Identification of a Streptococcal Octapeptide Motif Involved in Acute Rheumatic Fever

Katrin Dinkla1, D. Patric Nitsche-Schmitz1, Vanessa Barroso1, Silvana Reissmann1, Helena M. Johansson1, Inga-Maria Frick1, Manfred Rohde1, and Gursharan S. Chhatwal1,2

From the 1Department of Microbial Pathogenesis, Helmholtz Centre for Infection Research, 38124 Braunschweig, Germany and 2Department of Clinical Sciences, Lund University, 22184 Lund, Sweden

Acute rheumatic fever is a serious autoimmune sequela of pharyngitis caused by certain group A streptococci. One mechanism applied by streptococcal strains capable of causing acute rheumatic fever is formation of an autoantigenic complex with human collagen IV. In some geographic regions with a high incidence of acute rheumatic fever pharyngeal carriage of group C and group G streptococci prevails. Examination of such strains revealed the presence of M-like surface proteins that bind human collagen. Using a peptide array and recombinant proteins with targeted amino acid substitutions, we could demonstrate that formation of collagen complexes during streptococcal infections depends on an octapeptide motif, which is present in collagen binding M and M-like proteins of different β-hemolytic streptococcal species. Mice immunized with streptococcal proteins that contain the collagen binding octapeptide motif developed high serum titers of anti-collagen antibodies. In sera of rheumatic fever patients such a collagen autoimmune response was accompanied by specific reactivity against the collagen-binding proteins, linking the observed effect to clinical cases. Taken together, the data demonstrate that the identified octapeptide motif through its action on collagen plays a crucial role in the pathogenesis of rheumatic fever. Eradication of streptococci that express proteins with the collagen binding motif appears advisable for controlling rheumatic fever.

Acute rheumatic fever (ARF) is one of the most serious diseases caused by streptococci and occurs as an autoimmune sequela of untreated or inadequately treated group A streptococcal pharyngitis (1). ARF and the subsequent rheumatic heart disease (RHD) remain significant causes of cardiovascular disease today (2, 3). The most devastating effects are on children and young adults in their most productive years (2–4). According to a recent estimate more than 15 million people have RHD, more than 0.5 million acquire ARF each year, and about 0.25 million deaths annually are directly attributable to ARF or RHD (2). The fact that penicillin has clearly failed to eradicate ARF and that streptococcal vaccines are still years away from being available underlines the need for novel control strategies (5, 6).

Identification of the pathogenic mechanisms underlying ARF is a prerequisite for the development of the necessary diagnostic and preventive approaches.

Major virulence factors of streptococci that infect humans are the M and M-like proteins. They exert anti-phagocytic effects (7–10) and facilitate streptococcal survival within polymorph nuclear neutrophils (11). Variability in the N-terminal of M proteins generated more than 100 distinct M serotypes. Rheumatogenicity of *Streptococcus pyogenes* strains, which are also referred to as group A streptococci (GAS), was found to correlate with certain M serotypes in different parts of the world (12–14), indicating that such M proteins may be directly involved in the pathogenesis of ARF/RHD. Different autoimmune mechanisms have been proposed for pathogenesis of ARF (3, 8, 15) that bases on M proteins and other surface components of GAS as causative agents and results in autoimmune response against host proteins of cardiac tissues (3, 4, 8, 15–17).

One recent hypothesis is that human collagen IV, a major component of subendothelial basement membranes (18, 19), acts as such an autoantigen after forming a complex with *S. pyogenes* strains, which have a potential of causing ARF (15). Investigations on *S. pyogenes* strains of the rheumatogenic M serotypes M3 and M18 identified both M3 protein of the M3 strain and the hyaluronic acid (HA) capsule of the M18 strain as streptococcal surface components that interact with collagen. Immunization of mice with M3 protein led to the generation of collagen IV autoantibodies, which were also found in sera of patients with ARF or RHD. Direct binding of collagen to streptococcal surface components is, therefore, considered as one of the key mechanisms for induction of ARF (15).

