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T Helper 1 and T Helper 2 Cytokines in Atopic Children With Steroid-sensitive Nephrotic Syndrome

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Introduction. A higher incidence of allergic disorders has been documented in children with steroid-sensitive nephrotic syndrome (SSNS); however, the role of cytokines associated with T helper 1 and T helper 2 cells is not fully elucidated. This study aimed to evaluate the role of T helper 1 and T helper 2 cytokines in both remission and activity phases among atopic versus nonatopic children with SSNS.

Materials and Methods. Fifty-two children with SSNS (21 with atopic disorders and 31 nonatopics) and 60 healthy children were enrolled in the study. The healthy controls were comparable with the patients in terms of age and sex distribution. Serum levels of immunoglobulin E (IgE), interleukin (IL)-2, tumor necrosis factor (TNF)-α, IL-4, and IL-13 were measured in activity and remission phases of the disease and in controls.

Results. Serum levels of IgE, TNF-α, IL-4, and IL-13 were significantly increased in the children with SSNS during the active compared to remission phase and the controls. T helper 2 markers (IgE, IL-4, and IL-13) were also higher in the atopic patients with SSNS than those without atopy. No significant difference was observed in IL-2 levels between the SSNS children in activity and remission phases and the controls, or between atopic and nonatopic children with SSNS in activity and remission phases.

Conclusion**s.** Our findings suggested that type 2 immune response prevailed during the active stage in children with SSNS, atopic or not, with persistent elevation of IgE during remission. T helper 2 imbalance was markedly exaggerated in atopic children.

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INTRODUCTION

Idiopathic nephrotic syndrome (INS) is the most common glomerular disease in childhood, characterized by heavy proteinuria, edema, and hypoalbuminemia.[1] Minimal change disease is the most common cause of INS. Minimal change disease is often abrupt in onset. It can be dramatic in presentation, yet is one of the most rewarding diseases for a physician to manage because response to corticosteroids is often rapid and complete. Because kidney biopsy is usually not performed when the disease responds to corticosteroid therapy, the term *minimal change disease* has become synonymous with *steroid-sensitive nephrotic syndrome* (SSNS).[2] The pathogenesis of idiopathic SSNS is not well understood.[3] The role for the immune mechanism, proposed by many studies,[1,4,5] was supported by a favorable response to anti-inflammatory drugs.[6]

After the first report of hypersensitivity and

nephrotic syndrome (NS) by Hardwicke,[7] numerous studies have reported an association between NS and allergy.[2,8] Several studies have demonstrated elevated serum immunoglobulin E (IgE) levels in NS patients and hypothesized its active role in the pathophysiology of the disease[9]; however, a direct relationship between IgE and the pathogenesis of NS is controversial.

Recently, it has been proposed that alterations in the cytokine profile of INS patients might contribute to proteinuria and glomerular damage.[10] Idiopathic nephrotic syndrome was proposed to be a disorder of T-cell dysfunction.[11] Since then, many investigators have tried to identify the mechanism by which T cells might increase glomerular permeability.[12,13] It was suggested that this may be due to a factor (cytokine), which was released by activated T cells.[12] According to the cytokines which prevail, the immune response was functionally subdivided into type 1 and type 2. Type 1 response, which normally prevails, is dominated by interferon-γ and interleukin (IL)-2 and mainly has anti-infectious function, whereas type 2 response is dominated by IL-4 and IL-13 and favors atopic expression.[12] Although pathogenesis of INS remains to be elucidated, evidence suggests that it is a primary immune disease associated with immunoregulatory imbalance between T helper subtype 1 and T helper subtype 2 cytokines.[14]

One striking clinical association of NS is with a personal or family history of atopy,[15,16] and serum IgE is typically elevated in MCN, both in the acute phase and during remission.[15] In particular, IL-13 shares many biological activities with IL-4, which include IgE isotype switching, CD23 induction, stimulation of eosinophil activity, mucus production, and smooth muscle activity in the airway. This is due to the fact that they share a common IL-4 receptor alpha chain in the multimeric IL-4 and IL-13 receptor complexes.[17] Interleukin-4, which is produced by T cells and mast cells, is the key cytokine involved in the development of atopy,[18,19] being absolutely required for class switching of B cells to IgE production and also promoting eosinophil chemotaxis and adherence.[18]

For the last 2 decades, many studies investigated the cytokine pattern in SSNS with sometimes conflicting results. It is still questionable whether type 2 response is dominant in active SSNS. In view of the conflicting evidence of T helper 1 and T helper 2 cytokines profiles in this disease, this study aimed to evaluate serum levels of IL-2 and tumor necrosis factor (TNF)-α (T helper 1 cytokines), along with IL-4 and IL-13 (T helper 2 cytokines) in patients with SSNS in activity and remission to determine whether there is any privilege of T helper 2 over T helper 1 in the pathogenesis of SSNS in children with atopy.

