Turk J Med Sci
30 (2000) 289–295


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# Lymphocyte Subsets in Acute Post-Streptococcal Glomerulonephritis

Received: January 25, 1999

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**Abstract:** This prospective study was designed to investigate lymphocyte subsets in acute post-streptococcal glomerulonephritis (APSGN). Peripheral blood lymphocytes were analyzed using fluorescein isothiocyanate (FITC) labeled CD3, CD4, CD8, CD14, CD19, CD45, CD56, CD45RA and CDW29 monoclonal antibodies by flow cytometry. Twenty patients aged 14-22 years who fulfilled the diagnostic criteria for APSGN were evaluated during the 1st and 12th weeks of the of disease. Twenty–four age–matched healthy subjects served as controls. At the onset of the disease, all patients had macroscopic haematuria with red blood cell casts in urine, proteinuria (1.06±0.21 g/d), low complement 3 (C3) levels (<90mg/dl), increased antistreptolysin 0 titer (>200 IU/ml), edema, oliguria and high serum creatinine levels. Hypertension was recorded in 60% of the patients. Twelve weeks after onset, all patients had normal renal functions and serum C3 levels. Only one patient showed mild proteinuria (2+dipstick) and another had borderline hypertension. During the initial phase of the disease, patients demonstrated an increase in the total lymphocyte counts (CD3). The percentages of helper T cell (CD4), T helper 1 cells (Th1) and natural killer cells (CD56) were also significantly higher in the peripheral blood of APSGN patients during the acute stage than in the controls ($p<0.05$). The CD4/CD8 ratio was also significantly higher than in the controls during the initial stage of the disease ($p<0.05$). These findings indicate that T cell subsets and monocytes/macrophages might play a role in the pathogenesis of APSGN.

## Introduction

Immune-mediated glomerulonephritis accounts for a large fraction of acquired renal disease. The majority of cases are associated with the deposition of antibodies within the glomerular tuft, indicating dysregulation of humoral immunity, as in APSGN. Cellular immune mechanisms also contribute to the pathogenesis of antibody-mediated glomerulonephritis by modulating antibody production and through antibody-dependent cell cytotoxicity (1).

T cells act as a key in the immunological processes involved in any glomerulonephritis (1-3). They direct the intervention of the immune system and even the activation of the resident cells through the production and release of an array of cytokines (4). However, different subsets of T lymphocytes may play different pathogenetic roles in glomerular diseases (5). Although there are a few reports, the role of T cells in the mediation of glomerular injury has not been investigated in detail in APSGN so far. The aim of this study is to characterize the lymphocyte subsets in APSGN at presentation and 12 weeks after the onset and to correlate the results with the change in complement levels and renal function during the course.

## Patients and Methods

Between March 1995 and March 1997, 20 patients with APSGN treated at the nephrology unit of our hospital and 24 healthy age–matched controls were included in the study. Of these patients, 12 were male and 8 were female, aged14-22 years (17.0±2.1 years), and the controls were aged 17-22 years. All patients were sporadic cases and were admitted within the first week of the onset of the disease. Renal functions and serum C3 levels of all patients were improved at the 12$^{th}$ week after the onset.

Clinical evaluation included complete history, physical examination and laboratory investigation. Blood pressure (BP) was recorded regularly. Chest x-rays were obtained. Total and differential blood counts were performed.

Blood samples were taken regularly until 12th week for the evaluation of serum creatinine, urea, electrolytes, C3 and C4 levels and anti-streptolysine O titer (ASOT). Daily diuresis was measured until normalization of serum creatinine. Routine urinalysis was performed 2-5 times weekly, and 24-hour proteinuria at onset. Throat (and skin, if infected) swab and urine specimens were taken for culture. Renal biopsy was not performed in any of the patients.

All biochemical and serological measurements were made by standard methods. Serum C3 and C4 levels were determined by single radial immuno–diffusion technique using Behringwerke commercial plates (6).

