A Novel Monoclonal Antibody against FbaA Can Inhibit the Binding of the Complement Regulatory Protein Factor H to Group A Streptococcus

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Received 6 October 2010/Returned for modification 3 November 2010/Accepted 29 December 2010

Some microbial pathogens utilize human complement regulatory proteins, such as factor H (FH) and factor H-like protein 1 (FHL-1), for immune evasion. FbaA is an FHL-1 and FH binding protein expressed on the surface of group A streptococcus (GAS), a common agent of pharyngeal, skin, and soft tissue infections. In this study, we prepared monoclonal antibodies (MAbs) against FbaA, assayed them for specificity, and located their binding domains in FbaA. We found an MAb called FbaA MAb2, which demonstrated the highest affinity to GAS among all of the MAbs. Based on the binding with component peptides, the detected epitope, which was specific for FbaA MAb2, was the amino acid residues 95 to 118 of FbaA; on the other hand, it did not bind with the truncated protein of the internally deleted residues of the segment from 95 to 118 of FbaA. Furthermore, the predominant amino acids specific for FbaA MAb2 screened by phage display epitope library were I, T, P, D, and L, corresponding to the amino acid residues 101, 103, 105, 106, and 110 of FbaA, respectively. The binding location of FbaA with FH and FHL-1 was a 16-amino-acid region corresponding to amino acid residues 97 to 112 of FbaA, which overlapped the FbaA MAB2 binding domain, as confirmed by competitive inhibition enzyme-linked immunosorbent assay and immunofluorescence microscopy. Based on the results of the invasion assay, FbaA MAb2 can inhibit the binding of FH to GAS.

Group A streptococcus (GAS), a major human pathogen, can persist within the human nasopharynx and intestinal tract, causing a variety of purulent inflammations, scarlet fever, erysipelas, neonatal sepsis, meningitis, and allergic diseases, such as streptococcal allergic disease (6). Up to now, although antibiotics, e.g., penicillin, have been the major choice against streptococcus, some GAS infections still cannot be completely cured, and people may be infected by the pathogen repeatedly because of the increase in drug-resistant bacterial strains and the immune escape. The common pathway for the development of pathogen immunity is to evade complement attack and opsonophagocytosis, which is often influenced or dictated by a pathogen’s ability to bind complement regulatory proteins (1, 7, 8, 12, 13, 15, 17, 28). Cleary et al. (2) first proposed that GAS invades cells, shielding the bacterium from antibiotics and the immune system. In addition, Horstmann et al. (11) also confirmed that the acquisition of complement regulatory protein factor H (FH) by GAS contributes to the bacterium’s capacity to evade phagocytosis by polymorphonuclear leukocytes (PMNs).

Pandiripally et al. (26) have identified FbaA, which is expressed by a serotype M1 GAS isolate, 90-226, as the protein that mediates the binding of both human complement regulatory proteins FH and factor H-like protein (FHL-1) (25). FbaA is the first non-M-like protein of GAS that has been shown to bind these complement regulatory factors. Terao et al. have reported that the fba gene is present in GAS, such as the M1, 2, 4, 9, 13, 22, 28, 44, 49, 60, 67, 75, 77, 79, 80, 82, 87, and 89 serotypes; among different serotypes, FbaA protein has high homology (25). Pandiripally et al. (26) also confirmed the functions of FbaA in promoting the entry of GAS into the cytoplasm of human epithelial cells through the binding of FH and FHL-1, suggesting that the protein contributes to GAS survival in its host (27, 32). We have recently observed that FbaA has a strong immunogenicity and can induce protective immunization against GAS challenge in mice (5). Therefore, FbaA potentially plays an essential role in streptococci survival, virulence, and pathogenicity in vivo.