MATERIALS AND METHODS

Participants

This case-control study was conducted at the Pediatrics and Medical Biochemistry and Molecular Biology Departments; Faculty of Medicine, Zagazig University. The study included 52 SSNS patients (30 boys and 22 girls, aged from 3.3 to 9.1 years) and 60 community-based healthy children with no history of steroid therapy (24 boys and 26 girls, aged from 3.8 to 10.3 years). The two groups were comparable in terms of age, sex, and ethnic origin. The patients' first episode of SSNS according had been documented based on the criteria of the International Study of Kidney Diseases in Children,[20] during the period from April 2011 to August 2012. Among the SSNS group, there were 21 children with atopy and 31 without atopy. Active stage of SSNS was defined as increased urinary protein excretion (dipstick protein ≥ 2+ for at least 3 consecutive days or > 40 mg/m^2/h) and a serum albumin level of 2.5 g/dL and lower. Remission was defined as a serum albumin concentration of 3.5 g/dL and greater and normal protein excretion (dipstick protein, trace or negative for at least 3 consecutive days or ≤ 5 mg/m^2/h).[21] Atopy was diagnosed on the basis of positive family history, clinical varieties of atopy (including asthma, eczema, recurrent urticarial, and allergic rhinitis), and elevated serum IgE concentration. The results of the skin-prick tests were positive for all the atopic children.

All of the NS patients were treated with the standard initial steroid therapy, consisting of daily dosages of prednisone, 60 mg/m^2 body surface area, for 4 weeks, followed by 40 mg/m^2 given on alternate days, and finally various steps of tapering-off over the next 4 to 8 weeks. Relapses were treated with daily prednisone, 60 mg/m^2, until remission was achieved, followed by 40 mg/m^2 on alternate days.[22] Children with SSNS were evaluated during the active stage of the disease

(before steroid initiation) and during remission (while still on steroids at a dose of 40 mg/m^2 on alternate days).

None of the patients or controls were taking any immunomodulating drug which might affect interpretation of the cytokines levels (cyclosporine A, cyclophosphamide, levamisole, or mycophenolate mofetil) or other medications, such as verapamil or diltiazem, which might affect the clinical outcome of prednisone therapy. The participants had no history of recent (within the previous 6 months) infection, inflammatory conditions, or abnormal urinary sediments (abnormal casts or crystalluria). Other exclusion criteria were nephrotic patients suffering from any associated disease including parasitic infection, liver disease, heart disease, immunodeficiency disease, or any diseases elevating IgE.

The Ethics Committee of the Faculty of Medicine, Zagazig University, approved the study protocol. All parents of the patients and the controls were informed about the study, and written informed consent was obtained.

Blood and Urine Measurements

Blood samples were drawn from all participants without anticoagulant under sterile conditions. Sera were separated by centrifugation at 300 g for 10 minutes, and then divided in small aliquots and stored at -80°C for future serum cytokine and IgE assessments. Serum albumin was measured using the routine calorimetric methods (Spinreact, Girona, Spain). Twenty-four-hour urine samples were collected from each participant in sterilized urine containers and used to determine 24-hour protein excretion.

Immunochemical Assay

T helper 1 activity was assessed by measuring serum IL-2 and TNF-α, while T helper 2 activity was evaluated by assessing IL-4 and IL-13 both in activity and in remission. Serum cytokines were assessed by double antibody sandwich enzyme-linked immunosorbent assay (Biosource Europe SA, Belgium) according to the manufacturer's instructions. Briefly, standard dilutions and samples were pipetted into the wells, and the concentrated streptavidin-horseradish peroxidase solution was added to all the wells. After removing any unbound reagent by washing, a tetramethylbenzidine substrate solution was added to the wells and the intensity of the color was measured by a spectrophotometer at 450 µm.

Total IgE level was measured in the controls and in NS patients in activity and in remission using a commercial quantitative kit (AccuBind, Lake Forest, CA, USA) for enzyme-linked immunosorbent assay.

Statistical Analyses

Data were analyzed using the SPSS software (Statistical Package for the Social Sciences, version 17.0, SPSS Inc, Chicago, Ill, USA). The values were expressed as mean ± standard deviation. The nonparametric Wilcoxon signed-rank test was used to compare differences between the study groups with paired data. For nonpaired data, statistical significance was analyzed by the Mann-Whitney U test, independent Student *t* test, and the 1-way analysis of variance. *P* values less than .05 were considered significant.