The diagnosis of APSGN was established according to the six major criteria : 1) haematuria with red blood cell casts (RBC casts), 2) transient reduction of serum C3 level, 3) positive throat or skin culture for A-group ß hemolytic streptococcus and/or elevated ASOT>200 Todd's units; 4) Edema; 5) hypertension [(BP> 97% for age and sex (7)]; and 6) Oliguria (1). All patients showed an acute onset without a history of previous renal disease or a positive familial renal disease.

Isolation of peripheral blood mononuclear cells: Peripheral blood mononuclear cells (PBMC) were isolated from heparinised peripheral blood by Ficoll-Hypaque (Sigma, St. Louis, USA) density gradient centrifugation (8).

Aliquots containing approximately $1x10^6$ PBMC/ml were stained with fluorescein isothiocyanate (FITC)-labelled CD3 (total T-lymphocyte), CD4 (T-helper), CD8 (T-supressor), CD14 (myelomonocytic), CD19 (B lymphocyte), CDW29 (Th1), CD45RA (Th2), and CD56 (natural killer cell) monoclonal antibodies (mAbs, Clonal Biotest Diagnostics, Dreieich, Germany) for 30 minutes at 4°C. After staining, cells were washed with 0.01 M phosphate-buffered saline (PBS) pH 7.4, fixed with 4% formol PBS and analysed with a Coulter EPICS 541 flow cytometer (Coulter Electronics, Luton, UK). The percentage of lymphocyte subsets was obtained by flow cytometry (FCM) (9).

Follow-up procedure:

All patients were checked again 12 weeks after the onset. Evaluations were based on the interim history, physical examination including BP testing, blood and urine examination, serum electrolytes, creatinine and C3 level, and urine sediment. Hypertension was estimated according to the patient's age and sex (7). For adults, a BP of >140/90 mmHg was considered hypertension (7). Haematuria was evident when more than 5 RBC/HPF were detected in urine sediment. Serum creatinine was considered to be elevated when it was above 1 mg/dl (1).

The criterion for nephrotic proteinuria was accepted as > 3g/24h (10). Blood flow cytometric analysis of the 20 patients was conducted again during the 12th week.

The Mann Whitney-U test was used for statistical analysis. A p value of less than 0.05 was considered to be significant.

## Results

Initial: Initial clinical data of patients are presented in Table 1. At the onset of the disease, all patients had macroscopic haematuria with RBC casts and proteinuria, but in none was it in nephrotic range. C3 levels were low in all. Oliguria and edema were also noted in all patients. Pleural effusion was recorded in five patients and hypertension in 12 (Table 1). ASOT was elevated in all patients.

A positive history of antecedent upper respiratory tract infection was found in 95%, and skin infection in 5% of the patients. Positive bacteriology for ß-hemolytic streptococcus was found in 35%.

Table 1. Clinical data of the patients at onset.

| Clinical and laboratory findings | n | % |
|---|---|---|
| Haematuria (with RBC casts) | 20 | 100 |
| Low serum C3 levels: (< 90 mg/dl) | 20 | 100 |
| ASOT (>200 IU/ml) | 20 | 100 |
| Oedema | 20 | 100 |
| Oliguria | 20 | 100 |
| High Serum creatinine levels (>1.5mg/dl) | 20 | 100 |
| Hypertension | 12 | 60 |
| Positive throat culture | 7 | 35 |
| Pleural effusion | 5 | 25 |

Table 2. Percentage of lymphocyte subsets at onset.

