Induction of myocarditis in rabbits injected with group A streptococci

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Background & objectives: We have earlier proposed that group A streptococcal (GAS) immunoglobulin binding surface proteins (IgGBPs) might trigger anti-IgG production and immune complex formation leading to glomerulonephritis. In the present study, cardiac tissue material from rabbits injected with heat-killed GAS was investigated.

Methods: Rabbits were injected intravenously with 10^9 colony forming units of streptococci three times weekly for 8 wk. Cardiac tissue samples were obtained at different times and deposition of IgG, C3, TNF-α and IL-6 was studied.

Results: After 8 or more weeks of intravenous (iv) injections, minimal changes were seen in animals receiving an IgG non-binding GAS strain, type T27, whereas in those animals receiving either of two IgG binding GAS strains, types M1 or M22, strong inflammatory and degenerative myocardial changes accompanied by deposition of IgG and C3 were noted. Furthermore, on injecting rabbits with defined mutants of a type M22 strain, the development of myocardial tissue damage proved to be dependent on the presence streptococcal IgGBPs.

Interpretation & conclusion: The present data supported a role of streptococcal IgGBPs in the induction of myocardial tissue injury by GAS.

Key words Anti-IgG - experimental carditis - IgG binding protein - Streptococcus pyogenes

Acute rheumatic fever (ARF) continues to account for a major public health problem in developing countries and has also re-emerged recently in the US. As commonly suggested, rheumatic carditis may be an autoimmune phenomenon due to molecular mimicry between GAS and cardiac myosin or other tissue components. There is ample evidence that both M protein and the hyaluronate capsule are of importance in the pathogenic events leading to ARF. Many GAS M-related proteins are known to bind the Fc-part of IgG. A role of these in experimental acute glomerulonephritis triggered by GAS has been previously reported from our group. The present study was carried out to examine the capacity of IgG-binding proteins (IgGBPs) to induce myocardial inflammatory changes in rabbits injected with various GAS strains. The heart tissue of rabbits injected with various GAS strains was examined morphologically and immunohistochemically.

Material & Methods

The following streptococcal reference strains were included: group A, types M1 (40/58), M22 (10/69) and T27 (SF40), which were originally obtained from the WHO reference laboratory, Prague. In addition, the clinical type M22 isolate AL168 and the following mutants were used: AL168mrp-emm-, AL168mrp-, AL168emm. These mutants were isogenic derivates of AL168,
affected in either or both of the genes for the M family proteins Mrp22 and Emm22, and generated by transposon mutagenesis or gene replacement. All strains were from Dr G. Lindahl (Lund University, Sweden). Rabbits were injected intravenously with 10\(^6\) colony forming units (CFU) of streptococci in 1 ml PBS three times weekly for 8 wk. After one month’s rest, injections were continued in some rabbits for a period of 6 more weeks. Thirty female rabbits of Chinchilla species weighing 2.5-30 kg, obtained from the animal nursery “Rappolovo” (St. Petersburg) of the Russian Academy of the Medical Sciences, were used. All experiments with rabbits were carried out at the Department of Molecular Microbiology, Institute of Experimental Medicine according to Animal Welfare Assurance # A5243-01.

Heart tissue samples were obtained at different times from 7 animals immunized with M1(40/58), M22(10/69) or T27(SF40) and 23 rabbits injected with strain AL168 or its isogenic mutants by sacrificing randomly chosen rabbits after 4 and 8 wk of primary immunization or after 6 wk of reimmunization. Deposition of IgG was demonstrated, following fixation of the sample for 12 h in 4 per cent paraformaldehyde and deparaffinization, by goat anti-rabbit IgG antibodies followed by HRP-labelled rabbit anti-goat antibodies (Southern Biotechnology Associates, USA). Complement component C3 was detected by rabbit anti-human C3 (Daco, Denmark), cross-reacting with rabbit C3, followed by HRP-labelled goat anti-rabbit IgG antibodies (Sigma, USA). TNF-α and IL-6 were similarly detected by specific anti-rabbit TNF-α antibodies (AMS Biotechnology, USA) and goat anti IL-6 antibodies (Biosource International), respectively, followed by HRP-labelled rabbit anti-goat antibodies. The slides were viewed in an Axiomat (Opton) microscope. The details of these procedures were described previously.

For transmission electron microscopy (TEM), tissue samples were incubated with 2.5 per cent glutaraldehyde solution in 0.1 cacodylate buffer, pH 7.4, at 4° C for 3 h. Following washes in the same buffer for 24 h the blocks were fixed with 1 per cent OsO\(_4\) in cacodylate buffer, dehydrated in increasing concentrations of alcohol and propylene oxide, and embedded in araldite. The prepared material was visualized using a JEM 100B equipment.

