Immunohistochemical Analysis of Human Serum Sickness Glomerulonephritis

Snježana Ćužić, Mira Šćukanec-Špoljar, Dubravka Bosnić, Duško Kuzmanić, Mirna Sentic

Pliva Pharmaceuticals Inc.; 1Department of Pathology and 2Department of Medicine, Zagreb University Hospital Center and School of Medicine, Zagreb, Croatia

Aim. To analyze pathological and immunohistochemical characteristics of glomerulonephritis in human serum sickness.

Methods. Renal biopsy specimens from two female patients with serum sickness that ensued after application of anti-lymphocyte horse globulin for aplastic anemia were analyzed by light microscopy, immunofluorescence, and electron microscopy. To prove the depositions of foreign protein, frozen sections were incubated with fluorescein-conjugated anti-horse protein serum. Immunohistochemical analysis was performed on B5-fixed paraplast-embedded tissue or frozen acetone-fixed sections with the primary antibodies for molecules/cell markers CD35, CD43, CD45RO, CD68, CD2, lysozyme, L26, and S100.

Results. Diffuse proliferating and necrotizing glomerulonephritis with crescents was found. There were coarse granular mesangial, subepithelial, subendothelial, and intramembranous deposits of mainly horse globulin, C3, and IgG. Most mesangium infiltrating cells were macrophages and T-lymphocytes. Electron microscopy revealed hypertrophy of podocytes, but immunohistochemistry did not show their normal CD35 (C3b-receptor) staining. Apart from epithelial cells, main crescent forming cells were macrophages and T-lymphocytes. Rare dendritic cells and abundant infiltration of macrophages, T-lymphocytes, and neutrophiles were found in the interstitium.

Conclusion. In severe serum sickness, glomeruli and tubuli are destroyed beyond the range usually seen in other types of glomerulonephritis caused by immune complexes, except in cases with widespread crescents. Hypertrophy of podocytes and loss of their normal C3b-receptor staining has not yet been described in the literature. C3b-receptors on podocytes could play a role in pathogenesis of glomerular injury caused by immune complexes.

Key words: biopsy; glomerulonephritis; immunohistochemistry; microscopy; electron microscopy; fluorescence; nephritis, interstitial; receptors, complement; serum sickness

Serum sickness was first described by von Pirquet and Schick (1) at the beginning of the 20th century. The disease is characterized by fever, lymphadenopathy, skin lesions, arthralgias, glomerulonephritis, and vasculitis, although pericarditis and pleuritis may also occur. The severity of clinical manifestations varies among patients. Most patients who develop serum sickness have received a foreign globulin (2,3). The most common non-human globulin given to humans is anti-lymphocyte horse globulin, usually used in the treatment of chronic acquired bone marrow failure, especially in patients with aplastic anemia (4,5).

Serum sickness is an example of immune-complex disease (6). Pathogenic immune complexes can be formed either locally or in the systemic circulation and then deposited in the kidney. This happens because immune complexes containing IgG and IgM are able to activate the complement, fix C3b component, and attach to cells carrying C3b receptors. These complexes are largely cleared from circulation by phagocytes. Charge, size, antigen to antibody ratio, and antigen valence of immune complexes are common factors that influence their deposition in the kidney (7,8). Deposits of complement (C3) and immunoglobulins along the glomerular basement membrane and in mesangium can be revealed by immunofluorescent methods. On light microscopy, glomeruli show mesangial proliferative, focal proliferative, or diffuse proliferative glomerulonephritis often accompanied by extracapillary proliferation (6-8).

There are only a few reports (9,10) on immunohistochemical markers in renal tissue obtained from animals with experimentally induced serum sickness, but to the best of our knowledge none describes changes in humans. Therefore, we performed an immunohistochemical study of renal samples obtained from two patients who developed human serum sickness after administration of anti-lymphocyte horse globulin for the treatment of aplastic anemia. In both cases, pronounced renal lesions were found. Despite the therapy with corticosteroids, the patients died several months after receiving anti-lymphocyte horse globulin.
Material and Methods

The specimens were obtained by kidney biopsy, which was performed in both patients with severe serum sickness, and at autopsy in one patient.