| Patient No | CD3 | CD4 | CD8 | CD4/CD8 | CD14 | CD19 | CDW29 | CD45 | CD56 | CD45RA |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 88.9 | 49.5 | 20.5 | 2.4 | 8.8 | 8.5 | 46.5 | 95 | 4.1 | 61.4 |
| 2 | 86.5 | 51.8 | 22.5 | 2.3 | 7.8 | 9.6 | 44.9 | 96.5 | 4.7 | 60.5 |
| 3 | 87.5 | 51.5 | 21.8 | 2.3 | 9.2 | 9.1 | 50.3 | 95.6 | 3.6 | 55.5 |
| 4 | 86.8 | 52 | 21 | 2.4 | 10.2 | 8.8 | 51.7 | 98 | 3.8 | 61.8 |
| 5 | 88.5 | 52.3 | 23 | 2.2 | 9.5 | 7.9 | 50.5 | 97 | 3.4 | 62.5 |
| 6 | 83.4 | 50.5 | 21.6 | 2.3 | 7.8 | 8.3 | 46.8 | 96 | 2.9 | 63 |
| 7 | 87.5 | 49.8 | 21.3 | 2.3 | 10.5 | 9.2 | 47.5 | 95.2 | 3.6 | 64.2 |
| 8 | 86.6 | 48.2 | 20.1 | 2.3 | 9.5 | 8.6 | 52 | 96.6 | 3.8 | 63.5 |
| 9 | 86.5 | 47.8 | 19.8 | 2.4 | 9.9 | 8.5 | 48.7 | 97.2 | 3.9 | 64 |
| 10 | 86.9 | 50.5 | 22 | 2.2 | 8.5 | 9.5 | 49.5 | 98.4 | 4.5 | 56.8 |
| 11 | 86.5 | 51.9 | 21.5 | 2.4 | 8 | 8.3 | 50.5 | 96.7 | 4.5 | 57.5 |
| 12 | 88.9 | 53.9 | 22.6 | 2.3 | 8.9 | 10.5 | 51.5 | 95.5 | 5.5 | 57 |
| 13 | 87 | 50.9 | 20.9 | 2.4 | 11.1 | 10.6 | 50.9 | 97.3 | 5.5 | 58.5 |
| 14 | 85.5 | 54.2 | 23.5 | 2.3 | 8.3 | 9.1 | 52 | 98.2 | 5.8 | 59 |
| 15 | 89.5 | 53.3 | 23 | 2.3 | 9.5 | 8.9 | 50.2 | 96.1 | 5.9 | 60.5 |
| 16 | 89.9 | 55 | 24.1 | 2.2 | 10.0 | 9.5 | 46.8 | 95.5 | 6.5 | 62.8 |
| 17 | 88.5 | 54.2 | 22.1 | 2.4 | 8.4 | 9.5 | 47.2 | 98 | 4.6 | 60.9 |
| 18 | 87.8 | 52.5 | 22 | 2.3 | 8.5 | 10.1 | 48.5 | 96.6 | 4.5 | 63.2 |
| 19 | 90.5 | 50.8 | 21.2 | 2.3 | 9.2 | 10.3 | 49 | 95.6 | 3.9 | 61.5 |
| 20 | 86.5 | 53 | 22 | 2.4 | 8.6 | 9.6 | 45.5 | 97.5 | 3.8 | 59.2 |
| Mean | 87.4 | 51.6 | 21.8 | 2.3 | 9.1 | 9.2 | 49.0 | 96.6 | 4.4 | 60.6 |
| SD | 1.6 | 1.9 | 1.1 | 0.06 | 0.9 | 0.7 | 2.2 | 1.0 | 0.9 | 2.6 |

Two out of 20 patients showed abnormalities at the 12th week. In patient 1, a 22 year-old male, borderline hypertension was detected (138/90 mmHg). His other findings were normal. Patient 9 had mild proteinuria (2+ on the dipstick) with a normal glomerular filtration rate (GFR). C3 levels returned to normal in all patients. ASOT levels were higher than those obtained at onset.

Flow cytometric studies were carried out in all patients at presentation (Table 2) and 12 weeks later (Table 3), in controls (Table 4). A significant increase was found in CD3 and CD4, but not CD8 positive cells during the acute phase of the disease compared with healthy controls ($p<0.05$). The CD4/CD8 ratio was also found to be significantly higher ($p<0.05$). CD56, CD14 and CDW29 positive cells were found to be significantly increased at the acute period with decreased CD45RA levels ($p<0.05$). CD19 positive cells did not differ from those of controls during the acute period and at the 12$^{th}$ week ($p>0.05$) (Table 5).

Flow cytometric studies were again carried out in all patients at the 12$^{th}$ week (Table 3). There were no significant differences between the results of FCM analyses in patients at the 12$^{th}$ week and controls other than lower CD3 and CD4 positive cell counts in the patients group ($p<0.05$) (Table 5).

