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Case Report

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A case of endocapillary glomerulonephritis associated with peripheral blood natural killer cell proliferation

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Abstract
A 69-year-old male was admitted to our hospital due to rapidly progressive glomerulonephritis. A peripheral blood smear showed a marked increase in large granular lymphocytes. Flow cytometry analysis of the blood showed a marked increase in CD3-negative and CD56-positive natural killer (NK) cells. A renal biopsy showed a characteristic pathological pattern that involved endocapillary proliferation, a predominance of mononuclear cells and mesangiolysis. Prednisolone was administered, and the patient's renal function subsequently improved concomitant with the amelioration of NK cell proliferation. In our case, there was evidence of a strong association between NK cell proliferation and glomerulonephritis.

Keywords: CD56dim NK cell; endocapillary glomerulonephritis; NK cell proliferation

Background

Large granular lymphocytes (LGL) are a morphologically distinct lymphoid subset comprising 10 to 15 percent of normal peripheral blood mononuclear cells [1]. LGL cytosis arises from two major lineages, namely, CD3-positive and CD56-negative T cells or CD3-negative and CD56-positive natural killer (NK) cells. LGL cytosis is further divided into subgroups on the basis of the presence of clonal or non-clonal proliferation [2]. Renal diseases associated with non-clonal NK cell proliferation have rarely been described [3–6]. However, the association between NK cell proliferation and renal disease remains unclear. In the present case, NK cells were detected in each glomerulus, and a significant increase in NK cells was observed in the urine. These observations indicated a strong association between NK cell proliferation and glomerulonephritis.

Case report

In August 2006, a 69-year-old male was referred to our Department of Nephrology for acute exacerbation of renal function with proteinuria and hematuria. His serum creatinine rose from 1.1 mg/dL (83.9 μmol/L) in July 2006 to 2.4 mg/dL (183 μmol/L) in August 2006. He was afebrile, his blood pressure was 150/92 mmHg and peripheral edema was absent. A physical examination revealed no liver, spleen or lymph node enlargement. A urinalysis revealed 1.5 g/24 h proteinuria and 20–30 red blood cells per high power field. Scattered mononuclear cells and cellular casts containing the mononuclear cells were also evident (Figure 1A).

The peripheral white blood cell count was $13 \times 10^3/\mu L$ ($13 \times 10^9/L$) with lymphocytosis (61% lymphocytes). The blood urea nitrogen was 22.1 mg/dL (7.85 mmol/L), creatinine was 2.36 mg/dL (180 μmol/L). The total complement level and C_3 and C_4 components were within the normal ranges. Antistreptolysin O (ASLO) and antistreptokinase (ASK) antibody concentrations were not elevated. Antineutrophil cytoplasmic autoantibody was negative, antinuclear antibody was ×640 with a speckled pattern, but anti-DNA antibody, anti-smooth muscle antibody, anti-RNP antibody and anti-SSA, SSB antibody were all negative. Cryoglobulin and monoclonal protein were both negative. A renal biopsy was performed, and the specimen included the cortex with 16 glomeruli, one of which was sclerotic. Others exhibited endocapillary proliferation accompanied by mesangiolysis. Most of the endocapillary infiltrating cells were mononuclear cells. (Figure 1B and C). Immunofluorescent microscopy showed no significant immune deposits in the glomeruli. There was also no evidence of electron-dense deposits by electron microscopy.

The increased lymphocyte population in the peripheral blood comprised LGL characterized by abundant cytoplasm containing fine azurophilic granulations. Flow cytometry analysis revealed the phenotype of the increased peripheral lymphocyte population to be CD3-negative, CD16- and CD56-positive NK cells (Figure 2A). On the other hand, immunohistochemical analysis of peripheral blood revealed the phenotype of the increased population of mononuclear cells to be CD16-positive and CD56-negative .

