specific IgE antibodies were not related to disease activity, suggesting that IgE production might be co-incident in childhood NS. In summary, IL-5 and IL-13 levels were significantly higher in SSNS compared to controls, in both pre than posttreatment, and higher in atopic patients. Interestingly, IL-6 and IL-10 levels were significantly increased in SRNS pretreatment compared to controls. In conclusion, the increased production of IL-5 and IL-13 in atopic SSNS may indicate that these cytokines are involved in the enhanced production of sIgE while IL-4 have a role as controlling cytokine.

ACKNOWLEDGEMENT

This study was supported by a grant from the MIUR (Department University of Research Scientific-Technology) in the years 2001–2002.

References

- Shalhoub RJ. Pathogenesis of lipoid nephrosis: a disorder of T cell function. *Lancet* 1974; 556–60.
- Fanconi G, Kousmine C, Frisch Knecht W. Prognosis of the nephrosis syndrome. *Helv Paediatr Acta* 1951; 6: 219–224.
- Hardwicke J, Soothill JF, Squire JR, Holti G. Nephrotic syndrome and pollen hypersensitivity. *Lancet* 1959; I: 499–502.
- Gerber MA, Paronetto F. IgE in glomeruli of patients with nephrotic syndrome. *Lancet* 1971; I: 1097–9.
- Meadow SR, Sarsfield JK, Scott DG, Rajah SM. Steroid-responsive nephrotic syndrome and allergy: immunological studies. *Arch Dis Child* 1981; 56: 517–24.
- Wittig HJ, Goldman AS. Nephrotic syndrome associated with inhaled allergens. *Lancet* 1970; I: 542–3.
- Williamson DAJ. Nephrotic syndrome associated with inhaled allergens. *Lancet* 1970; 11: 778.
- Yap HK, Yip WC, Lee BW, Ho TF, Teo J, Aw SE, et al. The incidence of atopy in steroid-responsive nephrotic syndrome: clinical and immunological parameters. *Ann All* 1983; 51: 590–4.
- Meadow SR, Sarsfield JK. Steroid-responsive nephrotic syndrome and allergy: clinical studies. *Arch Dis Child* 1981; 56: 509–16.
- Hu JF, Liu YZ. Elevated serum IgE levels in children with nephrotic syndrome, a steroid-resistant sign? *Nephron* 1990; 54: 275.
- Laguerre G, Laurent J, Hirbec G, Ansquer JC, Noirot C, Laurent G, et al. Serum IgE in primary glomerular disease. *Nephron* 1984; 984: 5–9.
- Neuhaus TJ, Wadhwa M, Callard R, Barratt TM. Increased IL-2, IL-4 and interferon-gamma (IFN-gamma) in steroid-sensitive nephrotic syndrome. *Clin Exp Immunol* 1995; 100: 475–9.
- Daniel V, Trautmann Y, Konrad M, Nayir A, Scharer K. T-lymphocyte populations, cytokines and other growth factors in serum and urine of children with idiopathic nephrotic syndrome. *Clin Nephrol* 1997; 47: 289–97.
- Yap HK, Cheung W, Murugasu B, Sim SK, Seah CC, Jordan SC. Th1 and Th2 cytokine mRNA profiles in childhood nephrotic syndrome: evidence for increased IL-13 mRNA expression in relapse. *J Am Soc Nephrol* 1999; 10: 529–37.
- Matsumoto K. Decreased release of IL-10 by monocytes from patients with lipoid nephrosis. *Clin Exp Immunol* 1995; 102: 603–607.
- Cho BS, Yoon SR, Jang JY, Pyun KH, Lee CE. Up-regulation of IL-4 and CD23/Fc ϵ R2 in minimal change nephrotic syndrome. *Pediatr Nephrol* 1999; 13: 199–204.
- Kobayashi Y, Arakawa H, Suzuki M, Takizawa T, Tokuyama K, Morikawa A. Polymorphism of interleukin-4-related genes in Japanese children with minimal change nephrotic syndrome. *Am J Kidney Dis* 2003; 42: 271–6.
- Bacharier LB, Geha RS. Molecular mechanism of IgE regulation. *J Allergy Clin Immunol* 2000; 105: S547–58.
- International Study of Kidney Disease in Children Nephrotic syndrome in children: prediction of histopathology from clinical and laboratory characteristics at time of diagnosis. *Kidney Int* 1978; 13: 159–65.
- Schwartz GJ, Haycock GB, Edelmann CM, Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. *Pediatrics* 1976; 58: 259–63.
- Kemper MJ, Meyer-Jark T, Lilova M, Muller-Wiefel DE. Combined T- and B-cell activation in childhood steroid-sensitive nephrotic syndrome. *Clin Nephrol* 2003; 60: 242–7.
- Stachowski J, Barth C, Michalkiewicz J, Krynicki T, Jarmolinski T, Runowski D, et al. Th1/Th2 balance and CD45-positive T cell subsets in primary nephrotic syndrome. *Pediatr Nephrol* 2000; 14: 779–85.
- Fuke Y, Endo M, Ohsawa I, Satomura A, Hidaka M, Fujita T,