There were no significant differences between CD8, CD14, CD19, CDW29, CD45RA and CD56 levels in patients at the 12$^{th}$ week and controls ($p>0.05$). We found a significant increase in total lymphocytes (CD3) and in CD4 positive cells during the acute phase of the disease. The CD4/CD8 ratio was also found to be higher, probably due to normal levels of CD8 cells. CDW29 (Th1) cells were found to be significantly increased at the acute stage, with decreased CD45RA (Th2) levels ($p<0.05$).

## Discussion

Human glomerulonephritis remains one of the most important causes of end-stage kidney disease worldwide (2). Much effort has been expended over the last twenty years in trying to gain a better understanding of the

Table 3. Percentage of lymphocyte subsets 12 weeks later.

| Patient No | CD3 | CD4 | CD8 | CD4/CD8 | CD14 | CD19 | CDW29 | CD45 | CD56 | CD45RA |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 73.5 | 40 | 22.5 | 1.7 | 3.9 | 10.5 | 30.5 | 97 | 3.2 | 69.8 |
| 2 | 74 | 37 | 19.6 | 1.8 | 3.5 | 9.5 | 31.5 | 98.2 | 2.9 | 69.5 |
| 3 | 75.3 | 36.5 | 20 | 1.8 | 4.9 | 11.5 | 28.9 | 97.5 | 2.5 | 70.3 |
| 4 | 76.5 | 39 | 21.5 | 1.8 | 5.5 | 11 | 29.5 | 96.3 | 2.8 | 68.2 |
| 5 | 74.5 | 41 | 22.2 | 1.8 | 6.6 | 10.3 | 35 | 95.5 | 3.1 | 68.8 |
| 6 | 70.9 | 42.5 | 23 | 1.8 | 7.0 | 9.3 | 35.5 | 96 | 3.4 | 66.5 |
| 7 | 72 | 35 | 18.6 | 1.8 | 6.2 | 8.8 | 34 | 95 | 3.5 | 62.5 |
| 8 | 73 | 35.5 | 19 | 1.8 | 5.5 | 9.9 | 33.8 | 96.6 | 2.9 | 63.7 |
| 9 | 72.5 | 33.5 | 18 | 1.8 | 4.9 | 10 | 32.5 | 95.8 | 2.5 | 74 |
| 10 | 71.8 | 36 | 19.5 | 1.8 | 3.5 | 10.3 | 32 | 98 | 2.2 | 72.5 |
| 11 | 72 | 38 | 20.8 | 1.8 | 6.1 | 10.9 | 33.5 | 97.5 | 3.1 | 73.5 |
| 12 | 74 | 37.3 | 20.4 | 1.8 | 5.5 | 11.8 | 27.5 | 96 | 3.8 | 70.9 |
| 13 | 75 | 35 | 19.2 | 1.8 | 4.9 | 11.3 | 33.8 | 97.5 | 3.6 | 69.5 |
| 14 | 75.9 | 36.5 | 21 | 1.7 | 6.6 | 11.5 | 28.5 | 98 | 2.7 | 68.8 |
| 15 | 76 | 37 | 20.5 | 1.8 | 5.9 | 10.5 | 29.8 | 96 | 3 | 64.5 |
| 16 | 73 | 34 | 18.1 | 1.8 | 4.3 | 10.8 | 30.5 | 95.5 | 3.8 | 65.6 |
| 17 | 72.9 | 33.5 | 17.5 | 1.9 | 3.5 | 11 | 31.8 | 96.8 | 4.0 | 70.5 |
| 18 | 71.5 | 39 | 21.8 | 1.7 | 4.1 | 9.5 | 33.3 | 96 | 3.8 | 72.1 |
| 19 | 73.5 | 39 | 20.9 | 1.8 | 5.0 | 9.9 | 34.1 | 95 | 3.7 | 73.3 |
| 20 | 74 | 40.5 | 22.5 | 1.8 | 4.0 | 10.7 | 30.6 | 96.1 | 3.5 | 69.5 |
| Mean | 73.5 | 37.2 | 20.3 | 1.79 | 5.0 | 10.4 | 31.8 | 96.5 | 3.2 | 69.1 |
| SD | 1.5 | 2.5 | 1.6 | 0.04 | 1.1 | 0.8 | 2.2 | 0.9 | 0.5 | 3.2 |

mechanisms involved in the pathogenesis of the heterogeneous group of renal diseases (2).

