Streptococcal IgA-binding Proteins Bind in the Ca2-Ca3 Interdomain Region and Inhibit Binding of IgA to Human CD89*

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Certain pathogenic bacteria express surface proteins that bind to the Fc part of human IgA or IgG. These bacterial proteins are important as immunochemical tools and model systems, but their biological function is still unclear. Here, we describe studies of three streptococcal proteins that bind IgA: the Sir22 and Arp4 proteins of Streptococcus pyogenes and the unrelated β protein of group B streptococcus. Analysis of IgA domain swap and point mutants indicated that two loops at the Ca2/Ca3 domain interface are critical for binding of the streptococcal proteins. This region is also used in binding the human IgA receptor CD89, an important mediator of IgA effector function. In agreement with this finding, the three IgA-binding proteins and a 50-residue IgA-binding peptide derived from Sir22 blocked the ability of IgA to bind CD89. Further, the Arp4 protein inhibited the ability of IgA to trigger a neutrophil respiratory burst via CD89. Thus, we have identified residues on IgA-Fc that play a key role in binding of different streptococcal IgA-binding proteins, and we have identified a mechanism by which a bacterial IgA-binding protein may interfere with IgA effector function.

Human IgA is abundant in the seromucous secretions that bathe mucosal surfaces, such as those lining the lungs, gut, and genitourinary tracts. These surfaces represent major potential sites of invasion, and the immune protection offered by secretory IgA, as the predominant antibody at these sites, serves as a critical “first line of defense” against many bacteria and viruses (1). Moreover, evidence is accumulating that IgA present in serum plays an important role in a “second line of defense” against microorganisms that have penetrated the mucosal barrier (2, 3).

IgA performs the dual role of all antibodies, of both recognizing foreign invaders and triggering their elimination. For IgA, this latter effector function involves the interaction of its Fc region with an Fcε receptor (FceRI,1 CD89) expressed on neutrophils, eosinophils, or macrophages (4, 5). After binding to antigen, IgA can interact with CD89 and elicit an array of potent eradication mechanisms, including phagocytosis, superoxide generation, and release of enzymes and inflammatory mediators (5). The molecular basis of this important interaction between IgA and CD89 is now emerging, with the demonstration of the critical role played by two loops lying at the interface of the two domains of IgA-Fc (6, 7) and with the identification of the binding region in CD89 (8, 9).

Surface proteins that bind human IgA-Fc have also been identified in many strains of Streptococcus pyogenes (group A streptococcus) and group B streptococcus (GBS), two important human pathogens (10–12). Despite the importance of streptococci as pathogens, it is unclear what advantage the ability to bind IgA-Fc offers a bacterium during the establishment of an infection. However, even in the absence of information concerning their exact biological role, the IgA-binding proteins (IgA-BPs) are of considerable interest as immunochemical tools and model systems. A similar situation prevails for the well known bacterial IgG-binding proteins, staphylococcal protein A and streptococcal protein G, which have been extensively characterized (13), but whose biological function is unknown.

In S. pyogenes, the two IgA-BPs that have been studied in most detail are the Arp4 and Sir22 proteins (14, 15), which are members of the heterogeneous M protein family (16, 17). These streptococcal proteins have 29-residue IgA-binding regions that are related, but not identical, making comparisons of interest (18, 19). Importantly, these IgA-BPs bind human IgA of both subclasses and bind both serum IgA and secretory IgA (15, 20).

In GBS, binding of IgA is due to the β protein, which is unrelated to the IgA-BPs of S. pyogenes (21, 22) and has a 73-residue IgA-binding region that does not vary in sequence between strains (23). The β protein binds human serum IgA of both subclasses and has the remarkable property that it binds poorly to secretory IgA, the molecular form of IgA that predominates on mucous membranes (24).

Here, we report experiments aimed at analyzing the interaction between IgA-Fc and streptococcal IgA-BPs. Experiments with domain swap antibodies and mutant IgAs indicate that binding of the different IgA-BPs, and a 50-residue synthetic IgA-binding peptide derived from the Sir22 protein (25), depend on almost identical sites in the Fc interdomain region of IgA, the binding region also used by CD89. In agreement with these results, we demonstrate that the streptococcal IgA-BPs inhibit interaction of IgA with CD89, a property that may allow IgA-BPs to inhibit IgA effector function.