The specimens – two cylinders from each biopsy specimen – were halved and fixed in Dubosque-fixative for light microscopy, B5-fixative for immunohistochemical studies, 10% neutral formalin for electron microscopy, and frozen for immunofluorescence. Specimens fixed in Dubosque-fixative were embedded in paraffin blocks, cut in 3-5 μm-thick sections, and stained with hematoxylin-eosin, Mallory's trichrome (for staining connective tissue), and periodic acid-Schiff (PAS) reagents, and then impregnated with silver for basal membranes according to Jones. Frozen sections were cut 5 μm-thick, acetone fixed, and incubated with fluorescein-conjugated anti-IgA, IgG, IgM, C1q, C3, C4, and fibrin-fibrinogen serum (DAKO, Glostrup, Denmark). Additionally, sections were incubated with fluorescein-conjugated anti-horse protein serum produced and tested in Institute of Immunology, Zagreb, Croatia. Formalin-fixed tissue was embedded in Durcopan resin (Fluka, Buchs, Switzerland). Semi-thin sections were cut and stained with toluidine, whereas ultrathin sections were contrastained with uranylacetate and lead citrate. Opton EM 9A electron microscope (Opton, Oberkochen, Germany) was used.

Immunohistochemical analysis was performed on either B5-fixed paraffin-embedded tissue or frozen acetone-fixed sections. After abolishment of endogenous peroxidase activity, sections were treated with diverse primary antibodies CD35 (C3b receptor), CD2, CD43, and CD45RO for T-lymphocytes; CD68, and lysozyme for macrophages; L26 for B-lymphocytes; and S100 protein for dendritic cells (DAKO). A standard peroxidase-anti-peroxidase complex method (DAKO) was used. Slides were counterstained with hematoxylin.

Results

Two women, one 43 and the other 63 years of age, received anti-lymphocyte globulin for aplastic anemia over 6 days, together with methyl prednisolone (Solu-Medrol, Merck, Darmstadt, Germany) for 10 days. After application of the first dose of anti-lymphocyte globulin, one of the patients developed fever, malaise, and back pain; by the next day, all signs were gone. Three months after the administration of anti-lymphocyte globulin, both patients were admitted to the hospital because of severe proteinuria (more than 1.2 g/day), pretibial edema, and oliguria; their blood pressure was normal.

Both patients had increased erithrocites sedimentation rate (148/h; 152/h), elevated serum creatinine (833 mmol/L; 535 mmol/L), and serum urea (39 mmol/L; 40.2 mmol/L). One of the patients had sideropenic anemia. In both patients, C3 and C4 levels in

Figure 1. A glomerulus with disrupted Bowman’s capsule in the patient with serum sickness. There are necrotic remnants of the glomerulus with accumulation of inflammatory cell and a cellular crescent at one end. Pronounced inflammation with tubuli damage is visible in the interstitium. PAS (x600).

Figure 2. Human serum sickness glomerulonephritis. Necrosis in the renal parenchyma, with accumulation of macrophages, lymphocytes, and neutrophils. Mallory’s trichrome method (x400).

Figure 3. A. Human serum sickness glomerulonephritis. Coarse deposits of horse globulin along glomerular basal membrane. Immunofluorescence (x400). B. Negative control. IgA glomerulonephritis: renal tissue was incubated with fluorescein-conjugated anti-horse protein antibody and no fluorescence was found. Immunofluorescence (x100).
serum were normal. LE cells (phagocytes engulfing denatured nucleus), antineutrophil cytoplasmic antibodies, aspartate aminotransferase, rheumatoid factor, and cryoglobulins in serum were negative. One patient had negative antinuclear factor, whereas the other showed 1:16 titer. Sternal bone marrow smear was normal. Diagnostic ultrasound showed that kidneys of both patients were of average size.