The initial events triggering the development of the various forms of primary GN vary somewhat, as do their histological features and natural history. However, in the early phases, all GN are characterized by an inflammatory process mediated by a number of soluble factors released by activated resident cells and/or by infiltrating immune cells (11). If the inflammatory process persists, the renal damage will progress toward renal fibrosis at a constant rate, independently of the original events; otherwise a more or less complete recovery may be observed (12).

In all forms of primary glomerulonephritis, there are at least three groups of contributors: T cells, monocytes/macrophages and resident glomerular cells (mesangial, endothelial and epithelial) (3). T cells act as a key in the immunological process involved in any GN (3). They direct the intervention of the immune system and even the activation of the resident cells, through the production and release of an array of cytokines (4). However, different subsets of T lymphocytes may play different pathogenetic roles in glomerular diseases (5). CD4 and CD8 are the main markers of the two major subclasses of mature T cells, helpers and suppressors, which respond differently to antigens (4). Over the last few years it has been demonstrated that the cellular and humoral areas of the specific immune responses are regulated by distinct subsets of T helper cells, termed type 1 (Th1) and type 2 (Th2) cells (5). These cells can be demonstrated with specific antigen expressions, CDW29 for Th1 and CD45RA for Th2 (13,14). They both respond to antigenic stimulation with a transient release of cytokines, which differ in the two subsets (15). Th1 cells primarily secrete interleukin (IL)-2 and interferon (IFN)-γ, whereas Th2 cells produce IL-4, IL-5, IL-10 and IL-13 (5,15). Among the cytokines, IL-2 and IL-4 are the main messages directing the development of T helper cells toward the Th1 and Th2 phenotype, respectively (16). Th1 cells are responsible for cell-mediated inflammatory reactions characterized by a marked potentiation of phagocytic cell functions, and also provide help to some B cells. We found increased Th1 cells at

Table 4. Percentage of lymphocyte subsets in controls.