The patient had a clinically indolent course except for glomerulonephritis. Prednisolone was administered at a daily dose of 50 mg, and the patient's renal function and

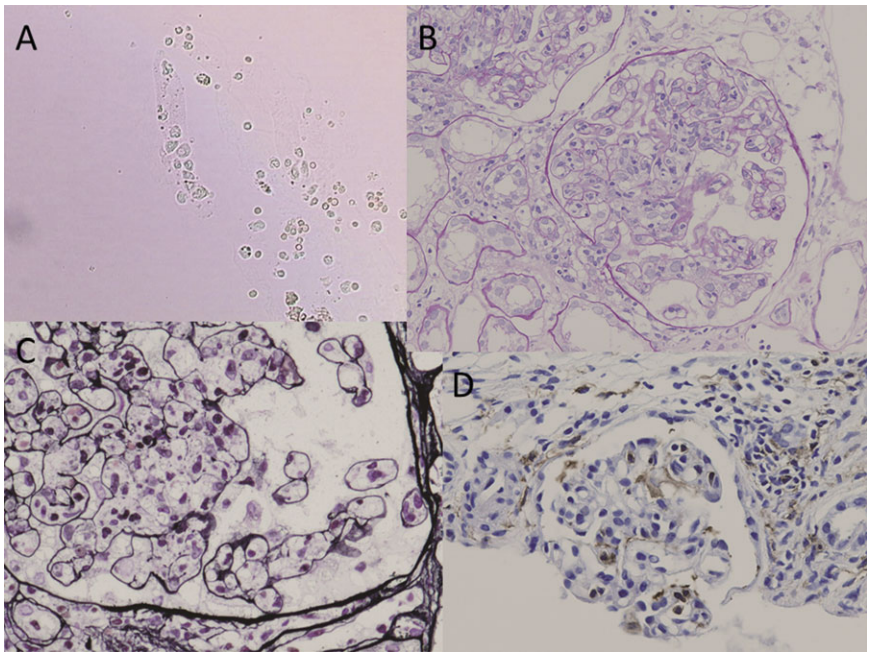

Fig. 1. (**A**) A cellular cast containing mononuclear cells in urine. (**B** and **C**) Renal biopsy evaluation by light microscopy. A renal biopsy showed a characteristic pathological pattern that involved diffuse endocapillary proliferation, a predominance of mononuclear cells, and mesangiolysis (B: Periodic acid-Sciff stain). A closer view of the area presenting mesangiolysis. (C: Periodic acid-methenamin-silver stain). (**D**) Immunoperoxidase staining with anti-CD16 antibody of renal biopsy specimen. CD16-positive cells are seen in glomerular tuft.

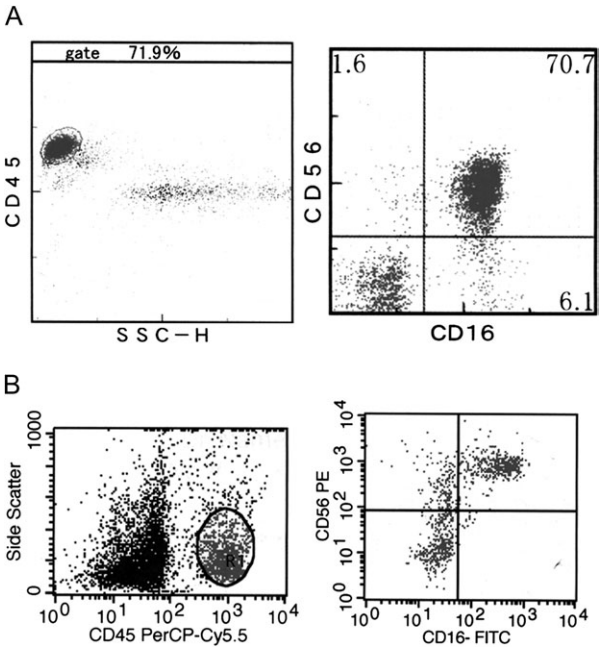

Fig. 2. (**A**) Flow cytometry analysis of peripheral blood. A gate is drawn to acquire lymphocytes and monocytes by size scatter/CD45 gating. Two color flow cytometry analysis shows an increase of CD16- and CD56-positive cells. (**B**) Flow cytometry analysis of the increased cells in urine. CD16-positive cells also express CD56.

urinary abnormalities subsequently improved concomitant with the amelioration of NK cell proliferation. Four months later, the urinary abnormalities had disappeared. However, a relapse of urinary abnormalities occurred as the dose of prednisolone was reduced.