To explore further the pathogenesis and therapy of GAS infections, we focused on preparing monoclonal antibodies (MAbs) against FbaA, characterizing the local sites of FbaA to which the MAbs are specific, and evaluating the biological functions of MAbs. We successfully isolated a hybridoma clone, hereafter referred to as FbaA MAb2, which showed a high affinity in binding FbaA+ GAS. We also found that the epitope specific for FbaA MAb2 overlapped the FH binding site in FbaA. The antibody, FbaA MAB2, could be a novel tool in the study of pathogenesis and could be a new target in the diagnosis and immunotherapy of GAS.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture media. The bacterial strains used in the present study were FbaA+ Streptococcus pyogenes strain, 90–226 emm1:km (4), and an isogenic FbaA− strain, DC276 (25), hereafter referred to as FbaA+ and FbaA− GAS, respectively. Streptococci were grown in Todd-Hewitt broth containing 1% yeast extract (Difco Laboratories, Detroit, MI). The solid media used for streptococci contained sheep blood agar. Escherichia coli DH5α (Ta-kara) was used for cloning the fbaA fragments and the maintenance of plasmids. E. coli strain BL21 (Stratagene) was used for protein expression; these samples were cultured in Luria-Bertani (LB) broth or on LB agar (29) containing 100 μg of ampicillin per ml, as appropriate.

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† Published ahead of print on 12 January 2011.
The FbaA 119-161 coding region was amplified with primer 2 and the reverse of FbaA68-161. PCR products were digested with BamHI and EcoRI, gel purified, and the reverse primers contained EcoRI restriction sites. The internally deleted amino acid residues 95 to 118 of FbaA68-161, hereafter referred to as FbaA95-118 with the forward and reverse primers of FbaA95-118. The forward and reverse primers of FbaA95-118 were constructed based on the structural and functional domains of FbaA (Fig. 1); these were then expressed and transferred to the polyvinylidene difluoride (PVDF) membrane (Bio-Rad) at 10 μg.

| Oligonucleotidea | Sequence (5’−3’) |
|------------------|-----------------|
| Fba57-324        | GGTCGGTGCAGTTTT |
| R                 | GCACCAGACAAAGATAAGCTA |
| Fba57-110        | TGTTGAAGGCAAGTAC |
| R                 | GAAACGTATAAACAAAAAATTA |
| Fba60-161        | R..................................GGCGGAATCATGGCTAAGTTT |
| F                 | R..................................GCCGG \_A \_A \_A \_A \_A \_A |
| Fba104-277       | R..................................GCCGGATCACAACAGAGGCTG |
| F                 | R..................................GCCGGATCACAACAGAGGCTG |
| Fba160-324       | R..................................GCCGGATCACAACAGAGGCTG |
| F                 | R..................................GCCGGATCACAACAGAGGCTG |
| Fba205-113       | R..................................GCCGGATCACAACAGAGGCTG |
| F                 | R..................................GCCGGATCACAACAGAGGCTG |
| Primer1          | R..................................GCCGGATCACAACAGAGGCTG |
| Primer2          | R..................................GCCGGATCACAACAGAGGCTG |
| Fba34-101        | R..................................GCCGGATCACAACAGAGGCTG |
| F                 | R..................................GCCGGATCACAACAGAGGCTG |
| Fba40-114        | R..................................GCCGGATCACAACAGAGGCTG |
| Primer1          | R..................................GCCGGATCACAACAGAGGCTG |
| Primer2          | R..................................GCCGGATCACAACAGAGGCTG |
| Fba101-118       | R..................................GCCGGATCACAACAGAGGCTG |
| F                 | R..................................GCCGGATCACAACAGAGGCTG |

a F, forward; R, reverse.

b Relevant restriction endonuclease cleavage sites are indicated in boldface, and the complementary oligonucleotides are underlined.

| Sequence (5’−3’) |
|-----------------|
| Fba119-161      | R..................................AGT |
| F                 | R..................................CGGG |
| Fba101-118      | R..................................CGCG |
| F                 | R..................................GCG |
| F                  | R..................................GCCGG |
| Fba37-110       | R..................................TATA |
| F                 | R..................................TTAA |