In geographical areas with a high incidence of ARF there is a widespread carriage of group C streptococci (GCS) and group G streptococci (GGS) (20–22). Traditionally, these serogroups are considered as important veterinary pathogens but are also involved in human infections. Their spectrum of diseases is very similar to the one of GAS, comprising pharyngitis, skin, and soft tissue infections as well as bacteremia and streptococcal toxic shock syndrome (23). Some GCS and GGS strains have
the potential to induce autoimmunity against cardiac myosin, suggesting contribution to the pathogenesis of ARF/RHD (20). This might at least partially explain the high incidence of ARF in certain indigenous populations of the Northern Territory of Australia, where pharyngeal isolation of GAS is rare but the prevalence of GCS and GGS is high (24). The mechanisms known today are not sufficient to fully explain the pathogenesis of ARF. In this study we investigated the potential of GCS and GGS to interact with collagen IV and identified the binding factors, one being the M-like protein FOG, which was described as an adhesin (25). Analysis of patient sera revealed contribution of FOG in clinical cases of ARF/RHD, indicating the role of GGS as a causative organism. By pinpointing the responsible binding motif we could gain deeper insight into the pathogenesis of ARF and discovered a promising diagnostic marker for the recognition of rheumatogenic strains.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Human Sera**—Group C and G streptococcal strains were collected in different geographic regions, with an emphasis on human isolates from the Northern Territory in Australia. The *S. pyogenes* control strain (A60) was obtained from the Institute for Medical Microbiology of the TU Aachen. Unless stated otherwise, streptococci were grown statically to late exponential phase in tryptic soy broth (Roth) at 37 °C. Human sera from patients with acute rheumatic fever and of healthy volunteers were collected in Chandigarh, India.

**Collagen Binding Assays**—Streptococci were suspended in phosphate-buffered saline (PBS) to give 10⁸ bacteria/ml. Bacteria (2.5 × 10⁷) were incubated with 30 ng (100,000 cpm) of ¹²⁵I-labeled collagen IV isolated from placenta (Sigma) for 45 min at room temperature. Bacteria were harvested by centrifugation, washed 3 times in 1 ml of PBS and incubated for 45 min at 37 °C. Human sera from patients with acute rheumatic fever and of healthy volunteers were collected in Chandigarh, India.

**Screening and Sequencing of emm and emm-like Genes**—The chromosomal DNA of all collagen binding isolates was tested by PCR for the presence of *emm* and *emm*-like genes using the primers 1 and 2 recommended by the Center of Disease Control. The obtained PCR products were subsequently sequenced using primer 1.

**Electron Microscopy**—G45 (a FOG-positive, HA-negative, and collagen binding strain) and G50 (a non-collagen binding strain) were grown statically to late exponential phase in Todd Hewitt broth supplemented with 0.5% yeast extract (THY). The bacteria (5 × 10⁷) were suspended in 500 μl of PBS and incubated with 40 μg of collagen IV at room temperature for 30 min. Samples were washed, fixed, and processed for field emission scanning electron microscopy as described before (15).

**Recombinant Proteins and Site-directed Mutagenesis**—For cloning of FOG constructs and of MLC1903, suitable PCR products were cloned into the pGEX-6P-1 vector (GE Healthcare) between the BamHI and the SalI or the BamHI and the EcoRI cleavage site. FOGfl (amino acid residues 1-557) represents the mature full-length protein. FOG1-B represents the first 278 amino acids, and FOG1-A represents the first 134 amino acids of the mature FOG protein (Fig. 2A). The plasmids coded for fusion proteins that carried an N-terminal GST tag. They were expressed in *Escherichia coli* HB101. Site-directed mutagenesis was performed on the plasmid pGEX-6P-1-FOGfl that coded for the GST-FOGfl fusion protein. *E. coli* clones containing the plasmids with the desired substitutions were generated using the GeneTailor™ site-directed mutagenesis system (Invitrogen) following the manufacturer’s recommendations. The tagged proteins from *E. coli* lysates were bound to glutathione-Sepharose 4B (GE Healthcare) and eluted with 10 mM reduced l-glutathione (Sigma) in 50 mM Tris-HCl (pH 8.0) after washing the matrix with PBS. When desired the GST tag was removed by digesting the proteins with PreScission™ protease (GE Healthcare) while bound to the affinity matrix, which eluted the untagged FOG proteins.