**Clinical Course**

The patients showed the course of rapidly progressive glomerulonephritis without signs of extrarenal vasculitis or dermal involvement. They were treated with a pulse dose of corticosteroids (3 doses of 1,000 mg methyl prednisolone intravenously every second day) and cyclophosphamide (500 mg/5% glucose twice a day) continuing with prednisone 1 mg/kg/day for next 6 weeks. The dose of cyclophosphamide was reduced due to renal insufficiency, and plasmapheresis and dialysis were performed.

The younger patient had bacterial pneumonia and developed sepsis. *Staphylococcus aureus* was isolated from hemocultures. The patient was given vancomycin (1 g on the first day; afterwards, 500 mg in infusion over 90 minutes every fourth day). She died five weeks after admission to the hospital, with clinical signs of severe sepsis.

**Kidney Biopsy**

Percutaneous needle biopsy of kidney was performed in both patients. There were up to 20 glomeruli in each biopsy sample. A few glomeruli were completely sclerotic, whereas others showed abundant mesangial proliferation and crescents. There was focal-segmental necrosis of glomerular tuft, with polymorphonuclear infiltration (Figs. 1 and 2). Tubular epithelium showed segmental necroses and some tubules were completely necrotic, with accumulation of neutrophils. The interstitium was densely infiltrated with mononuclears. Hyaline arteriolosclerosis was noticed.

Immunofluorescence showed granular coarse horse globulin (Fig. 3), C3, IgG, and, too a lesser extent, C1q, IgM, and fibrin deposits along glomerular basement membrane. C3, IgG, C1q, IgM, and fibrin deposits were less prominent in mesangium, whereas horse protein deposits were not found. Neither horse protein, nor immunoglobulin and complement deposits were visible in or around tubules or vessels.

**Electron Microscopy**

Electron microscopy revealed coarse osmiophilic deposits in mesangium and smaller subepithelial, subendothelial, and intramembranous deposits. Glomerular basement membrane showed segmental duplication with interposition of the mesangium. Podocytes were hypertrophied and some had no foot processes (Fig. 4), whereas endothelial cells were atrophic.

**Immunohistochemistry**

In the mesangium of non-necrotic glomeruli, a few CD45RO+ and CD2+ T-lymphocytes (Fig. 5) were found, as well as CD68 and lysozyme-positive cells (macrophages). Inside the cellular crescents, we detected macrophages, CD45RO+ and CD2+ T-lym-
phocytes, but the majority of the cells remained unstained. CD43+ T-cells were situated in fibrocellular parts of the crescents. Podocytes were CD35+, although podocytes in normal renal tissue control stained with anti-CD35 antibody (Figs. 6 and 7).

In the interstitium, rare S100 positive dendritic cells were present. Among mononuclear inflammatory cells at the sites of inflammation, macrophages and CD2+ (Fig. 5) CD43+ cells prevailed over L26+ B-lymphocytes. There were a few CD35+ cells (Fig. 6) seen among mononuclear interstitial inflammatory cells. Some epithelial cells of the proximal tubule were lysozyme positive.

**Autopsy**

The autopsy revealed that the cause of death of the younger patient were multiple myocardial abscesses (Fig. 8A) and bronchopneumonia. Kidneys showed residual extracapillary glomerulonephritis with fibrous crescents and mesangial widening without signs of acute inflammation (Fig. 8B).

The other patient died several months after release from hospital with signs of renal insufficiency and infection (data given by son). No autopsy was performed.

**Discussion**

Serum sickness was recognized as a medical entity soon after the introduction of heterologous hyperimmune sera for the treatment of various infectious diseases (11). Symptoms appear a few days after foreign protein administration and are usually mild. The most
frequent signs of serum sickness are malaise, fever, joint pain and swelling, cutaneous eruptions, myalgias, bone pain, gastrointestinal and ophthalmological complications, lymphadenopathy, and splenic tenderness (11-13). Deterioration of renal function during serum sickness in humans has been reported, but only a few studies provided the description of pathohistological changes, too (14-16). Most data presented in the literature on serum sickness glomerulonephritis were gained from animal models (6-10). In this study, we analyzed serum sickness glomerulonephritis in humans by standard histological methods – immunofluorescence, electron microscopy, and immunohistochemical methods. Our study showed that glomeruli and tubules were destroyed far beyond the range usually seen in other types of glomerulonephritis caused by immune complexes, but on the other hand, the interstitial inflammation shares some similarities. An unexpected finding was the loss of C3b receptor staining on podocytes.