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1 The abbreviations used are: FcεRI, Fcε receptor; GBS, group B streptococcus; IgA-βP, IgA-binding protein; NIP, 3-hydroxy-4-nitro-5-iodophenylacetate; PBS, phosphate-buffered saline; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay.
This monoclonal antibody was used, since the light chains of the IgGs (gen, San Diego, CA) diluted 1:2000 in PBS with 0.1% Tween 20 (PBST) were used as the value for nonspecific binding and was subtracted from total binding to give specific binding values. Results from different experiments were normalized such that the fractional binding of wild-type IgA at $1 \times 10^{-5}$ $M$ was 1.0.

**Binding Assays with Whole Bacteria**—Washed suspensions of bacteria ($5 \times 10^{9}$/ml) were prepared as described (26), and identical samples were added to each of a series of tubes. Different amounts of the Ig (in a volume of 50 ml) were added, giving the final concentrations indicated. After incubation for 2 h, the bacteria were washed twice with PBSAT (PBS with 0.02% NaN$_3$ and 0.05% Tween 20), and the presence of bound Ig was analyzed by the addition of $\sim 15,000$ cpm of $^{125}$I-labeled rat anti-mouse $\lambda$ light chain (Pharmingen). After incubation for 1 h and two washes with PBSAT, the radioactivity associated with the pelletted bacteria was determined in a y-counter. All incubations were performed at room temperature. Binding is expressed as a percentage of the radioactivity added to each tube. Binding to control bacteria ($\pm 1\%$) has been subtracted. All determinations were made in duplicate.

Binding to Sir22 on the bacterial cell surface was analyzed with $S$. pyogenes strain AL168, using the non-IgA-binding mutant Arp4 (22) as a negative control. Binding to Arp4 on the surface of $S$. pyogenes was performed with strain JRS145/pJRS264, with JRS145 as the negative control, and binding to $\beta$ on the surface of GBS was analyzed with strain A909, using a $\beta$-negative mutant of this strain as the negative control.

**Inhibition of Rosetting**—Human erythrocytes were derivatized with NIP and sensitized with wild-type IgA at 200 $\mu$g/ml as described (7). Neutrophils were isolated as described (7) and resuspended in PBS containing 0.1% (w/v) BSA (PBS/BSA). In the inhibition assay, which was essentially a modification of a previously described method (33), diluted coated erythrocytes and inhibitor protein (peptide, bacterial proteins, or their control peptide or proteins), both in PBS/BSA, were incubated at room temperature for 1 h (except for $\beta$ protein and protein $G$ which overnight incubation was used) in wells of a V-bottomed microtiter. Neutrophils ($\sim 50,000$) in PBS/BSA were added and mixed carefully; the plates were incubated for 10 min, centrifuged at $50 \times g$ for 5 min, and further incubated for 50 min. Following the addition of acridine orange solution (6 $\mu$g/ml final concentration) to stain nucleated cells, the suspensions were examined by fluorescence microscopy, defining a rosette as a fluorescent neutrophil with three or more erythrocytes attached.

The results were normalized so that the mean rosetting level seen in the absence of inhibitor gave 0% normalrosette inhibition. For each inhibitor, experiments were performed at least twice, each time using neutrophils from a different donor.

**Inhibition of Neutrophil Respiratory Burst**—The inhibition assay was essentially a modification of a previously described chemiluminescence assay of respiratory bursts (7). Wells of a chemiluminescence microtiter plate (Dynex Technologies, Ashford, UK) were coated with NIP-BSA with subsequent incubation with wild-type IgA1 in PBS (100 $\mu$g/ml) at 50 $\mu$g/ml for 1–2 h at room temperature. After washing, appropriately diluted inhibitor in Hanks’ buffered saline solution containing 20 ml HEPES and 0.1% (w/v) globulin-free BSA (Hanks’ buffered saline solution/BSA) was added. After incubation for 1 h at room temperature to allow prebinding of the inhibitor to the IgA, neutrophils in Hanks’ buffered saline solution/BSA containing 260 $\mu$g/ml luminol were transferred (giving a final suspension of 0.25 $\times 10^{6}$/ml), the plate was transferred to a Microlumat LB96P luminometer, and the chemiluminescence was measured at regular intervals.

