Streptococcal IgG Fc-Binding Proteins Are Factors Initiating Experimental Glomerulonephritis

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Immunomorphological analysis of renal tissue from rabbits immunized with group A streptococci differing in the expression of IgG Fc-binding proteins showed that only IgG Fc-positive streptococci induced destructive degenerative changes in the kidneys classified as membranous proliferative glomerulonephritis with symptoms of fibroplastic glomerulonephritis. These morphological changes in renal tissue are comparable to changes in patients with acute poststreptococcal glomerulonephritis. Depositions of IgG and C3 complement component on the basal membrane of renal glomeruli and secretion of antiinflammatory cytokines IL-1β, IL-6, and TNF-α by mesangial cells were revealed. No destructive changes were found in the kidneys of rabbits immunized with IgG Fc-negative streptococcus strain or isogenic mutant completely devoid of genes responsible for the expression of IgG Fc-binding proteins. Thus streptococcal IgG Fc-binding proteins determine the development of experimental glomerulonephritis in rabbits.

Key Words: *group A streptococci; IgG Fc-receptors; isogenic mutant; anti-IgG; experimental glomerulonephritis*

Serogroup A streptococci induce a wide spectrum of diseases in humans, some of which are acute infections (tonsillitis, pharyngitis, scarlet fever, suppurative lesions of the skin, necrotic fasciitis, sepsis, toxic shock syndrome), others are chronic (rheumatic fever, poststreptococcal glomerulonephritis).

M-protein and hyaluronic capsule were for a long time believed to be the main factors responsible for pathogenicity of serogroup A streptococci and their resistance to phagocytosis *in vivo* [7,14]. However, after the discovery of streptococcal ability to react with human and mammalian blood proteins, primarily with IgG Fc-fragment, special attention was paid to investigation of the receptor proteins and their role in the microbe biology and its interactions with the host.

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IgG Fc-binding proteins of group A streptococcus belong to the M-protein family. Genes regulating the expression of M-proteins and Fc-receptors are located in the same regulon responsible for expression of the bacteria virulence factors and denoted as virulence regulon [8,12].

IgG Fc-positive streptococci are often isolated from patients [1,9]. IgG Fc-binding proteins enhance the streptococcal virulence, improve their resistance to phagocytosis, deplete the complement system by binding C1q component, and induce the production of antiimmunoglobulins in experimental immunization of rabbits [2]. Anti-IgG were induced by immunization of animals with IgG Fc-positive streptococci and with preparations of purified streptococcal IgG Fc-binding proteins [6]. Deposition of IgG and C3 complement component in the renal tissue and production of antitissue antibodies were detected in rabbits with high titers of anti-IgG [4,5]. Tissue deposition of immune

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complexes IgG-anti-IgG together with C3 leads to destructive processes [5]. Reactive oxygen metabolites, cytokines, and other immune inflammation products released by mesangial, endothelial, epithelial, and other cells can be additional factors damaging renal glomeruli [3,10].

We investigated the role of streptococcal IgG Fc-binding proteins in the development of experimental glomerulonephritis. A complex immunomorphological study was carried out in order to determine the type of tissue injuries in rabbits immunized with IgG Fc-positive strains of group A streptococci and isogenic mutant completely devoid of genes responsible for the expression of IgG Fc-binding proteins.

MATERIALS AND METHODS

The following reference strains of serological group A streptococci were used: types M1 (40/58), M22 (10/69), T27 (SF 40); clinical isolate of type M22 strain, strain AL168, and its isogenic mutant AL168-sir-mrp- completely devoid of genes responsible for expression of IgG Fc-binding proteins [13].

Rabbits were immunized as described previously [6].

IgG-binding activity of bacteria was evaluated by binding of ^{125}I-labeled polyclonal human IgG [4].

The level of anti-IgG was evaluated in the passive hemagglutination test with human erythrocytes sensitized with anti-Rh antibodies [6].

Rabbit renal tissue was examined 8 weeks after immunization. Deposition of IgG and C3 complement component in renal tissue was evaluated by the immunohistochemical method, rabbit IgG in tissue sections was identified with goat monospecific antibodies to rabbit IgG followed by treatment with antibodies to peroxidase-conjugated goat IgG; C3 in sections was identified by treatment with rabbit anti-human C3 serum cross-reacting with rabbit C3 (Dako) and then with peroxidase-conjugated monospecific antiserum to rabbit IgG (Sigma).

The production of cytokines — interleukins (IL) IL-1β, IL-6, and tumor necrosis factor-α (TNF-α) in renal glomeruli of immunized rabbits was studied by treating tissue sections with polyclonal goat antirabbit TNF-α antibodies (AMS Biotechnology), goat polyclonal antihuman IL-6 antibodies (Biosource International), and murine antirabbit IL-1β antibodies, respectively. Second antibodies were peroxidase-conjugated antigoat IgG or antimurine IgG antibodies.