**Results**

Strains M1(40/58) and M22(10/69) bound 78 and 35 per cent of radiolabelled IgG, respectively, in uptake tests whereas strain T27(SF40) did not bind IgG (<3%). Both M1(40/58) and M22(10/69) induced circulating anti-IgG at agglutination titres exceeding 1:80 using Ripley-coated cells, thus in contrast to T27(SF40) (<1:10). In three animals injected with T27(SF40), no tissue deposition of IgG or C3 was found. In contrast, four rabbits injected with M1(40/58) or M22(10/69) showed a high degree of deposition, located in the sarcolemma, intermyofibrillar spaces and on the capillary basement membrane and reaching a maximal intensity already at 4 wk of immunization. In the same animals, monocytes/macrophages stained for IL-6, but not for TNF-α, whereas in the animals given T27(SF40) no staining was observed.

Strain T27(SF40) did not induce any tissue alterations (Fig. d). In contrast, after 4 wk of injections with

![Fig. Degenerative changes of rabbit myocardial tissue after 8 wk immunization and 6 wk of reimmunization with GAS type M1 (40/58). (a) Strongly hypertrophic mitochondria with partial or complete disintegration of cristae; (b) destruction of reticulum of reticulum and extension of channels into sarcoplasm; (c) disintegration and ruptures of myofibrils; (d) normal myocardial morphology of rabbit immunized with IgG Fc-nonbinding GAS strain, type T27 (TEM X16,000 in a, b, d and X24,000 in c).](image-url)
M1(40/58), myocardial changes characterized by congestion and swelling of the endomysium, and at 8 wk, intense degenerative changes involving sarcoplasm and myofibrils were found. Furthermore, disintegration of mitochondria, slight degeneration of myofibrils, and a pronounced cellular inflammatory reaction were noted. At 6 wk of reimmunization, mitochondrial hypertrophy with distintegration of cristae, destruction of the sacroplasmatic reticulum, and rupture of myofibrils were noted (Fig. a,b,c). In addition, a strong cellular reaction in capillary and precapillary vessels, with accumulation of lymphocytes, granulocytes, and activated monocytes, and a picture of myofibrosis, including proliferation of perivascular connective tissue, damage of the capillary basement membrane and endothelial cells, emerged. Similar tissue changes, compatible with severe myocarditis, were observed in a rabbit receiving M22(10/69).

At 6 wk of reimmunization, mitochondrial hypertrophy with pronounced cellular inflammatory reaction were noted. Mitochondria, slight degeneration of myofibrils, and a picture of myofibrosis were found. Furthermore, disintegration of cristae, destruction of the sacroplasmatic reticulum, and rupture of myofibrils were noted (Fig. a,b,c).

Isogenic mutants of AL168, defective in the genes encoding one or both FcBs were then tested. The parent strain yielded tissue deposition patterns and myocardial changes in agreement with M1(40/58) and M22 (10/69). In contrast, there were no morphological alterations in four animals receiving the double mutant AL168 mrp-; emm-. In those animals receiving mutants AL168 mrp- or AL168 emm-, both positive for binding of IgG though to a lower level than AL168, development of myocarditis was observed in 6 out of 8 and 3 out of 7 rabbits injected, respectively. Of interest, in the animals given either of the single mutants but which did not develop myocarditis, circulating anti-IgG was not detectable, whereas in those developing pathological changes anti-IgG titres ranged between 1:20 and 1:320.

**Discussion**

In rabbits injected by either of two IgG binding GAS strains, pronounced myocardial changes, accompanied by heavy deposits of IgG and C3 and involving myofibrils, sarcolemma and other structures, were found. An IgG non-binding strain did not elicit any tissue damage. Moreover, using isogenic mutants of a type M22 IgG binding strain, induction of experimental myocarditis was found dependent on the presence of at least one IgGBP in the test strain.

Though serotype M1 may be sometimes associated with ARF, M22 and other OF serotypes are usually considered not to be rheumatogenic. Furthermore, IgG binding capacity of GAS strains is more common in OF than OF serotypes, and may therefore not be required for rheumatogenicity. Nevertheless, a role of streptococcal IgGBPs in the induction of myocardial tissue injury by GAS, was strongly supported by our data. Though ARF and APSGN are distinct complications of acute streptococcal infection and probably linked to different strains of GAS, our results suggest that, in both cases, bacterial binding of IgG might be of pathogenic importance. Furthermore, previous and present data indicate that induction of anti-IgG formation may be crucial in this context. By such triggering, streptococcal IgGBPs thus appear to be involved in the development of tissue inflammatory damage common to these streptococcal sequelae.

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**References**