RESULTS

Children With Nephrotic Syndrome Versus Controls

Among the children with SSNS during the active phase, the levels of IgE, TNF-α, IL-4, and IL13 were significantly increased compared to the remission phase ($P < .001$). No significant differences were observed in TNF-α, IL-4, and IL13 serum levels between the controls and the SSNS patients in remission phase. There was no significant difference in IL-2 levels between the SSNS children in the two disease stages and the controls (Table 1). The IgE levels were significantly higher in the SSNS patients in remission phase than in the controls ($P < .001$; Table 1).

Atopic and Nonatopic Children With Nephrotic Syndrome

Among the NS patients in activity phase, there were no significant differences in TNF-α and IL-2 levels between the atopic and nonatopic SSNS children. On the other hand, there were significantly higher levels of T helper 2 markers (IL-4, and IL-13) and IgE in the atopic SSNS patients than those without atopy, both of which had significantly higher values than the controls (Table 2).

In remission phase, there were no significant differences between atopic and nonatopic SSNS children with regard to all markers tested, while

Table 1. Comparisons of Mean Values Between Patients With Nephrotic Syndrome and Controls

Parameter	Patients With Nephrotic Syndrome		Controls
	Remission Phase	Activity Phase	
Age, y	5.6 ± 1.9	5.8 ± 2.0	5.4 ± 2.1
Proteinuria, mg/m^2/h	…	95.1 ± 19.8	…
Serum albumin, mg/dL	3.9 ± 0.4	1.8 ± 0.7*†	4.0 ± 0.2
Immunoglobulin E, IU/mL	62.7 ± 39.3*	186.0 ± 83.6*†	28.8 ± 12.7
Tumor necrosis factor-α, pg/mL	2.6 ± 0.3	6.2 ± 0.4*†	2.4 ± 0.3
Interleukin-2, pg/mL	7.9 ± 0.6	8.7 ± 1.0	8.6 ± 1.0
Interleukin-4, pg/mL	8.2 ± 2.5	25.9 ± 11.0*†	7.9 ± 2.6
Interleukin-13, pg/mL	16.2 ± 4.0	83.2 ± 39.2*†	15.9 ± 4.8

*Significantly different compared to controls
†Significantly different compared to patients with nephrotic syndrome in remission

Table 2. Comparisons of Mean Values Between Atopic and Nonatopic Patients With Nephrotic Syndrome

Parameter	Patients in Activity Phase			Patients in Remission Phase		
	Nonatopic	Atopic	*P*	Nonatopic	Atopic	*P*
Age, y	6.1 ± 2.0	5.4 ± 2.0	.32	5.3 ± 2.1	5.6 ± 1.8	…
Proteinuria, mg/m^2/h	97.9 ± 23.7	98.1 ± 24.7	.65	…	…	…
Serum albumin, mg/dL	1.9 ± 0.8	1.7 ± 0.7	.59	4.0 ± 0.3	3.8 ± 0.5	.14
Immunoglobulin E, IU/mL	141.6 ± 62.5	251.9 ± 65.8	< .001	37.8 ± 23.0	99.2 ± 22.9	< .001
Tumor necrosis factor-α, pg/mL	6.4 ± 0.4	6.3 ± 0.4	.63	2.6 ± 0.3	2.5 ± 0.2	.37
Interleukin-2, pg/mL	8.7 ± 0.9	8.4 ± 1.0	.77	8.2 ± 0.7	8.9 ± 0.5	.23
Interleukin-4, pg/mL	19.2 ± 4.2	35.6 ± 5.9	< .001	8.1 ± 2.8	8.4 ± 2.2	.75
Interleukin-13, pg/mL	66.5 ± 35.0	107.8 ± 31.7	< .001	15.3 ± 3.9	17.5 ± 3.8	.08

the level of IgE was significantly higher in those with atopy versus nonatopics, both of which yet significantly higher than the controls (Table 2).

DISCUSSION

The pathogenesis of INS is still controversial. The immune system is thought to play a pivotal role, and there is ample evidence that supports this theory.[4-6] Lymphocytic dysfunction, increased cytokines production, and release of free radicals have been suggested to be the pathogenic mechanisms for kidney injury in childhood INS.[23] Therefore, inhibiting the activation of renal inflammation could be the therapeutic target of protecting renal lesions in NS. Several cytokines have a pathological association with SSNS,[10] inducing proteinuria or regulating T cells.[8,24] Studies of the type 1 and type 2 cytokine patterns in the sera of patients with SSNS have generally been variable and inconsistent. These conflicting results may be due to the differences in the immunologic techniques used to assess cytokine synthesis.[25] Therefore, it remains unclear whether there is a type 2 response dominance in active SSNS.