Paraformaldehyde-fixed tissue sections were used in enzyme immunoassay. Tissue sections were incubated with first antibodies diluted 1:50 for 1 h at 18-20°C and after washout with peroxidase-conjugated second antibodies for 1 h. Diaminobenzene tetrahydrochloride (0.05%) and 0.03% H_2O_2 were used as the substrates. Tissue sections were stained with hematoxylin-eosin and examined under an Axiomat microscope (Opton).

For transmission electron microscopy ultrathin sections were fixed in 2.5% glutaraldehyde for 3 h and then incubated for 3 h in 0.1 M cacodylate buffer (pH 7.4) at 4°C. After washing in the same buffer, the sections were fixed in 1% OsO_4 in 5% cacodylate buffer, dehydrated in ascending alcohols and then in propylene oxide, embedded in araldite, and examined under a JEM-100B microscope at 75 mV.

RESULTS

By the end of immunization, high titers (1:160-1:320) of circulating anti-IgG were found in all 10 rabbits challenged with M1 and M22 streptococci. No antiimmunoglobulins were detected in 8 rabbits immunized with type T27 group A streptococcus and isogenic mutant AL168 sir-mrp-.

Depositions of IgG and C3 (mainly on the glomerular basal membrane) were detected only in rabbits immunized with IgG Fc-positive streptococcal strains. In rabbits immunized with IgG Fc-negative strain T27 and AL168 sir-mrp- mutant there were virtually no depositions of IgG and C3.

Tissue specimens from 10 rabbits were examined under electron microscope and by immunohistochemical methods. Degenerative destructive changes developed in the renal glomeruli after 8-week immunization with types M1 and M22 streptococci (both the reference strain 10/69 and clinical strain AL168) (Fig. 1, *a, b*). Destructive changes in glomeruli were seen in the basal membrane and intracapillary structures. Destruction of epithelial cells and their complete lysis were detected in the efferent tubules and accumulations of monocytes and basophils were seen in the lumen. Depositions of IgG and C3 were seen on the glomerular basal membrane; production of TNF-α (Fig. 2, *a*), IL-1β (Fig. 2, *b*) and IL-6 was observed in mesangial and endothelial cells. Local thickening of the basal membrane, interposition of mesangial processes into membranous substance, destruction of the epithelium, and intense proliferation of mesangial cells were observed in glomeruli. Mesangial cell processes were surrounded with membranous substance. The first signs of capillary sclerosis were seen at their periphery. Hypertrophy, vacuolation, and disintegration of podocytes and their processes, destruction of epithelial cells, and aggregation of cellular debris were found in all rabbits immunized with IgG Fc-positive streptococci. Pronounced hypertrophy of endothelial cells with complete capillary obliteration was observed at sites of their contact with the basal

membrane. Degenerative changes and necrosis of epithelial cells were observed in the tubules. These morphological changes induced by all IgG Fc-positive strains of group A streptococci used for immunization can be classified as membranous proliferative glomerulonephritis leading to fibroplastic glomerulonephritis, one of the most typical complications in humans.

By contrast, none of the rabbits immunized with strain T27 and isogenic mutant AL168 sir-mrp- developed degenerative changes in the kidneys (Fig. 1, *c, d*).