**Immunization of Mice**—For immunization with FOGfl and FOGB2-C2, groups of pathogen-free 8-week-old female C3H mice were injected intraperitoneally with 200 μl of an emulsion of the appropriate recombinant protein (10 μg) in PBS (2/3) and Freund’s incomplete adjuvant (1/3) at days 1, 14, and 21. Emulsion devoid of FOG was injected into mice of the control group. Serum samples were taken at day 28.

In experiments with M3.5 and FOG1-B, respectively, groups of five pathogen-free 8-week-old female BALB/c mice were immunized intraperitoneally with an emulsion of 25 μg of the regarding recombinant protein in 50 μl of PBS and 50 μl of Freund’s incomplete adjuvant per dose at days 1, 7, and 14. Control mice were injected with 50 μl of PBS and 50 μl of Freund’s incomplete adjuvant per dose. At day 21, the serum samples were collected and tested in enzyme-linked immunosorbent assay (ELISA) as described below. To absorb FOG-reactive antibodies, 10 μl of serum pool was diluted 1:50 in PBS and incubated for 1 h with 1 mg of GST-FOGfl protein immobilized on glutathione-Sepharose 4B. Antibodies that bound to this affinity matrix were removed by centrifugation. The supernatant was subjected to ELISA analysis.

**ELISA**—To determine anti-collagen IV antibody titers, 96-well plates (Greiner, Frickenhausen, Germany) were coated overnight at 4 °C with anti-human collagen IV rabbit polyclonal antibody (Progen, Heidelberg, Germany) diluted 1:100 in 0.1 M NaHCO₃, pH 9.6, blocked with 2% bovine serum albumin in PBST, and incubated with collagen IV (2 μg/ml in PBS) for 1 h at 37 °C. After washing with PBST, mouse sera or human sera diluted 1:50, 1:158, 1:500, and 1:1580 in PBS were added to the wells and incubated overnight at 4 °C. After washing, bound antibodies were detected using suitable horseradish peroxidase-coupled secondary antisera (goat anti-mouse IgG and IgM, Jackson Laboratories; rabbit anti-human IgG, IgA, and IgM, Sigma-Aldrich) and 2,2-azino-di-[3-ethylbenzthiazoline sulfonate] diaminonitrobenzene salt (ABTS tablets, Roche Applied Science) as substrate. The absorbance was determined at 405 nm. To determine anti-M3 or anti-FOG antibody titers, M3.5 or FOGB1-A (4 μg/ml in 0.1 M NaHCO₃, pH 9.6) were immobilized overnight at 4 °C followed by blocking with 1% bovine serum albumin in PBST. Wells were washed before human sera

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placenta (Sigma, 1 mg/ml in 0.1M sodium acetate) was diluted with carbodiimide hydrochloride in water. Collagen IV from human skin was activated by a 4-min injection of 0.05M N-hydroxysuccinimide, 0.2 M N-ethyl-N-(3-dimethylaminopropyl)-carbodiimide hydrochloride in water. Collagen IV from human placenta (Sigma, 1 mg/ml in 0.1 M sodium acetate) was diluted 1:25 in 10 mM sodium acetate (pH 5.2). Injection of 3 μg of C. dysgalactiae subsp. equisimilis, were examined in this study. A S. pyogenes strain (A60) of the rheumatogenic M3 serotype was included as a positive control (15). All strains were studied in a BIAcore 2000 system (BIAcore AB) using 10 mM HEPES, 100 mM NaCl (pH 7.4) as running buffer. A CM5 sensor chip was activated by a 4-min injection of 0.05 M N-hydroxysuccinimide, 0.2 M N-ethyl-N-(3-dimethylaminopropyl)-carbodiimide hydrochloride in water. Collagen IV from human placenta (Sigma, 1 mg/ml in 0.1 M sodium acetate) was diluted 1:25 in 10 mM sodium acetate (pH 5.2). Injection of 3 μl at a flow rate of 5 μl/min led to immobilization of 400–550 response units of collagen IV. Residual reactive groups were inactivated by a 6-min injection of 1 M ethanolamine, 0.1 M NaHCO₃, 0.5 M NaCl, 5 mM EDTA (pH 8.0). Collagen I was immobilized as described previously (25). Interaction measurements with FOG1-A and FOG1-B were carried out at a flow rate of 60 μl/min measurements with GST-FOGfl, mutated GST-FOGfl, and GST at a flow rate of 35 μl/min. Surface regeneration was achieved by injection of two 30-s pulses of 0.2% SDS in water. The Blaovulation 3.0 software was used for further analysis of the data. The curves shown represent the difference between the signal of the collagen-immobilized surface and of a deactivated control surface devoid of protein. They were further corrected by subtraction of the curve that was obtained after injection of buffer alone. Buffer injection led to responses less than five response units.