**RESULTS**

**Immunochromatographic Comparison of Streptococcal IgA-binding Proteins**—The $\sim 40$-kDa Arp4 and Sir22 proteins from $S$. pyogenes are both members of the M protein family and share structural similarities, including a high degree of residue identity in the 29-residue IgA-binding region (Fig. 1). As expected, antibodies to Sir22 were found to cross-react with Arp4 (Fig. 1). However, the $\sim 125$-kDa $\beta$ protein of GBS lacks residue identity with Arp4 and Sir22 and did not react with anti-Sir22 antibody; nor did anti-$\alpha$ antibodies recognize Arp4 or Sir22. These data confirm that the IgA-BPs of $S$. pyogenes and GBS are unrelated, underlining the interest in comparing their functional properties.

**Contribution of IgA-Fc Domains to Interaction with IgA-binding Proteins**—To analyze the contribution of the Co2 and Cg2 domains of IgA-Fc to the binding of streptococcal IgA-BPs, we used two domain swap antibodies in which homologous domains are exchanged between IgA1 and IgG1. These domain swaps are designated y12o23 (constant domain structure Cg1, Cg2, Cg3), and y12o23 (constant domain structure Co1, Co2, Cg3) (Table 1). The ability of the constructs to bind the three streptococcal IgA-BPs and the IgA-binding peptide derived from Sir22 was analyzed by ELISA (Fig. 2).
proteins of Western blot analysis of purified preparations of the Sir22 and Arp4 proteins. Domain also makes a contribution, since regions in the Cg molecules, we observed that the interaction site. For all of these three IgA-binding molecules, we tested in purified form. The A442R mutant displayed strongly decreased binding to the Sir22-derived peptide, and more markedly decreased binding to intact Sir22 and to the Arp4 protein. Together, these data suggest that residues in the Co2 region may play a role in the binding of streptococcal IgA-BPs and that the Arp4 and Sir22 proteins, which are closely related, may not have completely identical IgA-binding properties when tested in purified form.

When the two Co2 mutants were analyzed for binding to IgA-BPs expressed on the bacterial cell surface, a similar pattern emerged (Fig. 4B). Thus, G259R bound to bacteria expressing Sir22, Arp4, or β with apparent affinities similar to those of wild-type IgA1. In contrast, L257R displayed strongly decreased ability to bind to bacterial whole bacteria. However, it should be noted that L257R is not a mutant bound all three molecules with affinity generally similar to those of wild-type IgA1.

Use of Point Mutations in the Co2/Co3 Interdomain Region of IgA-Fc for Characterization of Sites That Bind Streptococcal Proteins—Since the binding regions for the IgG-binding bacterial proteins staphylococcal protein A and streptococcal protein G have been localized to the Fc interdomain region of IgG (35, 36), we analyzed whether the interdomain region of IgA-Fc might be directly involved in interaction with the streptococcal IgA-binding proteins. Studies of this region in IgA were also of interest because recent work has implicated two loops at the Co2/Co3 interface in the binding of IgA to human CD89 (6, 7). We used a panel of IgA1 antibodies (7), each with a single or double amino acid substitution located in either of two predicted loops in the interdomain region, corresponding to Leu257–Gly259 in the Co2 domain and Pro440–Phe442 in the Co3 domain (Table I). These two predicted loops will be referred to as the LLG and PLAF loops. Molecular modeling (37) suggests that these two loops occupy positions in IgA analogous to interdomain loops in IgG that are essential for binding of staphylococcal protein A (35). The IgA proteins mutated in the two loops have Arg substitutions, since conversion to this bulky side chain in a critical residue was thought likely to be sufficient to ablate binding. However, several lines of evidence indicate that no gross conformational changes have been introduced into the IgA mutants (7).

Effect of Mutations in the Predicted LLG Loop of the Co2 Domain—Two mutants with single point mutations in the LLG loop, the L257R and G259R mutants, were assessed by ELISA for binding to the four streptococcal IgA-binding molecules (Fig. 4A). The G259R mutant had apparent affinities similar to those of wild-type IgA1. In contrast, L257R displayed slightly decreased binding to the Sir22-derived peptide, and more markedly decreased binding to intact Sir22 and to the β protein, but was not affected in its ability to bind the Arp4 protein. Together, these data suggest that residues in the Co2 region may play a role in the binding of streptococcal IgA-BPs and that the Arp4 and Sir22 proteins, which are closely related, may not have completely identical IgA-binding properties when tested in purified form.