In the present study, we found higher significant levels of serum IgE in children with SSNS in both activity and remission phases compared to the control group. Additionally, SSNS patients in the active phase had higher levels of serum IgE than those in remission. Interestingly, we observed that serum IgE levels were higher in atopic SSNS patients than those nonatopic children in both activity and remission. Similar to our findings, many studies have shown a strong association between idiopathic NS and atopic disorders.[2,8] The IgE levels were significantly higher in the NS patients with atopy than in nonatopic patients, especially during the relapse phase of NS compared with the remission phase, implicating IgE as a barometer of disease severity and giving it prognostic value. It is not certain whether the increased levels of IgE in INS are pathogenic or coincident. However, Youn and coworkers[26] revealed that some nephrotic children had persistently normal serum IgE levels, indicating different etiologies in the pathogenesis of NS. Abdel-Hafez and colleagues[2] suggested that the elevated serum IgE in some patients could not cause their NS, but rather reflected allergic responses to the perturbation of their humoral immunity.

In order to evaluate immunologic pattern and their modulating serum cytokines, we evaluated serum levels of T helper 1 cytokines (IL-2 and

TNF-α) and T helper 2 cytokines (IL-4 and IL-13) in the SSNS patients in activity and remission phases. We found no significant differences in serum IL-2 levels between the controls and SSNS children or between children with SSNS in different stages. Notably, no significant difference was found in IL-2 serum levels between atopic and nonatopic SSNS children in neither of the activity nor remission phases of the disease. In harmony with our findings, previous studies reported no significant difference in IL-2 serum levels between children with SSNS in different stages.[25,27,28] This is consistent also with Yap and colleagues[29] who found no significant differences in IL-2 mRNA expression in patients with INS. In Contrast, Zachwieja and colleagues[30] demonstrated the presence of a higher intracellular expression of IL-2 using a 3-color flow cytometric assay. The discrepancy between our findings and Zachwieja and colleagues' could be explained partially by the physiological fact that increased intracellular production does not usually result in increased secretion.[28,31]

In the present study, TNF-α level was significantly decreased in SSNS children during the remission phase compared to SSNS patients in active phase. However, we did not observe any significant difference in TNF-α level between SSNS patients in remission and controls or between atopic and nonatopic SSNS children in neither activity nor remission. Our findings are in agreement with those of a previous study by Suranyi and colleagues[32] who reported significantly high TNF-α level in INS children. They suggested that TNF-α might play a pathogenic role in the induction or maintenance of glomerular barrier dysfunction in humans. This is consistent also with Bakr and colleagues[33] who found elevated TNF-α production in INS patients during the active phase compared with controls. However, they revealed that remission was associated with normalization of TNF-α production.[33]

The shift to type 2 immune response in our SSNS children is further demonstrated by the increase of both serum levels of IL-4 during the active phase and also in atopic SSNS patients than those without atopy. Our findings are in harmony with those of previous studies that reported an increased synthesis of IL-4 by stimulated peripheral mononuclear cells in patients with active SSNS.[34-36] In contrast, Cheung and coworkers were unable to demonstrate any increase in the percentage of IL-4-producing T cells.[9] Studies of the type 1 versus type 2 cytokine patterns in the sera of patients with SSNS have generally been variable and inconsistent. This conflicting result may be due to the differences in immunologic techniques which were used to assess cytokine synthesis.[10]

Another piece of evidence for the shift to type 2 immune response in our SSNS patients was demonstrated by the increase in IL-13 serum levels during the active phase and also in atopic SSNS children than those without atopy. These results are in agreement with previous some reports.[9,29] Yap and colleagues used a semiquantitative reverse trancriptase polymerase chain reaction technique and showed an elevated expression of IL-13 mRNA.[29] In a recent study, Lai and colleagues showed that the IL-13 overexpression in the rat might lead to podocyte injury, inducing a minimal change-like nephropathy.[37] Recently, several studies have reported that serum IL-13 level is significantly higher in the active stage of SSNS compared with the remission phase and it remains elevated during both activity and remission phases compared with the controls.[25,28,31]

Increased IL-4, IL-13, and IgE levels during activity phase of NS in atopic and nonatopic children, together with the persistent elevation of IgE even during remission reflect that common immunoregulatory imbalance predisposes to both diseases and this imbalance is markedly exaggerated in atopic nephrotic children. These findings, however, should be interpreted considering the limitations of the study; the small number of patients might introduce uncertainty into the findings. Furthermore, although we considered that changes of cytokines were due to disease and not steroid (as these changes were found in activity even before starting steroid), we recommend the changes of cytokines levels be studied in a larger multicenter study with patients on different dosages of steroid to analyze the effect of steroid on cytokines levels in these children.

CONCLUSIONS

The results of our study indicate that a type 2 immune response prevails during the active stage of SSNS and also in atopic patients with SSNS than those without atopy. However, the mechanism leading to this type 2 cytokine upregulation awaits further elucidation.

CONFLICT OF INTEREST

None declared.

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