- et al. Implication of elevated serum IgE levels in minimal change nephrotic syndrome. *Nephron* 2002; 91: 769–70.
24. Levy F, Kristofic C, Heusser C, Brinkmann V. Role of IL-13 in CD4 T cell-dependent IgE production in atopy. *Int Arch Allergy Immunol* 1997; 112: 49–58.
 25. Lama G, Luongo I, Tirino G, Borriello A, Carangio C, Esposito Salsano M. T-Lymphocyte populations and cytokines in childhood nephrotic syndrome. *Am J Kidney Dis* 2002; 39: 958–65.
 26. Tain YL, Chen TY, Yang KD. Implication of serum IgE in childhood nephrotic syndrome. *Pediatr Nephrol* 2003; 18: 1211–25.
 27. Cheung W, Wei CL, Seah CC, Jordan SC, Yap HK. Atopy, serum IgE, and interleukin-13 in steroid-responsive nephrotic syndrome. *Pediatr Nephrol* 2004; 19: 627–32.
 28. Valanciu   A, le Gouvello S, Solhonne B, Pawlak A, Grimbert P, Lyonnet L, et al. NF-  B p65 antagonizes IL-4 induction by c-maf in minimal change nephrotic syndrome. *J Immunol* 2004; 172: 688–98.
 29. Kimata H, Fujimoto M, Furusho K. Involvement of interleukin (IL)-13, but not IL-4, in spontaneous IgE and IgG4 production in nephrotic syndrome. *Eur J Imm* 1995; 25: 1497–501.
 30. Tain YL, Chen TY, Yang KD. Implication of serum TNF-   and IL-13 in the treatment response of childhood nephrotic syndrome. *Cytokine* 2003; 21: 155–9.
 31. van der Velden VH, Laan MP, Baert MR, de Waal Malefyt R, Neijens HJ, et al. Selective development of a strong Th2 cytokines profile in high-risk children who develop atopy: risk factors and regulatory role of IFN-gamma, IL-4 and IL-10. *Clin Exp Allergy* 2001; 31: 997–1006.
 32. Garin EH, Laflam PF, Muffly K. Proteinuria and fusion of podocyte foot processes in rats after infusion of cytokine from patients with idiopathic minimal lesion nephrotic syndrome. *Nephron Exp Nephrol* 2006; 102: e105–12.
 33. Araya CE, Wasserfall CH, Brusko TM, Mu W, Segal MS, Johnson RJ, et al. A case of unfulfilled expectations. Cytokines in idiopathic minimal lesion nephrotic syndrome. *Pediatr Nephrol* 2006 May; 21: 603–10.

Supplementary material

The following supplementary material is available for this article:

Table S1 Clinical features of patients with idiopathic nephrotic syndrome at the onset

Table S2 T-lymphocyte subpopulations (%) in children with nephrotic syndrome and controls

Table S3 Serum levels (mean $\pm$ SD) of Th-1 and Th-2 cytokines in nephrotic syndrome and controls

Table S4 Serum levels (mean $\pm$ SD) of Th-1 and Th-2 cytokines in atopic and nonatopic steroid-sensitive nephrotic syndrome

Table S5 Serum levels (mean $\pm$ SD) of Th-1 and Th-2 cytokines in atopic and nonatopic steroid-resistant nephrotic syndrome

This material is available as part of the online article from: <http://www.blackwell-synergy.com/doi/abs/10.1111/j.1651-2227.2007.00154.x>

(This link will take you to the article abstract).