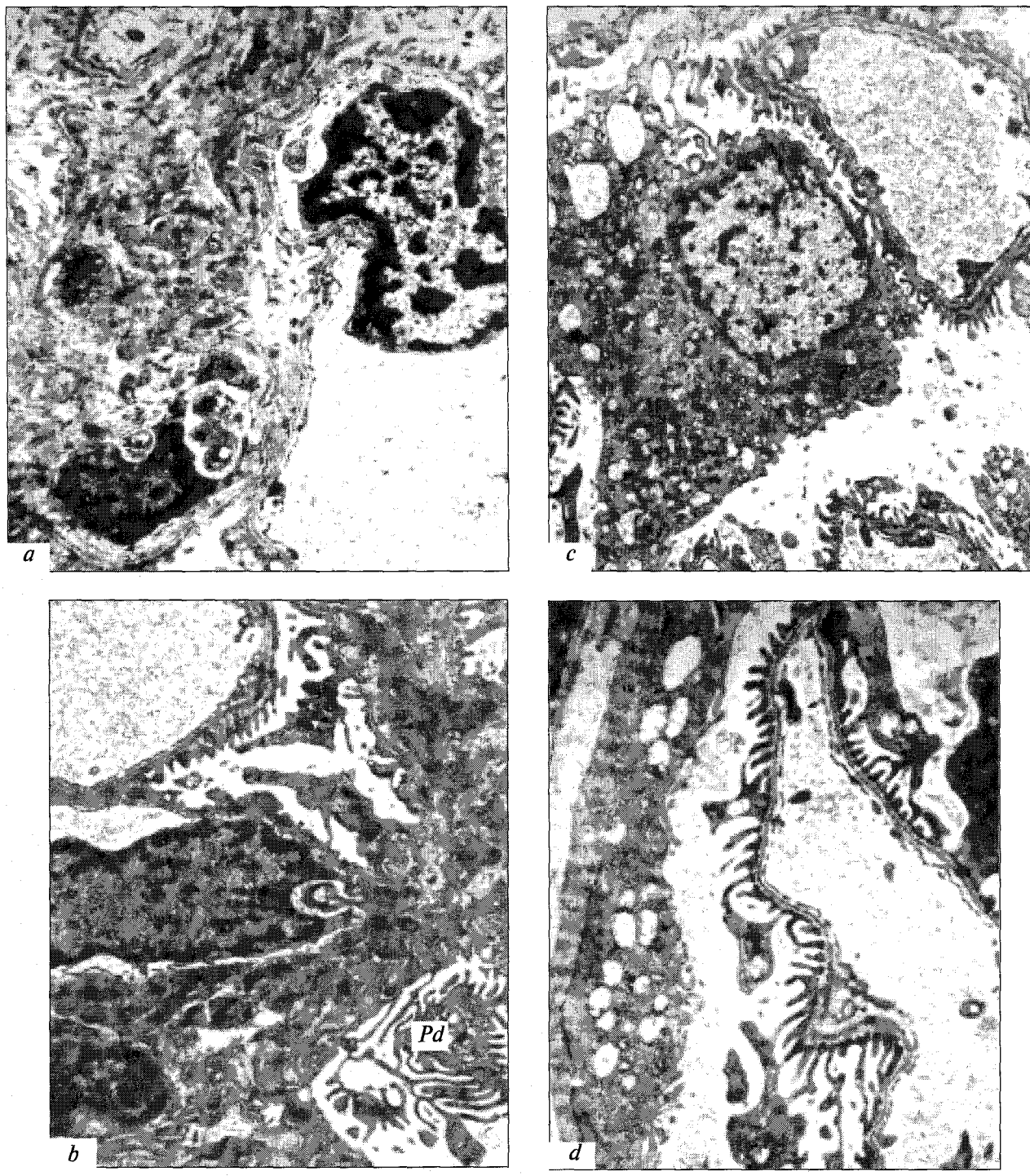

Fig. 1. Morphological changes in renal glomeruli of rabbits after 8-week immunization with IgG Fc-positive group A type M22 streptococcus, strain AL168 (*a, b*), its isogenic mutant AL168 sir-mrp- (*c*), and IgG Fc-negative type T27 streptococcus(*d*). *a, b*) Extension of the glomerular capsule, sharp thickening of basal membrane, interposition and proliferation of mesangial cells, atrophy of individual podocytes (*Pd*), ×12,000; *c*) partial hypertrophy of podocyte without signs of degenerative and proliferative changes, ×12,000; *d*) no morphological changes, ×16,000.

Morphological structure of renal tissue in these animals did not differ from normal.

Membranous glomerulonephritis developed at sites of IgG and C3 deposition with simultaneous activation and proliferation of mesangial and endothelial cells (proliferative glomerulonephritis). Presumably, activated cells express antiinflammatory cytokines IL-1β, IL-6, and TNF-α which in turn can activate production of surface antigens by these cells promoting neutrophil adhesion and stimulating procoagulant activity. Macrophages activated by TNF-α and other cytokines can involve monocytes and T cells in cell reaction and