Because intact Sir22 binds both IgA and IgG (15), the IgA/IgG domain swap antibodies could not be used to provide information on the IgA binding requirements of Sir22. However, the Sir22-derived peptide, Arp4, and β protein did not bind wild-type IgG1 in the ELISA, so this approach was useful to illustrate the relative contributions of the two IgA-Fc domains to the interaction site. For all of these three IgA-binding molecules, we observed that the γ1γ2α3 antibody bound with an apparent affinity generally comparable with wild-type IgA1 (Fig. 2). In contrast, no binding was observed for the α1ο2γ3 antibody. Several streptococcal proteins/peptides that do not bind IgA were used as controls, and all were unable to bind to either wild-type IgA1 or the swap antibodies. Together, these results suggest that the Co3 domain makes a major contribution to the binding site for these IgA-binding molecules.

To analyze whether the findings using purified bacterial IgA-binding proteins give a true reflection of the reactivity of the proteins when expressed on the bacterial cell surface, we performed binding studies with whole bacteria. Due to the IgG binding ability of the S. pyogenes strains expressing Sir22 and Arp4 (34), we were unable to analyze whole S. pyogenes bacteria for ability to bind the IgA/IgG domain swap antibodies but could perform studies with whole GBS bacteria expressing the β protein (Fig. 3). Neither IgG1 nor the domain swap α1ο2γ3 bound to the β-expressing GBS, while both wild-type IgA1 and the γ1γ2α3 antibody bound to these bacteria. Indeed, the γ1γ2α3 construct bound even better than IgA1 in this test. None of the constructs showed significant binding to an isogenic GBS mutant lacking expression of the β protein (data not shown). These results are consistent with a major role for the Co3 domain of IgA in binding to the β protein.

Although the data described above indicate that the Co3 domain is of major importance for the binding of streptococcal IgA-BPs to IgA, they do not rule out the possibility that the Co2 domain also makes a contribution, since regions in the Cγ2 domain in the γ1γ2α3 antibody may be able to adequately substitute for the corresponding parts of the Co2 domain in the binding process. Data reported below suggest that this is indeed the case.

**Use of Point Mutations in the Co2/Co3 Interdomain Region**

**Fig. 1. Comparison of streptococcal IgA-binding proteins.** A, Western blot analysis of purified preparations of the Sir22 and Arp4 proteins of S. pyogenes and the β protein of group B streptococcus. Blotting membranes were incubated with anti-Sir22 serum or anti-β serum, as indicated, and bound antibodies were detected with radiolabeled protein A or protein G, as described under “Experimental Procedures.” No bands were seen in control blots incubated with preimmune serum. An equivalent Coomassie-stained SDS gel is shown on the left. B, alignment of the IgA-binding regions of Sir22 and Arp4.
domain swap antibodies
\[\alpha1\beta2\gamma3\n\gamma1\beta2\alpha3\n\]

Table I
Mutant antibodies used

| Antibody                  | Structural notes                                      |
|---------------------------|-------------------------------------------------------|
| CH1, hinge and CH2 of human IgA1, and CH3 of human IgG1 (IgA-derived sequence ends at residue Ser^{415}) | CH1, hinge and CH2 of human IgG1, and CH3 of human IgA1 (IgA-derived sequence starts at Gly^{342}) |
| Leu^{257} of human IgA1 replaced by Arg | Gly^{258} of human IgA1 replaced by Arg |
| Pro^{440} of human IgA1 replaced by Arg     | Pro^{440} of human IgA1 replaced by Ala   |
| Ala^{442} of human IgA1 replaced by Arg     | Phe^{443} of human IgA1 replaced by Arg       |
| Leu^{441} and Ala^{442} replaced by Met and Asn, respectively |

FIG. 2. Binding of wild-type IgA1, wild-type IgG1 and domain swaps to streptococcal IgA-binding molecules. The antibodies were diluted as indicated and analyzed by ELISA for ability to bind to the Sir22-derived IgA-binding peptide and to the Arp4 and \(\beta\) proteins, immobilized in microtiter wells. Results were normalized by expressing ELISA absorbance as a fraction of mean absorbance seen with wild-type IgA1 at \(1 \times 10^{-6}\) M. The controls for the IgA-binding peptide was the M5-N peptide, the control for Arp4 was the Arp44451 mutant, and the control for \(\beta\) was the Rib protein from GBS. All of these control molecules lack ability to bind IgA. The control binding shown is in each case the mean of the fractional binding seen for the panel of antibodies, all at \(1 \times 10^{-6}\) M. This value varied very little from antibody to antibody (S.D. values of ±0.02 for M5-N, ±0.06 for Arp44451, and ±0.19 for Rib). The experiment was performed twice with very similar results.