| Patient No | CD3 | CD4 | CD8 | CD4/CD8 | CD14 | CD19 | CDW29 | CD45 | CD56 | CD45RA |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 75 | 41.5 | 22.1 | 1.8 | 3.5 | 9.8 | 30.8 | 98 | 3.5 | 68.5 |
| 2 | 74.5 | 43.1 | 23.2 | 1.8 | 3.9 | 10.5 | 32 | 97.5 | 3.3 | 69.6 |
| 3 | 73.5 | 40 | 21 | 1.9 | 2.5 | 11.2 | 34.2 | 99 | 2.9 | 68 |
| 4 | 74 | 39.5 | 20.5 | 1.9 | 4.2 | 9.2 | 33.5 | 97 | 2.5 | 70.5 |
| 5 | 75 | 38 | 21 | 1.8 | 4.2 | 9 | 32.8 | 96.8 | 2.4 | 71 |
| 6 | 76.5 | 37.5 | 20.3 | 1.8 | 3.5 | 8.8 | 35 | 98.3 | 2.3 | 70.2 |
| 7 | 75.5 | 41.5 | 23 | 1.8 | 4.6 | 8.9 | 30.5 | 98 | 3 | 70 |
| 8 | 74.5 | 43 | 23.5 | 1.8 | 5.3 | 8.9 | 30.8 | 98.5 | 3.9 | 65 |
| 9 | 72.5 | 42 | 21.6 | 1.9 | 3.9 | 9.9 | 29.8 | 99 | 3.8 | 71 |
| 10 | 73 | 37 | 19.5 | 1.8 | 5.5 | 10 | 30.5 | 97.7 | 4.1 | 70.8 |
| 11 | 76 | 38.5 | 20.6 | 1.8 | 6.3 | 8.5 | 32.6 | 96.8 | 4.3 | 65.5 |
| 12 | 72 | 40.5 | 22.8 | 1.7 | 4.5 | 11.2 | 29 | 97.9 | 4.2 | 66.8 |
| 13 | 71.9 | 42.5 | 20.5 | 2 | 3.5 | 11 | 31.5 | 98.2 | 3.9 | 69.5 |
| 14 | 74.5 | 36 | 18.2 | 1.9 | 5.5 | 10.3 | 31.9 | 96.9 | 3.8 | 66.3 |
| 15 | 75 | 39 | 21 | 1.8 | 6 | 9 | 31.3 | 98.7 | 3.5 | 67.5 |
| 16 | 76 | 38 | 19.5 | 1.9 | 4 | 8.5 | 30.8 | 99 | 4 | 68.9 |
| 17 | 77 | 43 | 24.5 | 1.7 | 3.9 | 10.2 | 31.5 | 98.4 | 3.3 | 69.5 |
| 18 | 76.5 | 40 | 22 | 1.8 | 4.4 | 11.2 | 29.6 | 96.1 | 3 | 70.8 |
| 19 | 77.5 | 36.5 | 19 | 1.9 | 5.6 | 10.9 | 29.3 | 96.8 | 4.1 | 69.2 |
| 20 | 78 | 37 | 20 | 1.8 | 6.2 | 10.6 | 30.2 | 97.3 | 3.9 | 66.3 |
| 21 | 75.2 | 39.4 | 22.4 | 1.7 | 5 | 11.5 | 34.2 | 98 | 3.8 | 65.9 |
| 22 | 74.3 | 41.5 | 20.9 | 1.9 | 4.5 | 9.3 | 34.5 | 96.9 | 2.9 | 68.5 |
| 23 | 73.5 | 39.5 | 21 | 1.8 | 5.5 | 8.98 | 31.9 | 98.5 | 2.8 | 67.3 |
| 24 | 75 | 40.9 | 22.3 | 1.8 | 4.1 | 10.5 | 32 | 99.1 | 2.9 | 68 |
| Mean | 74.8 | 39.8 | 21.2 | 1.82 | 4.5 | 9.9 | 31.6 | 97.8 | 3.4 | 68.5 |
| SD | 1.6 | 2.1 | 1.5 | 0.07 | 0.9 | 0.9 | 1.6 | 0.8 | 0.6 | 1.8 |

onset, which returned to normal after 12 weeks. This may indicate that the Th1 cell plays an active role during the acute period of APSGN. Th2 cells, on the other hand, promote the synthesis of antibodies and inhibit several macrophage functions. Thus, Th2 cells seem, to a certain extent, to play a protective role against the damaging effects of Th1-mediated immune reactions (5). We found a decreased ratio of Th2 cells initially, which became normal at the 12$^{th}$ week. We suggest that this may be related to a good prognosis of APSGN, at least in the short term. In proliferative and progressive GN, in which a delayed hypersensitivity reaction is taking place in the glomeruli, the T cells involved in the immune process are mainly of the Th1 type (17). The Th2 cell response appears to play a pathogenetic role in animal models of GN associated with systemic auto-immunity. They can also be considered as the real effectors of the immune system in the pathogenesis of glomerular diseases (18). Once in the glomerulus or within the interstitium, monocytes can mediate the injury through different pathways. They can synthesize and release a veriety of cytokines (IL-1, IL-6, IL-8) and growth factors (GM-CSF, ICAM-I, TNF, PGs) that can, in turn, attract more infiltrating immune cells and activate resident cells (19). Besides the soluble factors, the activated monocytes/macrophages have a considerable number of weapons for directly damaging the glomerular and the interstitial structures (19). Although it is now clear that macrophages have an important part in the pathogenesis of GN, the mechanisms underlying their influx into the glomeruli and/or the renal interstitium are still largely unknown. However, it can be hypothesized that they move through the endothelial barrier attracted by the presence of specific chemotactic factors produced and

Table 5. Flow cytometoic analyses of peripheral blood presentation, 12 weeks later and in controls (mean±SD).