Table 1. Sequences of the oligonucleotides used in this study

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Cloning and expression of recombinant FbaA proteins. Plasmids encoding truncated or internally deleted FbaA proteins were constructed. Regions of fbaA (accession number AB046356) were amplified by PCR using pGEX-2T-FbaA as the template (5). The oligonucleotide primers used in the present study are listed in Table 1. Forward primers for cloning of truncated FbaA proteins contained BamHI restriction sites, and the reverse primers contained EcoRI restriction sites. The internally deleted amino acid residues 95 to 118 of FbaA119-161, hereafter referred to as FbaA95-118, were also constructed. The forward primer of FbaA95-118 with the forward and reverse primers of FbaA95-118 were used to amplify the coding region for FbaA95-118. The FbaA119-161 coding region was amplified with primer 2 and the reverse primers of FbaA119-161. DNA fragments of both FbaA95-118 and FbaA119-161 were used as templates to amplify FbaA95-118. With the forward and reverse primers of FbaA95-118, PCR products were digested with BamHI and EcoRI, gel purified, and ligated with BamHI- and EcoRI-restricted pGEX-2T. All constructs were verified by DNA sequencing. The recombinant proteins were expressed as fusions to GST, which was removed by thrombin cleavage. Thrombin was removed by chromatography on benzamidine-Sepharose (GE Healthscience) (5). The proteins were dialyzed against phosphate-buffered saline (PBS). Protein concentrations were determined by using the bicinchoninic acid method (Pierce Chemical Co., Rockford, IL).

Immunization of mice. In all, five adult female BALB/c mice, 8 to 10 weeks old, were subcutaneously injected with purified FbaA (approximately 50 μg per mouse), which was emulsified in a 1:1 ratio with complete Freund adjuvant (Sigma-Aldrich). The mice were screened for immune responsiveness by purified FbaA through indirect enzyme-linked immunosorbent assay (ELISA) 10 days after the third immunization.

Indirect ELISA. Briefly, 96-well microtiter plates were coated with purified FbaA (10 μg/ml) in carbonate buffer at 4°C overnight and then blocked with 3% bovine serum albumin (BSA) in PBS overnight. Serial dilutions of sera from immunized mice were added to each microtiter well with 100 μl and incubated at 37°C for 2 h. After incubation, the wells were washed thrice with PBS and 0.05% Tween (PBST). Upon removing the unbound antibody, wash buffer containing a horseradish peroxidase (HRP)-labeled second antibody was added, and the incubation was repeated. Finally, the wells were washed, after which o-phenylenediamine (OPD; 10 mg of OPD in 10 ml of 0.1 M sodium acetate buffer [pH 6] with 10 ml of 30% H2O2 was added. The reaction was stopped with 1 M H2SO4 (50 μl/well), and the plate was read at 490 nm. Positives were indicated by a fold increase in absorbance at 490 nm between the sample and negative control. Afterward, the IgG titers were expressed as the reciprocal of the last sample dilution, giving an absorbance at least 2-fold higher than that of the preimmune sample with an optical density of ≥0.15.

Cell fusion and hybridoma preparation. Prior to cell fusion, mice with the highest antibody titer were boostered with 50 μg of FbaA proteins without adjuvant for 3 successive days; these mice were then sacrificed by cervical dislocation. Spleens were removed from the mice, and single-cell suspensions were prepared. Splenocytes and SP2/0 myeloma cells were mixed at a 10:1 ratio and centrifuged to form a cell pellet for cell fusion as described in a previous study (9). Upon reaching 75% cell confluance, the culture supernatants were isolated and tested for the presence of FbaA immune responsiveness by indirect ELISA (see above for a description).

Screening hybridoma supernatants. FbaA immune responsive cell cultures of interest were cloned in limiting dilution cultures at one cell per microtiter well (9). After cell expansion, the supernatants were screened by indirect ELISA. Of the nearly 2,000 supernatants that were screened, 4 positive clones were identified. The positive clones were subcloned on 96-well microtiter plates at 0.3 cells per microtiter well. Supernatants from the subclones were also screened for verification. Positive subclones were expanded and cryopreserved. BALB/c spleen cells served as a feeder (9 layers for fusions (5 × 10^6 per well), as well as cloning and subcloning (3 × 10^4 cells/well).