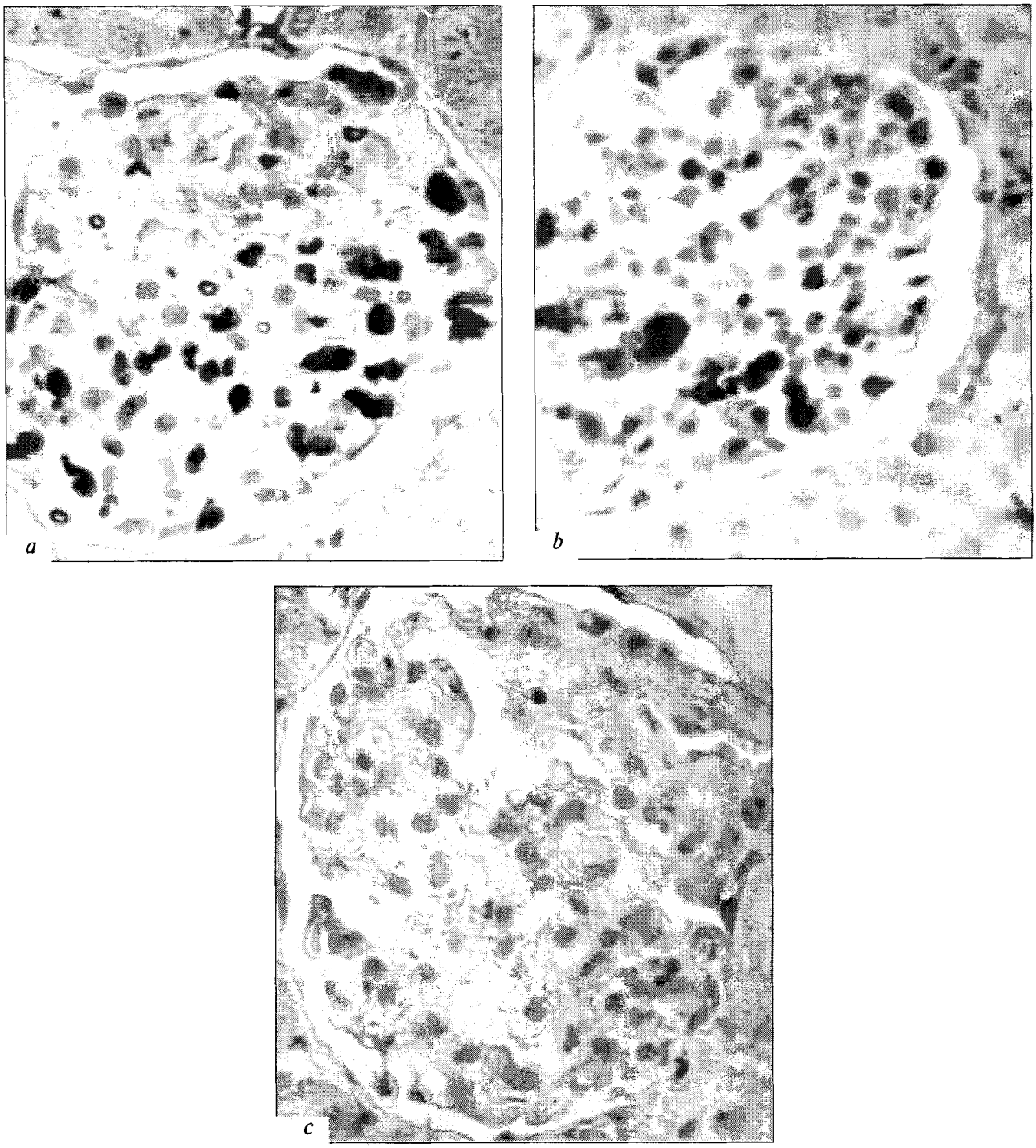

Fig. 2. Immunohistochemical changes in renal glomeruli of rabbits after 8-week immunization with IgG Fc-positive group A type M22 streptococcus, strain AL168 (*a, b*), and its isogenic mutant AL168 sir-mrp- (*c*). Coons' staining with appropriate polyclonal antibodies, ×1000. *a*) tumor necrosis factor-α (TNF-α)-positive staining mainly in mesangial cells; *b*) interleukin-1β-positive staining in mesangial cells; *c*) TNF-α-negative staining.

generate cytotoxic free radicals $O_2^{\bullet}$, $OH^{\bullet}$, and nitric oxide promoting the development of fibroplastic glomerulonephritis, which eventuates in complete atrophy of the glomerulus. Tissue neoantigens can serve as additional factors promoting the development and chronization of the immunopathological process in the kidneys.

These results and the data on the IgG-binding capacity of the majority of nephritogenic streptococci [11] suggest that streptococcal IgG Fc-binding proteins play an important role in the initiation and development of poststreptococcal glomerulonephritis. This concept was also confirmed in experiments on rabbits immunized with type M22 streptococcal strain AL168 and its isogenic mutant AL168 sir-mrp- completely devoid of genes responsible for the expression of immunoglobulin-binding Sir- and Mrp-proteins. Immunization with the original AL168 strain induced changes characteristic of membranous proliferative glomerulonephritis with deposition of IgG and C3 and positive reaction to IL-1β, IL-6, and TNF-α in mesangial cells, while after immunization with AL168 sir-mrp- mutant only functional reversible changes were noted in renal tissue without deposition of IgG and C3 or positive staining for TNF-α, IL-1β, and IL-6.

In further experiments we are planning to use various mutants of group A streptococci, devoid of genes regulating the expression of a wider spectrum of surface M proteins.

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