**Spot-synthesized Peptides**—The 15-mer peptides were synthesized on an amine-terminated cellulose membrane (AAMS Scientific Products) as described previously (26). After washing in ethanol (96%) and thereafter in PBS, the membrane was incubated for 1 h with blocking buffer (2% blocking solution from Genosys and 0.5% sucrose in PBST) at room temperature. Binding was tested by incubating the membrane with 3 μg of radiolabeled collagen IV (1,000,000 cpm) in PBST for 4 h at room temperature. Non-bound ligand was removed by washing with PBST for 1 h. Bound collagen IV was detected using an x-ray film.

**RESULTS**

**Collagen Binding Factors on GCS and GGS Isolated from Human Infections**—A total of 70 GCS and GGS strains isolated from human infections, all belonging to the species Streptococcus dysgalactiae subsp. equisimilis, were examined in this study. A S. pyogenes strain (A60) of the rheumatogenic M3 serotype was included as a positive control (15). All strains were tested for binding to radiolabeled collagen IV, revealing that 27 strains (38%) of the isolates had an ability to bind collagen IV, similar to the control strain. All strains that interacted with collagen were subjected to further analyses. Because HA capsule and M3 protein have been reported as collagen binding factors of GAS (15), the presence of such factors in the GCS and GGS strains was investigated (Table 1). Collagen binding via the HA capsule was tested by hyaluronidase treatment that abolished HA-mediated interaction. HA was found in seven of the isolates and was distributed equally among GGS and GCS. The presence of emm-related genes, coding for M-like proteins, was investigated by PCR using primers of highly conserved sequences (see “Experimental Procedures”) and subsequent DNA sequencing of the PCR products. All isolates possessed emm-related genes, which is in accordance with the observation that the presence of such genes is frequent in human isolates (27). Four of the strains were found to possess the emm-related gene fog that codes for the M-like fibrinogen binding protein of GGS (FOG) (25, 28). Because FOG was previously described as an adhesin that binds collagen I (25), these strains became the subject of further investigations. The gene fog was present in GGS isolates only. Of the four strains that were found to possess fog, two had an HA capsule that contributed to collagen binding. The interaction of streptococci with collagen IV was also analyzed using field emission scanning electron microscopy (Fig. 1), revealing that a FOG-positive human isolate negative for HA bound collagen IV and aggregated it on its surface (Fig. 1A). Taken together, the experiments allowed the conclusion that encapsulated GCS and GGS bind collagen IV via HA, but direct interaction between M-like protein FOG and collagen IV remained to be investigated.