Isotype of MAbs. The four positive FbaA MAbs were isotyped using IsoStrip, a mouse MAb isotyping kit (Roche Molecular Biochemicals, Indianapolis, IN). Briefly, 150 μl of supernatant from each of the four FbaA MAbs was added to the bottom of individual test tubes containing latex beads coated with anti-mouse kappa or lambda antibodies. An IsoStrip containing bands of goat anti-mouse IgG, IgM, and IgA isotyping antibodies was added. Mouse IgG, IgM, and IgA bands were identified by a blue band on the IsoStrip at a specific subclass and light chain indicated the antibody isotype.

Preparation of mouse ascites fluids containing MAbs. We pretreated mice by intraperitoneal injection of Pristane (2,6,10,14-tetramethylpentadecane; Sigma-Aldrich) 1 week later, we injected the positive mice with the positive FbaA monoclonal hybridoma cell line cells, at 10^6 cells per mouse, into the peritoneal cavities of mice. After 2 weeks, the ascites fluid was drawn from the mouse peritoneal cavities, centrifuged (1,500 × g, 10 min), and stored at −80°C.

Purification of MAbs. Chromatography was performed on a Biologic DuoFlow FPLC system (Bio-Rad) (9). Briefly, Hybridoma supernatants and ascites were filtered and loaded onto 5 ml of protein G- and 5 ml of protein A-Sepharose FF HiTrap columns (GE Healthscience) connected in series and equilibrated in phosphate buffer (pH 7.0, loading buffer). The flowthrough media were saved, and the columns were washed with loading buffer until the absorbance at 280 nm reached baseline. IgG was eluted with 100 μM glycine (pH 2.7) into tubes containing 1 M Tris (pH 9) for pH neutralization. The IgG fractions were buffer exchanged into PBS on a HiPrep desalting column 26/10 (GE Healthscience). Western blot. The FbaA truncated proteins—FbaA37-110, FbaA95-118, FbaA104-277, and FbaA160-324—were constructed based on the structural and functional domains of FbaA (Fig. 1); these were then expressed and transferred to the polyvinylidene difluoride (PVDF) membrane (Bio-Rad) at 10 μg. The filter was blocked overnight with 5% albumin (Sigma Chemical Co.) in PBS. The filter was incubated successively with purified MAbs and HRP-labeled second antibody after a washing step. The reaction was developed with enhanced chemiluminescence (ECL) reagents (Amersham Biosciences) and exposed to X-ray film.

To analyze the MAb specificity further, three segments containing FbaA37-110, FbaA95-118, and FbaA101-118 were expressed by pGEX-2T as fusions to GST. Afterward, these...
proteins were transferred to a PVDF filter for Western blot analysis as described above.

**Bacteria solution ELISA.** FbaA+ or FbaA− GAS strains were grown in an optically dense at 560 nm (OD560) of 0.5. Bacteria were isolated by centrifugation, washed twice with PBST, and then suspended in carbonate buffer to ~10^9 CFU/ml. Each well of the 96-well plates was coated with 0.1 ml of bacteria solution in carbonate buffer at 4°C overnight. Upon removal of unbound cells, the wells were blocked with PBST for 60 min at 37°C. To detect the binding of MABs, the plates were incubated successively with MABs and HRP-labeled second antibody to perform indirect ELISA as described above. The data were folded from three independent experiments, where each strain was assayed thrice.

**Screening epitope of MAB with phage display library.** To define the MAB epitopes, we screened a library of circularized 7-mer peptides for high-affinity binding to random peptides that were specific for individual MABs. The displayed heptapeptides were expressed at the N terminus of the III minor coat protein of M13 Phage (New England Biolabs, Inc.). Screening of the phage library was performed according to the method described in the manufacturer’s instruction manual. Candidate peptides were selected by three rounds of iterative affinity selection, after which the specificity of phage for individual MAB was assessed by competitive ELISA. Briefly, the ELISA plate wall was coated with 0.1 μg of purified FbaA, blocked with 1% BSA in PBS, and then washed with PBST. Serial dilutions of phages, including 2 x 10^6, 2 x 10^7, and 2 x 10^8 PFU, were respectively mixed with 0.0025 g of MAb. Subsequently, mixed solutions were added to the plates, and control wells were included that contained the antibody solution. After a washing step, the plates were incubated with a HRP-conjugated second antibody as described above in the indirect ELISA. Phages that specifically bound individual MABs were identified. The random inserts were then sequenced.