A urinalysis at the time of relapse showed a marked increase in the number of mononuclear cells. An immunohistochemical examination of the urine revealed the phenotype of the increased mononuclear cells in urine to be CD16-positive and CD56-negative cells. However, flow cytometry analysis for the urine revealed that CD16-positive cells also expressed CD56 (Figure 2B).

An immunohistochemical analysis of biopsy specimens also identified CD16-positive cells in the glomerular tufts (Figure 1D). The immunoreactivity for CD56 was also negative.

The patient was treated with methylpresnidolone 'pulse' therapy (0.5 g daily during 3 days), and the urinary abnormalities subsequently improved and the NK cell proliferation disappeared.

Discussion

Renal diseases associated with non-clonal NK cell proliferation have rarely been reported [3–6]. Rabbanni *et al.* [3] reported that vasculitic glomerulonephritis developed in one patient after follow-up of 16 patients with chronic NK cell lymphocytosis, but the details of the case are indistinct. The case reported by Bassan *et al.* [4] showed mild renal insufficiency with proteinuria. The renal biopsy documented focal and segmental glomerulosclerosis associated with interstitial inflammation and tubular atrophy and primarily involved lymphocytes containing cytoplasmic multivesicular bodies, like NK cells. Vargas *et al.* [5] reported another case in which the renal biopsy revealed focal proliferative glomerulonephritis with focal and segmental sclerosis and marked interstitial fibrosis. Each glomerulus had several lymphocytes, but the

authors could not determine whether the cells were NK cells due to the unavailability of reagents to detect CD16 or CD56. In these case reports, the association between NK cell proliferation and renal damage remains unclear. In our case, the renal biopsy showed diffuse endocapillary proliferation accompanied by mesangiolysis. The majority of the endocapillary-infiltrating cells were mononuclear cells. Post-streptococcal acute glomerulonephritis was unlikely, because there was no evidence of either hypocomplementemia or a rise in the ASLO or ASK value. The patient was positive for anti-nuclear antibody but negative for specific antibodies. Immune complex-mediated glomerulonephritis, including connective tissue disease, was unlikely, as evidenced by the results of the immunofluorescent study. Hotta [6] reported a similar case in which the renal biopsy showed diffuse mesangiolysis with severe endocapillary proliferation. Immunohistochemical analysis revealed that CD57-positive LGL were present predominantly within the glomerular tufts. In our case, an immunohistochemical analysis revealed that the increased population of mononuclear cells in peripheral blood, urine and glomerular tufts expressed CD16. The immunoreactivity for CD56 was negative. However, flow cytometry analysis for the peripheral blood and urine revealed that the CD16-positive cells also expressed CD56.

The cause of this discrepancy between CD56 positivity of the mononuclear cells in cytometry and immunohistochemistry is uncertain. Cronin [7] examined a series of cases of myeloid leukemia cutis by immunohistochemical staining and compared these findings with the flow cytometric findings in the corresponding bone marrow specimens. They noted discordance of expression of CD56 in skin lesions relative to the immunophenotype of the bone marrow, and one of the reason for this discrepancy may be related to sensitivity differences (a greater sensitivity of flow cytometry).

Human NK cells can be divided into two subsets with distinct phenotypic properties based on the cell-surface density of CD56. The CD56dim NK cell subset is more naturally cytotoxic and expresses higher levels of immunoglobulin-like NK receptors and FCγ receptor III (CD16) than the CD56bright NK cell subset. In contrast, the CD56bright subset has the capacity to produce abundant cytokines following the activation of monocytes but has low NK cytotoxicity and is CD16dim or CD16 negative [8].

It is also possible that the negative result of immunoperoxidase staining with anti-CD56 antibody was due to the cell-surface density of CD56 and the increased CD16-positive cells may be CD56dim NK cells.

These observations indicated a strong association between NK cell proliferation and glomerulonephritis. We hypothesize that cytotoxic CD56dim NK cells caused renal injury. Hotta reported that NK cells were also observed in the urine of patients with active IgA nephropathy [9]. NK cells may therefore play a crucial role in some types of glomerulonephritis.

Conflict of interest statement. None declared.

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