**The N Terminus of FOG Binds Collagen IV**—For investigation of the interaction between FOG and collagen IV, three different recombinant proteins, FOGfl (amino acids 1–557),
FOG1-B (amino acids 1–278), and FOG1-A (amino acids 1–134) (Fig. 2A), were subjected to SPR measurements using collagen IV as the immobilized ligand. FOGfl (Fig. 2B) as well as the shorter fragments FOG1-B (Fig. 2C) and FOG1-A (Fig. 2D) showed concentration-dependent binding with dissociation constants of 6 nM, 3 nM, and 1.3 μM, respectively. Coherent with data on collagen I (25), these experiments indicated that the N-terminal serotype-specific region of FOG is a major collagen binding factor found in GGS isolates from human infections. Binding of FOG to collagen IV, which was shown here for the first time, is an interaction that suggests a role of FOG in ARF pathogenesis (15). Binding of FOG to collagen I (25) indicates a low selectivity within the collagen family. This is corroborated by the observation that FOG also binds to collagen type II and type III. The specificity for collagens, however, is proven by the inability of FOG to bind C1q (37), which is a serum protein with an extensive collagenous moiety (30). Moreover, FOG was found negative for binding to fibronectin (data not shown), which is, like collagens, another abundant protein of the extracellular matrix (31).

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The N Termi of FOG and M3 Induce Collagen Autoimmunity—In the case of GAS the surface protein M3 binds and aggregates collagen, leading to collagen IV autoantigenicity (15). We, therefore, immunized mice with FOGfl and a FOG fragment that lacks the collagen binding region (FOGB2-C2; amino acids 187–415). Analysis of anti-collagen IV serum titers by ELISA revealed significantly higher autoimmunity in the group immunized with FOGfl as compared with the control group immunized with PBS. Immunization with FOGB2-C2 led to serum titers at the level of the control group (Fig. 3A). The results suggested that the collagen binding N-terminal region of FOG is crucial for the induction of collagen autoimmunity. To test whether the N-terminal fragments of M3 protein or FOG were sufficient for induction of collagen IV autoimmune response, sera from mice immunized with M3.5 (amino acids 4–134) were analyzed by ELISA (Fig. 3B). The results indicated that the N-terminal region of M3 and FOG is sufficient for the induction of collagen IV autoantibodies. The results further suggested that the collagen binding motif of M and M-like proteins is conserved and plays a crucial role in the induction of collagen autoimmunity.
were analyzed. As compared with the buffer control, both groups produced substantially higher titers of anti-collagen IV antibodies. To examine the specificity of the antibodies, the collagen-IV-reactive mouse sera were pooled, and the antibody titers against collagen IV and FOG1-A, determined before and after pre-absorption with FOGfl protein, were compared. Although reactivity against FOG1-A was completely eliminated (Fig. 3C), the serum titer against collagen IV remained almost unchanged after pre-absorption (Fig. 3D), indicating that antibodies against FOG and collagen were distinct populations that did not cross-react. This demonstrated that the observed collagen autoimmunity was not caused by structural similarities of FOG (molecular mimicry) but that interaction with FOG had rendered host collagen IV an antigen. In summary, the experiments indicate that collagen binding motifs in the N-terminal serotype-specific region of FOG and M3 induce collagen IV autoimmunity.

Collagen-binding M Proteins Possess a Common Binding Motif—To map the motif of FOG protein that is responsible for the interaction with collagen, a set of 15-mer peptides was synthesized on a membrane (26) that represented the sequence of FOG1-A. Radiolabeled collagen IV as a soluble ligand was tested for binding to the membrane-bound peptides. A strong signal was obtained with peptides 17–20 that identified the common sequence ARYLQK as a collagen binding site (Fig. 5, A and B). The sequence of peptide 19 was selected for mutational analysis, where different amino acids were substituted before
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FIGURE 5. Identification of a collagen-binding peptide motif in FOG. A depicts a spot-membrane experiment with radiolabeled collagen IV as the soluble ligand. Spots 1–40 are immobilized 15-mer peptides that, shifted by 3 amino acids, represent the first 132 amino acids of the FOG protein. Strong signals were obtained with peptides 17–20, the sequences of which are displayed in B. The box indicates the amino acid motif that is present in all four peptides. The mutational analysis based on peptide 19 is depicted in C. Mutations in the given peptide sequences are indicated in bold and underlined letters. In peptide 50 the regarding sequence was rearranged arbitrarily. In D peptide 19 is shown together with its position in the mature protein (superscript). Amino acids found to be crucial for collagen IV binding in the spot membrane experiments are indicated in bold letters.