**Competitive ELISA.** Competitive inhibition of FH binding to immobilized GAS by FbaA MAB2 was assayed as previously described in the bacterial ELISA with some modification. Bacteria were harvested from log-phase cultures, collected by centrifugation, and suspended to an OD_560 of 0.05 in carbonate buffer. Wells of the microtiter plates were coated with suspensions of bacterial cells after a blocking step with gelatin. Afterward, 100 μl of wash buffer containing 3 μg of purified MAB was added to each well. For a control, only the wash buffer was added to wells coated with bacterial cells. After a washing step, 100 μl of wash buffer containing 1 μg of FH was added to all of the wells, except for the blank control, and the plates were incubated for 2 h at room temperature. After the wells were washed, HRP-labeled anti-FH antibody was added to the wells, and the absorbance values were determined. The data were collected from three independent experiments, in which each strain was assayed thrice.

**Immunofluorescence microscopy.** To estimate further the relationship between FH and FbaA MAB2 binding sites on the surfaces of streptococci, bacteria were harvested from log-phase cultures, washed twice with PBS, and diluted to ~10^9 CFU/ml. The suspensions were applied to the empty wells of the chamber slides (Nalge Nunc International, Naperville, IL), and unbound bacteria were removed by washing with PBS. Slides were incubated with 10 μg of FH/ml, whereas the control was incubated only with PBS. Upon removal of the unbound FH, all of the slides were incubated successively with 5 μg of FbaA MAB2 and goat anti-mouse IgG labeled with fluorescein isothiocyanate (FITC)ml. The specimens were examined by fluorescence microscopy for FbaA MAB2 binding on GAS.

**Invasion assay.** A 24-well plate was inoculated with A549 cells at approximately 5 x 10^5 cells/well, followed by incubation at 37°C in 5% CO2 for 2 days. FbaA+ GAS diluted to approximately 5 x 10^6 CFU were incubated with 6 μg of FbaA MAB2 overnight at 4°C and then with 10 μg of FH per ml for 1 h at room temperature and then centrifuged to remove the supernatant. FbaA+ GAS were incubated with FH or PBS in the same conditions as the FH or the no-FH control. The pellets were suspended in appropriate RPMI 1640, transferred to one well of the plate, and then incubated for 2 h at 37°C. Some of the no-FH control cells were harvested after being washed twice with PBS; the trypsinized cells were transferred to sterile water as an attached bacteria control, and other cells were further incubated with RPMI 1640 containing 1,000 U of penicillin and 500 U of gentamicin/ml for 2 h at 37°C and subsequently harvested and transferred to sterile water as described above. The lysates were diluted and plated onto blood plate overnight at 37°C. The number of bacteria CFU was determined the next day. Indeed, the attached bacteria control was expressed as the number of bacteria CFU from the cells harvested prior to incubation with antibiotics minus the CFU number from the no-FH control.

**RESULTS**

**Production and screening of MABs.** Full-length FbaA fused to an N-terminal GST tag was purified by chromatography on glutathione-Sepharose, followed by GST reduction with thrombin and was used to immunize BALB/c mice. After fusing the splenocytes of the mice and SP2/0 myeloma cells and screening more than 2,000 single-cell supernatants, four positive hybridomas were selected, cloned, and purified based on the secreted antibody recognition of FbaA, which was performed by indirect ELISA. The four MABs (i.e., FbaA MAb1, FbaA MAb2, FbaA MAb3, and FbaA MAb4) were isotype as IgG1k. The IgG titers of the supernatants from the four hybridomas were 1:426 (FbaA MAb1), 1:586 (FbaA MAb2), 1:533 (FbaA MAb3), and 1:533 (FbaA MAb4). The IgG titers of the ascites were 1:128,000, 1:341,000, 1:426,000, and 1:213,000, respectively.

**Detection of MABs binding to FbaA+ GAS or FbaA− GAS.** The ability of the four MABs to bind with FbaA+ GAS or FbaA− GAS was determined by bacteria ELISA. FbaA MAB2 bound with FbaA+ GAS with the highest specificity, whereas FbaA MAB2 did not bind with FbaA− GAS specifically.