wild-type IgA1, while the LA441-442MN mutant displayed binding consistent with a decrease in affinity of around 10-fold for Sir22 and the peptide and around 2–5-fold for Arp4. Mutant P4440R showed only weak binding to the three IgA-binding molecules originating from \(S. \) pyogenes, with apparent reductions in affinity of greater than 100-fold, while the P4440A and F4443R mutants showed essentially no binding. Analysis of binding of these mutants to Sir22 or Arp4 expressed on the surface of \(S. \) pyogenes produced a similar picture (Fig. 5B). Thus, the binding of A442R to whole \(S. \) pyogenes bacteria was similar to that observed with wild-type IgA1. In contrast, P4440R and F4443R displayed markedly reduced binding, while no binding was observed for P4440A, i.e. the effect on binding was even more dramatic for P4440A than for P4440R. Together, these results suggest that the PLAF loop in Co3, and residues Pro^{440} and Phe^{443} in particular, are critical for binding of Sir22, its peptide derivative, and Arp4. As described above, some contribution to binding is also apparently made by the close-lying Leu^{257} residue in the LLG loop of Co2.

Mutations in the PLAF loop of Co3 also had dramatic effects on binding to \(\beta\) protein. The results were reminiscent of those obtained with the \(S. \) pyogenes proteins/peptide but with important distinctions. As observed for the \(S. \) pyogenes proteins, mutant A442R had an apparent affinity similar to that of wild-type IgA1, and LA441-442MN showed decreased binding consistent with a drop in affinity of around 10-fold (Fig. 5A). However, unlike the \(S. \) pyogenes proteins, mutant P4440R was almost completely negative, while mutant F4443R retained some binding, with an apparent reduction in affinity of around 10-fold. Thus, both of the P4440R and P4440A mutations appeared to virtually ablate binding to \(\beta\) protein, suggesting that the Pro^{440} residue plays a highly critical role in binding of \(\beta\) to IgA. Binding tests with \(\beta\) expressed on the bacterial cell surface confirmed the ELISA data (Fig. 5B).

Streptococcal IgA-BPs Inhibit Binding of IgA to Human CD89—The data reported above revealed that the PLAF and LLG loops at the IgA-Fc interdomain region, which are critical for CD89 binding (6, 7), also appear to be important for binding to the bacterial IgA-BPs. Therefore, we investigated the capacity of the bacterial proteins to inhibit the ability of IgA to bind to and activate CD89.

Using a rosetting assay, we found that Sir22 and the Sir22-derived peptide inhibited binding of IgA1 to human CD89, the former producing half-maximal inhibition at concentrations of

FIG. 3. Use of Ig domain swap mutants to analyze binding of IgA to \(\beta\) protein expressed on the surface of GBS. The analysis was performed with whole A909 bacteria and purified Ig proteins, as described under “Experimental Procedures.” Each value represents the average of duplicate determinations. This experiment was performed twice, with very similar results.
whole streptococci expressing one of the IgA-binding proteins Sir22, the experiment was performed twice with very similar results.

The following non-IgA-binding streptococcal peptide/proteins served as negative controls: M5-N peptide for the IgA-binding peptide, Arp4, and Rib for β protein. The control binding shown is in each case the mean of the fractional binding seen for the panel of antibodies, all at 1 × 10⁻⁶ M. This value varied very little from antibody to antibody (S.D. values of ±0.01 for M5-N, ±0.04 for Arp4, and ±0.08 for Rib). The experiment was performed twice with very similar results, B, binding to whole streptococci expressing one of the IgA-binding proteins Sir22, Arp4, or β protein. The protein expressed and the bacterial species are indicated above each graph. Each graph shows results obtained with the IgA1 wild type protein (IgA1) and different mutant proteins, as indicated. Each value represents the average of duplicate determinations, and each experiment was performed at least twice, with very similar results.

As an additional, physiologically relevant test for function, we did observe that Arp4 was capable of inhibiting the IgA-triggered oxidative burst at concentrations greater than 5 × 10⁻⁶ M (Fig. 7). This inhibition appears to be due to the ability of Arp4 to bind IgA, since the two non-IgA-binding Arp4 deletion mutants Arp4Δ4450 and Arp4Δ451 caused little or no inhibition. Moreover, Arp4 did not inhibit a phorbol 12-myristate 13-acetate-stimulated respiratory burst (data not shown). Together, these data indicate that Arp4 can inhibit a respiratory burst triggered by the binding of IgA-Fc to CD89.