Please note: Blackwell Publishing is not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

Table S1. Clinical features of patients with idiopathic nephrotic syndrome at the onset

	Total NS	Steroid-sensitive	Steroid- resistano	P
No. patients	72	58 (80.6%)	14 (19.4%)	<0.05
(M/F)	52M/20F	43M/15F	9M/5F	
Age at diagnosis, yrs	4.6 ± 3.2	4.2 ± 2.6	5.8 ± 3.8	<0.1
No. patients atopic	35/72 (48.6%)	30/58 (52%)	5/14 (36%)	<0.05
(M/F)	27M/8F	24M/6F	3M/2F	
No. pts atopic sympt	14/35 (40%)	12 (85.7%)	2 (14.3%)	<0.05
No. pts atopic asympt	21/35 (60%)	18(85.7%)	3 (14.3%)	<0.05
Creatinine clearance (ml/min)	116 ± 16	113 ± 15	100 ± 11	<0.1
Blood pressure (mmHg)	109/60	100/58	113/62	<0.1

Table S2. T-lymphocyte subpopulations (%) in children with nephrotic syndrome and controls

	Steroid-sensible		Steroid-resistant		Controls
	Pre-treatment (N=58)	Post-treatment (N=58)	Pre-treatment (N=14)	Post-treatment (N=10)	
CD 3	77 $\pm$ 5	75 $\pm$ 2	75 $\pm$ 5	73 $\pm$ 4	75 $\pm$ 7
CD 4	45 $\pm$ 7 ^a	36 $\pm$ 6 ^{a, b}	32 $\pm$ 9 ^b	34 $\pm$ 5 ^b	45 $\pm$ 10 ^b
CD 8	29 $\pm$ 11	27 $\pm$ 5	41 $\pm$ 13 ^c	26 $\pm$ 9	28 $\pm$ 8
CD4/CD8	1.3 $\pm$ 0.2 ^b	1.2 $\pm$ 0.1 ^b	1.2 $\pm$ 0.2 ^b	1.4 $\pm$ 0.3 ^b	1.9 $\pm$ 0.3 ^b
NK	7 $\pm$ 3	6 $\pm$ 5	15 $\pm$ 3 ^b	10 $\pm$ 5	10 $\pm$ 0.3 ^b
CD 19	17 $\pm$ 6 ^b	16 $\pm$ 6 ^b	16 $\pm$ 6 ^b	18 $\pm$ 8 ^b	10 $\pm$ 0.3 ^b
CD 25	7 $\pm$ 6	6 $\pm$ 6	6 $\pm$ 5	8 $\pm$ 4	5 $\pm$ 3

Legend:^a P < 0.005 compared pre and post-treatment^b P < 0.005 compared with controls^c P < 0.01 compared pre-treatment between SS and SR NS

Table S3. Serum levels (mean $\pm$ SD) of Th-1 and Th-2 cytokines in nephrotic syndrome and controls

	Steroid-sensible		Steroid-resistant		Controls
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	
	(N=58)	(N=58)	(N=16)	(N=10)	(N=42)
IFN-γ pg/ml	40 $\pm$ 15	30 $\pm$ 11	21 $\pm$ 12	26 $\pm$ 10	25 $\pm$ 11
IL-4 pg/ml	0.13 $\pm$ 0.1	0.05 $\pm$ 0.1	0.06 $\pm$ 0.1	0.05 $\pm$ 0.12	0.05 $\pm$ 0.04
IL-5 pg/ml	102 $\pm$ 54 ^{a b}	48 $\pm$ 23 ^{a b}	99 $\pm$ 65 ^b	83 $\pm$ 70 ^b	8.5 $\pm$ 3.4 ^b
IL-6 pg/ml	6 $\pm$ 5 ^c	6 $\pm$ 4	12 $\pm$ 4 ^{a b c}	6 $\pm$ 4 ^a	5 $\pm$ 3 ^b
IL-10 pg/ml	1.7 $\pm$ 0.8 ^c	1.2 $\pm$ 0.7	4.2 $\pm$ 2.5 ^{a b c}	1.4 $\pm$ 0.7 ^a	1.61 $\pm$ 1 ^b
IL-13 pg/ml	32 $\pm$ 25 ^{a b}	20 $\pm$ 15 ^{a b}	32 $\pm$ 25 ^b	28 $\pm$ 20 ^b	9 $\pm$ 3 ^b