synthesis on the membrane (Fig. 5C). The binding experiment indicated that the sequence ARYLKX is essential for the interaction with collagen IV (Fig. 5D). Sequence comparison revealed considerable homology between the N termini of FOG1-A and M3 protein (25.4% identity, 118-amino acid overlap), a region that is hypervariable between different *emm* types. Notably, the collagen binding peptide of FOG belongs to a patch of conserved amino acids (Fig. 6A). Because the surrounding protein structure may influence the binding site, we examined the collagen binding property of this motif in its native structural environment also. Targeted substitution of the amino acids conserved between FOG and M3 was performed using site-directed mutagenesis to generate mutants of the full-length protein FOGfl. Collagen binding of the mutants formed using site-directed mutagenesis to generate mutants of the amino acids conserved between FOG and M3 was performed using site-directed mutagenesis to generate mutants of the full-length protein FOGfl. Collagen binding of the mutants was compared with the wild type protein in dot blot and SPR analysis (Table 2). Individual substitution of Ala^{38}, Tyr^{60}, Leu^{64}, Asn^{65}, LND^{66}, and YL^{61} strongly diminished or completely abolished binding to collagen IV. Moreover, comparison with wild type FOG in SPR measurements demonstrated that binding to collagen I also was abolished by the YL^{61} mutation (Table 2), indicating that both collagen I and IV bind to the same site. Taken together, the results of spot membrane and site-directed mutagenesis led to the identification of an octapeptide AXYLZZLN as the collagen binding consensus motif (X, Z: variable amino acid). This finding was strongly supported by the *emm* sequence analysis of the collagen binding isolates. All strains found positive for collagen binding after hyaluronidase treatment possess a PARF (peptide associated with rheumatic fever) motif. Strains that did not bind collagen were negative for PARF. Searches in public data bases also discovered several M proteins that contained such sequences, among them type *emmG1*, stg4545, *emmLC1903*, and *emmLC1904* from GGS and GCS and *emm55* from GAS.

To test our prediction, a GCS strain of *emm*-type LC1903 (AEYLQRLN) was examined and found positive for collagen binding. The respective M protein MLC1903 was expressed recombinantly and found positive for binding to collagen IV both in dot blot and SPR measurements (data not shown).

The fact that MLC1903 binds to collagen allows further conclusions about the nature of the collagen-binding peptide motif. In both FOG and M3 one of the positions denoted by Z is occupied by lysine (Fig. 6). In MLC1903 lysine is replaced by arginine, which has a similar character. Alanine substitution demonstrated that Lys^{61} was important for an efficient interaction of the synthetic peptide 19 (Fig. 5, C and D), suggesting that lysine in one of the Z positions is required to form a collagen binding motif. Binding of collagen IV to MLC1903 indicates that lysine can be substituted by arginine without a loss of collagen binding.

### TABLE 2

| Recombinant protein | Binding to collagen IV | Dot blot | SPR |
|---------------------|------------------------|----------|-----|
| FOGfl               | + + + +                | ++       | ++  |
| Glu^{38} → Ala      | −                      | −        | −   |
| Ala^{38} → D        | +                      | −        | −   |
| Tyr^{60} → Ala      | −                      | −        | −   |
| Leu^{64} → Ala      | + +                    | +        | +   |
| Asn^{65} → Ala      | −                      | −        | −   |
| Asp^{66} → Ala      | + +                    | +        | +   |
| LND^{66} → AAA      | −                      | −        | −   |
| YL^{61} → AA        | −                      | −        | −   |

| Binding to collagen I | Recombinant protein |
|----------------------|---------------------|
|                      | FOGfl               |
|                      | YL^{61} to AA       |

*ND: not determined.*
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DISCUSSION