**Characterization of antibody binding sites.** To identify the binding sites of the monoclonal antibodies, FbaA proteins divided into four overlapping peptides based on the structural domains, were expressed. The four truncated peptides were amino acids 37 to 110, 68 to 161, 104 to 277, and 160 to 324, respectively (Fig. 2A). Western blot analysis showed that two antibodies (i.e., FbaA MAb2 and FbaA MAb3) only recognized the truncated FbaA_104-277 and not FbaA_104-277 (Fig. 2B). Thus, the epitopes for FbaA MAb2 and FbaA MAb3 were both located in a narrow region and encom-
The major binding site of FH in FbaA is a linear 16-amino-acid region encompassing FbaA residues 95 to 118 (26), indicating that the FH binding site overlaps the FbaA MAb2 binding site (positions 95 to 118) in FbaA. Therefore, to verify whether FbaA MAb2 affects the binding of FH to FbaA, a competitive-inhibition ELISA was performed. The results revealed that FbaA MAb2 could block the binding of FH with GAS (Fig. 4).

To verify the presumption that the FbaA MAb2 binding site is located in the amino acid residues 95 to 118 of FbaA, a fragment of the fbaA gene that deleted the amino acid residues 95 to 118 of FbaA_MAb2 was amplified by PCR and expressed in E. coli (Fig. 1F and Fig. 3A). As expected, FbaA MAb2 was unable to recognize the recombinant FbaA_MAb2 protein by Western blot analysis (Fig. 3B), whereas the FbaA MAb2 showed an insignificant blot with the positive control FbaA_MAb4.

The role of FbaA MAb2 inhibition in FbaA+ GAS epithelia cell invasion was also evaluated. FbaA+ GAS were incubated successively with FbaA MAb2 and FH and then centrifuged to remove the supernatant. FbaA+ GAS were only incubated with FH or PBS at the same condition as the FH or no-FH control. After these pellets were incubated with A549 cells for 2 h, some cells of no-FH control were harvested prior to incubation with 10-fold antibiotics as an attached bacterial

To define further the predominant amino acids specific for FbaA MAb2, phage 7 peptide library was used to screen the random peptides that showed a high-affinity binding to FbaA MAb2. The predominant amino acids specific for FbaA MAb2 were I, T, P, D, and L, which were located in the region from amino acid residues 100 to 110 of FbaA. Residues 101, 103, 105, 106, and 110 were predicted as essential for FbaA MAb2 recognition.
through intracellular invasion (6). Particularly, streptococcal organism to evade ingestion and/or killing by host phagocytes. Therefore, a major virulence trait of GAS is the capacity of the bacteria to evade immune factors and antibiotics (3, 22, 31), thereby protecting GAS from the innate immune system. This results in GAS dissemination in an infected host (20).

The persistence within mammalian cells (4, 18, 21, 24, 25) is important and highly versatile in GAS disease by affording partial protection against the human complement regulatory proteins, such as FH, FHL-1, and C4b binding protein (11, 14, 16), to its surface proteins; among these, FbaA protein and M protein play potential roles that mediate binding both FH and FHL-1 (25).

To further investigate the biological function of FbaA and to explore the pathogenesis of GAS, we prepared the MAbs against FbaA protein and detected their binding domains after Western blotting. Based on the structural and functional domains (Fig. 1), FbaA proteins were divided into four overlapping peptides, expressed and blotted with the four selected antibodies respectively by Western blotting, and the results suggested that two of the antibodies recognized the N terminus (amino acids 68 to 161) and two others identified the C-terminal region between amino acids 161 and 277 (Fig. 2). These antibodies are useful in clarifying FbaA protein localization and future experimentation in determining the biological function of the FbaA protein itself. FbaA MAb2 showed the highest affinity with FbaA + GAS, leading us to locate precisely the epitope for FbaA MAb2. Thus, the segments of FbaA (amino acid residues 95 to 114 and 101 to 118), control peptides (amino acids 84 to 101), and FbaA X95-118 (Fig. 1F) with deleted corresponding epitopes for FbaA MAb2 were constructed, expressed according to the Western blot results (Fig. 2B) and bioinformatics prediction, and blotted with FbaA MAb2 using Western blotting again. The result showed that FbaA MAb2 recognized the amino acid residues 95 to 114 and 101 to 118 but could hardly bind amino acid residues 84 to 101 and could not recognize FbaA X95-118 at all. Thus, the epitope specific for FbaA MAb2 was found to be located in the region from amino acids 95 to 118 of FbaA.