Legend:

^a P < 0.01 compared pre and post-treatment

^b P < 0.05 compared with controls

^c P < 0.01 compared pre-treatment between SS and SR NS

Table S4. Serum levels (mean $\pm$ SD) of Th-1 and Th-2 cytokines in atopic and non-atopic steroid-sensitive nephrotic syndrome

	Pre-treatment		Post-treatment		Controls
	Atopic	Non Atopic	Atopic	Non Atopic	
IFN-γ pg/ml	45 $\pm$ 11	42 $\pm$ 10	39 $\pm$ 12	40 $\pm$ 11	25 $\pm$ 11
IL-4 pg/ml	0.12 $\pm$ 0.17	0.10 $\pm$ 0.03	0.06 $\pm$ 0.1	0.13 $\pm$ 0.1	0.05 $\pm$ 0.04
IL-5 pg/ml	122 $\pm$ 90 ^{a bc}	1.1 $\pm$ 2 ^a	52.3 $\pm$ 33.7 ^{a b}	2.3 $\pm$ 3.8 ^a	8.5 $\pm$ 3.4 ^c
IL-6 pg/ml	4.5 $\pm$ 2.7	7 $\pm$ 5.7	5.2 $\pm$ 4.5	6.6 $\pm$ 4.9	5 $\pm$ 3
IL-10 pg/ml	1.4 $\pm$ 0.9	1.2 $\pm$ 1.7	0.9 $\pm$ 1.4	0.9 $\pm$ 0.4	1.6 $\pm$ 1
IL-13 pg/ml	44.7 $\pm$ 16.3 ^{a b c}	3.9 $\pm$ 2.7 ^a	35.9 $\pm$ 18.5 ^{a b c}	6.5 $\pm$ 4.6 ^a	9 $\pm$ 3 ^c

Legend:

^a P < 0.01 compared atopic with non-atopic patients

^b P < 0.05 compared pre and post-treatment

^c P < 0.05 compared with controls

Table S5. Serum levels (mean $\pm$ SD) of Th-1 and Th-2 cytokines in atopic and non-atopic steroid-resistant nephrotic syndrome

	Pre-treatment		Post-treatment		
	Atopic	Non Atopic	Atopic	Non Atopic	Controls
IFN-γ pg/ml	40 $\pm$ 15	35 $\pm$ 12	30 $\pm$ 10	33 $\pm$ 11	25 $\pm$ 11
IL-4 pg/ml	0.13 $\pm$ 0.1	0.06 $\pm$ 0.1	0.05 $\pm$ 0.1	0.08 $\pm$ 0.1	0.05 $\pm$ 0.04
IL-5 pg/ml	94.5 $\pm$ 65.5 ^c	94.4 $\pm$ 75.8 ^c	85.5 $\pm$ 64.8 ^c	83.1 $\pm$ 60.8 ^c	8.5 $\pm$ 3.4 ^c
IL-6 pg/ml	10.6 $\pm$ 3.3 ^{b c}	10.4 $\pm$ 7.3 ^{b c}	8.4 $\pm$ 4.5 ^{b c}	3.9 $\pm$ 1.8 ^{b c}	5 $\pm$ 3 ^c
IL-10 pg/ml	5.1 $\pm$ 0.3 ^{a bc}	1.2 $\pm$ 1.3 ^{a bc}	1.8 $\pm$ 0.2 ^{a bc}	0.7 $\pm$ 0.2 ^{a bc}	1.6 $\pm$ 1 ^c
IL-13 pg/ml	38.7 $\pm$ 22.3 ^{5 c}	21.2 $\pm$ 27.85 ^c	29.5 $\pm$ 21.4 ^{5 c}	26.5 $\pm$ 24.65 ^c	9 $\pm$ 3 ^c

Legend:

^a P < 0.01 compared atopic with non-atopic patients

^b P < 0.05 compared pre and post-treatment

^c P < 0.05 compared with controls