ARF and the subsequent RHD are well known autoimmune sequelae of GAS infections of the upper respiratory tract (32). For GAS, epidemiological data suggest that M proteins may have a crucial role in their common pathogenesis since rheumatic potential can be attributed to certain M serotypes (14). This was corroborated by the finding that M proteins of ARF-associated strains such as M5 and M6 can cause autoimmune responses in the host through molecular mimicry by reacting against cardiac myosin and other extracellular matrix proteins with a coiled-coil structure (17, 33). ARF-associated strains of serotype M3 bind collagen directly via M3 protein, thereby inducing autoimmunity against collagen IV. The clinical relevance of collagen IV autoimmunity in ARF was indicated by high titers against collagen IV in patient sera (15). Here we show that not only GAS but also other β-hemolytic streptococci are capable of causing ARF by induction of collagen autoimmunity. The M-like protein FOG was identified as the group G streptococcal surface protein that is capable of binding different members of the collagen family (Fig. 2, Table 2, and Ref. 25), thereby inducing the collagen autoimmunity response in mice. The low selectivity of FOG within the collagen family suggests that the autoimmune response is not caused by collagen IV only. Other collagen types that interact with FOG may be involved. Considering the high similarity between collagens, it seems likely that the autoimmunity against collagen IV at least partially depends on cross-reactivity being part of a broader anti-collagen response. The collagen antibodies, however, did not cross-react with FOG, indicating that they were not the result of molecular mimicry (Fig. 3, C and D). Evidence for the clinical relevance of FOG-induced collagen autoimmunity came from the analysis of human sera. The majority of sera from ARF patients with increased levels of collagen autoantibodies also had elevated titers against FOG (Fig. 4), which indicated the contribution of FOG-positive strains in ARF in Chandigarh, India during the period of sample collection. Moreover, it presents the first evidence of group G streptococcal involvement in clinical cases of ARF, broadening the spectrum of the causative bacteria.

Previous data (15) and the data presented here indicate that M and M-like proteins are active in ARF pathogenesis but do not exclude crucial roles of other streptococcal factors. GAS strains of serotype M18 were temporally associated with repeated outbreaks of ARF in Utah (34). Such strains were highly encapsulated by HA that, because of its ability to bind collagen (15), could also be a rheumatogenic factor. Experimental evidence for the induction of collagen autoimmunity by HA, however, is missing hitherto. Our data demonstrate that some strains of GCS and GGS are able to bind collagen via their HA capsule, but a rheumatogenic potential of such strains remains to be studied.

Early work has shown that ARF is almost invariably caused by strains of certain M serotypes when infecting the upper respiratory tract. For certain populations with a high incidence of ARF, i.e. indigenous populations in the Northern Territory of Australia, it has so far not been possible to get a clear-cut picture as described above. Pharyngeal carriage of GAS was found to be rare (35) as compared with throat carriage of GCS and GGS (21). The M serotypes previously reported as rheumatogenic (14) were rarely isolated (36). Skin infections with GAS, however, are common in this region, raising speculations about their possible contribution in causing ARF that are controversial to what is observed in other parts of the world (29, 32). Previous work on streptococcal isolates from the indigenous population that examined GAS pyoderma isolates as well as GCS and GGS isolated from healthy children, however, indicated that none of the GAS isolates, but some strains of GGS and GCS, induced autoimmunity in mice. Thus, GCS and GGS may be causative pathogens for ARF in the indigenous population (20). By studying patient sera and the effects of M proteins on collagen autoimmunity in mouse experiments, we could show that GGS and GCS, like GAS, have a rheumatogenic potential. Moreover, we provided evidence that a herein identified octapeptide motif plays a crucial role in rendering collagen an autoantigen and, subsequently, in the pathogenesis of rheumatic fever. The peptide was, therefore, designated PARF. It is yet to be seen if the surface proteins of other bacterial species possess PARF and thereby play a role in rheumatic fever. Re-evaluations of existing data or new epidemiological studies, which take these findings into account, may shed more light on regional characteristics of ARF pathogenesis and help to identify the causative bacteria.

We conclude that successful prevention of ARF requires thorough treatment not only of GAS infections but also of infections with GCS and GGS. We recommend PARF as a diagnostic marker for the early detection of rheumatogenic strains.

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