The Ph.D-7 phage display peptide library (10, 30) was used to determine the predominant amino acids of the epitope for FbaA MAb2, which were I, T, P, D, and L, corresponding to amino acids 95 to 118 (Fig. 2F). This suggests that these amino acid residues may be essential for FbaA MAb2 recognition. Through the bioinformatics prediction, and blotted with FbaA MAb2 using Western blotting again. The result showed that FbaA MAb2 recognized the epitope for FbaA MAb2 was found to be located in the region from amino acids 95 to 118 of FbaA.

The Ph.D-7 phage display peptide library (10, 30) was used to determine the predominant amino acids of the epitope for FbaA MAb2, which were I, T, P, D, and L, corresponding to amino acids 95 to 118 (Fig. 1F). This suggests that these amino acid residues may be extremely essential for FbaA MAb2 recognition. Through the bioinformatics prediction, and blotted with FbaA MAb2 using Western blotting again. The result showed that FbaA MAb2 recognized the amino acid residues 95 to 114 and 101 to 118 but could hardly bind amino acid residues 84 to 101 and could not recognize FbaA X95-118 at all. Thus, the epitope specific for FbaA MAb2 was found to be located in the region from amino acids 95 to 118 of FbaA.

The major FH binding site in FbaA is the sequence YK QIKKTAPDKDLFF (amino acids 97 to 112), a putative coiled-coil domain in the N-terminal half of the protein (26). Here, we determined the epitope specific for FbaA MAb2 located in the amino acid region from residues 95 to 118 of FbaA, which overlapped the FH binding sites. Activation of the complement system in response to infectious agents is a key component of the innate immune response,
resulting in opsonization and/or lysis of invading pathogens. The intermediate product of the complement activation, C3b, is the critical component in the three-complement pathway. Particularly, C3b deposited on the surface of a pathogen serves as a nucleus for the formation of the alternative complement pathway C3 convertase C3bBb, which can activate additional complement components and form a membrane attack complex to lyse the invading pathogens. On the other hand, C3b can bind the complement receptors on the surfaces of PMNs to mediate opsonization and/or complement-dependent cytotoxin of invading pathogens. Recruitment of FH or FHL-1 by microbes facilitates factor I-mediated cleavage of cell surface-bound C3b to iC3b, thereby decreasing the deposition of C3 fragments on bacterial cells. In addition, this limited amount of C3 deposited on cells can lessen the probability that a pathogen becomes phagocytosed or lysed (11, 33). The FbaA binding site in FH and FHL-1 has been localized to SCR7, which is a domain common to both proteins (19, 33). In the present study, the epitope of FbaA MAb2 was located in the amino acid region from residues 95 to 118 of FbaA, which overlapped with the binding sites of FH and FHL-1, suggesting that FbaA MAb2 and SCR7 possibly have a similar, if not the same, domain to bind FbaA (Fig. 7). We speculate that FbaA MAb2 may competitively inhibit FH and FHL-1 to bind FbaA protein.

The results of the invasion assay and competitive inhibition ELISA verified that FbaA MAb2 could inhibit drastically FH binding to GAS.

In summary, FbaA MAb2 can competitively inhibit FH binding to the surface proteins of GAS. Thus, the inhibition decreases the amount of GAS invading epithelia, giving an opportunity to the host to eliminate GAS. Hence, both FbaA MAb2 and the corresponding epitope are promising starting points for pharmacologic targeting and further development of new therapeutic agents for GAS.
ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (grants 30872399 and 30901350), the Natural Science Foundation of Hebei Province (C2009001091), and the Scientific Research Foundation of the Health Bureau of Hebei Province (grant 08054).

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