**DISCUSSION**

Surface proteins that bind to the Fc part of human IgA or IgG are expressed by many pathogenic bacteria, in particular by Gram-positive pathogens (38). While IgA-binding proteins have been less extensively studied than those binding IgG, more is known about their biological properties. For example, the IgA-binding Sir22 and Arp4 proteins of S. pyogenes are known to be important virulence factors (26), and the IgA-binding β protein of GBS has been shown to be a target for protective antibodies (39). However, the exact role of IgA-BPs in streptococcal pathogenesis remains unknown. This situation, and the potential usefulness of IgA-BPs as immunochemical tools and model systems, prompted us to characterize the binding site in IgA-Fc
for different IgA-BPs.

To analyze regions in IgA-Fc critical for interaction with streptococcal IgA-BPs, we employed domain swaps and point mutants. As discussed before (7), these mutant proteins are unlikely to have undergone any gross structural aberrations, allowing conclusions to be drawn on the relative contributions of different domains and mutated residues to the binding of streptococcal proteins. Our results indicate that the Ca3 domain of IgA-Fc makes a major contribution to binding for all the streptococcal proteins, with the Ca2 domain possibly playing a less important role. This result is in agreement with a previous study, which implicated the Ca3 domain in the binding of the Arp4 protein (40). The present study indicates that the PLAF loop (residues 440–443), predicted to lie on the surface of the Ca3 domain, is of particular importance for the binding of the bacterial IgA-BPs, but the LLG loop in Ca2 also appears to contribute. The data on proteins/peptide from S. pyogenes suggest that they bind to identical or very similar sites in IgA-Fc, and binding of the β protein from GBS appears to depend on essentially the same residues. However, Phe443 is less critical for interaction with β than with the IgA-binding molecules from S. pyogenes. Thus, the IgA-BPs of S. pyogenes bind to a site that appears to be very similar to the site used by the unrelated β protein of GBS, but the sites are probably not identical. This conclusion is in good agreement with previously reported inhibition experiments, which indicated that the Arp4 and β proteins bind to the same region in IgA (24).

It may be argued that mutations in the PLAF loop of Ca3 and the LLG loop in Ca2 produced their effects either by perturbing direct binding interactions or by triggered alterations in the conformation of close-lying residues that provide the binding contacts. The finding that mutation of Gly405 in the LLG loop and of Ala442 in the PLAF loop did not reduce binding, while that of adjacent residues produced marked effects, may indicate that the latter possibility is less likely. In either case, these interdomain loops may be considered as important markers of the binding sites for the streptococcal IgA-BPs.

The PLAF loop in Ca3 and the LLG loop in Ca2 are predicted to lie close in three-dimensional space, as highlighted on a molecular model of IgA based on solution structural studies (37) (Fig. 8). These loops are proposed to play a key role for binding of IgA-Fc to CD89 (6, 7) and to streptococcal proteins (this study), but there are clear differences between the sites interacting with the human and bacterial proteins. In particular, domain swap experiments indicated that binding of CD89 to IgA-Fc requires both of the Ca2 and Ca3 domains, while the Ca2 domain can be replaced with the Caγ domain without affecting binding of the streptococcal proteins. Further, mutation in the PLAF loop of Ala442 to Arg was associated with loss

**Fig. 6.** Inhibition of binding of IgA1 to CD89 on neutrophils, assessed by rosette formation. A, inhibition test with Sir22 and the Sir22-derived IgA-binding peptide and with the non-IgA-binding M5-N peptide. B, inhibition test with Arp4 and with the non-IgA-binding deletion mutants Arp4Δ450 and Arp4Δ451. C, inhibition test with β protein and with the non-IgA-binding protein G. The results were normalized and expressed such that rosette formation in the absence of IgA1 coating was used to provide the value for 0% rosettes (equivalent to 100% inhibition), while that in the absence of inhibitor was used as the value for 100% rosettes (equal to 0% inhibition).