Serum sickness is an example of glomerular injury caused by deposition of immune complexes, which can be formed locally or in circulation. IgG- and IgM-containing immune complexes are able to activate the complement, fix C3b component, and attach to cells carrying C3b receptors (6). Podocytes in normal kidney express C3b receptors (17, and our own observation) (Fig. 7), but in renal biopsies of the two patients with serum sickness, no C3D5+ podocytes were present (Fig. 6). It is possible that podocytes became CD35 “negative”, while C3b receptors were occupied by immune complexes containing C3b fragment of complement. Phagocytes bearing C3b receptor (6) usually clear immune complexes. Although podocytes were hypertrophied and without foot processes, as described in experimental serum sickness (9,18,19), they did not seem to be able to phagocyte immune complexes like macrophages. In addition, electron microscopy revealed no phagocytic vacuoles within podocytic cytoplasm. It has been suggested that C3b receptors on podocytes could limit the damage caused by complement activation in glomerulus (7). However, since the examined cases revealed a full-blown picture of serum sickness it was hard to draw definite conclusions upon its pathogenesis.

Electron microscopic studies of renal changes in the patients with serum sickness (9,18,19) revealed that mesangial and subepithelial immune deposits were coarse, as opposed to subtle subendothelial and intramembranous deposits (Fig. 4). Subepithelial deposits destroy the cytoskeleton of podocytes by inducing effacement of the foot processes. It has been postulated that the severity of proteinuria is related to the degree of podocytic damage (18). As shown by immunofluorescence, the main components of glomerular immune deposits were horse globulin, C3, and IgG.

The effacement of foot processes is one of the crucial steps towards glomerular sclerosis. Autopsy performed in one of the patients revealed extensive glomerular sclerosis. Although each of the described glomerular alterations has broad etiology, it seems that podocytes play a pivotal role in serum sickness glomerulonephritis.

The main mesangium-infiltrating cells seemed to be macrophages (CD68 and lysozyme-positive cells) and T-lymphocytes (CD2+ and CD45RO+ cells). Since experimental models have shown that macrophages infiltrate the mesangium after immune complex formation and early T-cell infiltration (8,10), it has been suggested that macrophages might phagocyte mesangial immune complexes. However, there is no firm experimental evidence that macrophages eliminate electron-dense mesangial deposits (7).

Crescents are mainly built of proliferating Bowman’s capsule epithelial cells. Macrophages, granulocytes, and lymphocytes make about 20% of cells in crescent (20). Our results are in concordance with those observations. Also, it seems that lymphocytes in crescent belong mainly to the T-cell subset.

In the cases of serum sickness reported here, glomeruli were largely destroyed, beyond the range usually seen in other types of glomerulonephritis caused by immune complexes. A certain proportion of tubules were necrotic and surrounded by neutrophils, but the exact etiology of severe tubular damage remained uncertain. Cunningham and colleagues (14) described granular deposits of horse IgG and C3 around the basement membrane of some tubules and suggested immunological etiology of interstitial inflammation. We, on the other hand, could not determine any immunodeposits around the tubular basement membrane.

Interstitial granulomas and abscesses due to Candida albicans were reported in patients with serum disease (15). In our two cases, there was no mycotic superinfection, but the tubular changes could be a consequence of bacterial infection. Otherwise, our results show that in serum sickness, interstitial inflammation shares the characteristics of inflammatory reactions seen in other types of proliferative glomerulonephritis, where macrophages and T-lymphocytes predominate in interstitial inflammatory infiltrate (21). B-lymphocytes represent a smaller population (22) in interstitial inflammatory infiltrate, and rare dendritic cells are constantly found in interstitial inflammation, regardless of the type of glomerular injury (23).

References


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Correspondence to:
Snježana Ćužič
Pliva Pharmaceuticals Inc.
Baruna Filipovića 25
10000 Zagreb, Croatia
snjezana.cuzic@pliva.hr