| Patient No | CD3 | CD4 | CD8 | CD4/CD8 | CD14 | CD19 | CDW29 | CD56 | CD45RA |
|---|---|---|---|---|---|---|---|---|---|
| Onset (n=20) | | | | | | | | | |
| Mean | 87.4[a] | 51.6[d] | 21.8[2] | 2.3[j] | 9.1[m] | 9.2[p] | 49.8[t] | 4.4[w] | 60.6[l] |
| SD | 1.6 | 1.9 | 1.1 | 0.06 | 0.9 | 0.7 | 2.2 | 0.9 | 2.6 |
| 12 Wks Later (n=20) | | | | | | | | | |
| Mean | 73.5[b] | 37.2[e] | 20.3[h] | 1.7[k] | 5.0[n] | 10.4[r] | 31.8[u] | 3.2[x] | 69.1[2] |
| SD | 1.5 | 2.5 | 1.6 | 0.04 | 1.1 | 0.8 | 2.2 | 0.5 | 3.2 |
| Controls (n=24) | | | | | | | | | |
| Mean | 74.8[c] | 39.8[f] | 21.2[i] | 1.8[l] | 4.5[o] | 9.9[s] | 31.6[v] | 3.4[y] | 68.5[3] |
| SD | 1.6 | 2.1 | 1.5 | 0.07 | 0.9 | 0.9 | 1.6 | 0.6 | 1.8 |

Onset-12 weeks later p: a-b, p-r, d-e, g-h, j-k, m-n, w-x, t-u, 1-2 <0,05
Onset-Control p: a-c, p-s, d-f, j-l, m-o, w-y, 1-3, t-v, <0.05
12 weeks later-Control p: b-c, e-f, <0.05

released by resident cells or by infiltrating T cells (18). We found a transient increase in CD14 cells (monocytes) in our study, which might indicate an active role of these cells in the pathogenesis of APSGN.

Humoral and cellular immunity may induce glomerular damage through the release of cytokines and growth factors. Proteinuria consequent to glomerular damage or cytokines released by infiltrating cells and diffusing from hilar areas of glomeruli may determine tubular cell activation with release of chemoattractant substances and fibrogeneic factors.

Finally, cytokines (IL-1, IL-6, IL-8) and growth factors (GM-CSF, PGs, PDGF) have been released due to the activation of interstitial fibroblasts. They may induce the increased production of extracellular matrix and fibrosis.

There are very limited and conflicting data on this issue in the current literature, mainly concerning the change of CD4/CD8 lymphocyte subtypes in different types of glomerular diseases. Topaloğlu et al. demonstrated a moderate decrease in the total absolute lymphocyte count and CD8+ T-lymphocytes in a group of pediatric patients with minimal change nephrotic syndrome (20). In contrast, Fiser et al. had showed an increase in CD8+ T-lymphocytes and a decrease in total lymphocyte count and CD4+ T-cells in children with an acute relapse of steroid responsive nephrotic syndrome (21). Lin et al. studied T cell subtypes in different types of glomerulonephritis and revealed significant changes only in certain types: a decreased total lymphocyte count in patients with chronic glomerulonephritis; increased CD8+ T-cells count in patients with mesangial cell proliferative nephropathy; and increased CD8+ and CD4+ T-lymphocytes in HBC membranous nephropathy. They did not find any significant change in patients with poststreptococcal glomerulonephritis (22).

In our study we demonstrated a significant increase in total and CD4+ T-cells in patients with acute PSGN and also showed an increased CD4/CD8 ratio, all of which returned to normal at the 12$^{th}$ week. This indicates a direct role of CD4+ T-lymphocytes in the pathogenesis of acute PSGN. Moreover we revealed that this increase of CD4+ T-cells was mainly associated with an increase of Th1 cells while Th2 cells tended to decrease during the acute phase. This is the first report of such an event in the current literature.

In this study, it was indicated that T cell subsets and monocytes/macrophages played a key role in the immunopathogenesis of APSGN. We think that helper T cells, monocytes, Th1 cells, and natural killer cells, but not B cells, suppressor T cells and Th2 cells, are activated in the initial period of APSGN and contribute to the renal damage in APSGN.

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