**Fig. 7.** Analysis of inhibition of an IgA1-mediated neutrophil respiratory burst by Arp4 and its non-IgA-binding deletion mutants Arp4Δ450 and Arp4Δ451. Chemiluminescence (CL, arbitrary units) was induced by IgA1 alone (●) or in the presence of bacterial inhibitor, as indicated. The negative control (○) shows chemiluminescence observed in the absence of IgA1 and inhibitor. Inhibitor at 5 × 10^{-15} M, 5 × 10^{-14} M, 5 × 10^{-13} M, 5 × 10^{-12} M, 5 × 10^{-11} M. The results of representative experiments are shown. The experiments were performed at least twice with very similar results.
of detectable binding to CD89 (7), while it had no impact on binding to the bacterial proteins. Together, these data indicate that CD89 and the bacterial IgA-BPs have overlapping, but not identical, binding sites in IgA-Fc.

The proposed overlapping nature of the binding sites in IgA-Fc for CD89 and bacterial IgA-BPs is strongly supported by the observed ability of the IgA-BPs to inhibit the binding of IgA to CD89, as measured by rosetting, and by the ability of the Arp4 protein to inhibit an IgA-triggered respiratory burst in CD89-expressing neutrophils. It could be argued that the observed blockade is rather a gross effect, since intact IgA-BPs might be expected to mask an appreciable area of the IgA-Fc surface due to their molecular size. However, we found that the much smaller 50-residue IgA-binding peptide, representing an isolated IgA-binding domain (25), was still capable of specifically blocking interaction with CD89. This peptide would be anticipated to adopt a three-dimensional structure of relatively small size, so its ability to inhibit CD89 binding is most likely explained by close proximity of their respective binding sites on IgA-Fc.

The ability of streptococcal IgA-BPs to inhibit binding of IgA to CD89 suggests that such disruption may also occur during a bacterial infection. We can therefore propose a mechanism by which possession of an IgA-BP may confer on a bacterium the ability to evade clearance mediated by specific IgA antibodies. According to this mechanism, specific binding of an IgA molecule to a bacterial surface antigen is followed by binding of the Fc part of the IgA molecule to a bacterial IgA-BP also present on the bacterial surface, thereby allowing the bacterium to evade the elimination processes that would normally be triggered via binding of IgA-Fc to CD89 (3). Such bridging of a bound Ig molecule has also been proposed to explain the mechanism of action of the IgG-Fc receptor of herpes simplex virus type 1, but it is not known if this viral Fcy receptor blocks binding of IgG to human Fcy receptors or if it exerts its function by some other mechanism (41, 42).

The finding that unrelated IgA-BPs, expressed by S. pyogenes or GBS, bind to similar sites in IgA-Fc is reminiscent of the situation described for the two unrelated bacterial IgG-binding molecules, staphylococcal protein A and streptococcal protein G, both of which bind to the Fc domain interface in IgG (13, 35, 36, 43). Taken together, these data imply that convergent evolution has favored the appearance of bacterial proteins that bind to the CH2/CH3 interdomain region in IgA or IgG. Interestingly, accessibility and sequence comparison analyses and a recent study exploiting random peptides indicate that the interdomain region of IgG-Fc has intrinsic properties that favor binding to other proteins (44, 45). This conclusion is supported by evidence that the Fcγ receptor of herpes simplex virus type 1 binds at the Cγ2/Cγ3 domain interface (46, 47) and by localization of the binding site for CD89 to the interdomain region of IgA-Fc (6, 7). These data raise the question whether one biological function of protein A, protein G, and the Fcγ receptor of herpes simplex virus type 1 might be to inhibit IgG effector function in a manner analogous to that proposed here for bacterial IgA-BPs, i.e. by interfering with the binding of IgG to Fcγ receptors on phagocytic cells. However, the majority of available evidence appears to argue against such a mechanism, since protein A has been shown not to inhibit the binding of IgG to human FcγRI and FcγRII (48) and all FcγR receptors bind at the N-terminal end of the Fc, well away from the Fc interdomain region (49–54). Further work will be required to clarify the various mechanisms that may afford evolutionary advantages to microbes that possess proteins that bind IgA or IgG.

In summary, we have demonstrated that unrelated bacterial IgA-BPs bind in the interdomain region of IgA-Fc, at sites overlapping with that used by the human IgA-receptor CD89. These findings have allowed us to propose a possible mechanism by which bacterial IgA-BPs may interfere with IgA effector function, thereby contributing to bacterial virulence. This study also highlights the potential of the bacterial IgA-BPs, and the IgA-binding peptide in particular, as tools for studies of the structure and function of IgA.

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