Michael W. Russell[○+], Jesper Reinholdt[○] and Mogens Kilian[○]

Department of Oral Biology[○], Royal Dental College, Aarhus and Department of Microbiology[+], University of Alabama at Birmingham, Birmingham

Anti-inflammatory activity of human IgA antibodies and their Fabα fragments: inhibition of IgG-mediated complement activation*

The interaction of human IgA antibodies with the classical pathway of complement activation was investigated in a homologous human system, by means of two IgA1 and three IgG1 myeloma proteins having antibody activity against a defined antigen, staphylococcal α-toxin. In a solid-phase antigen-dependent C3b-binding ELISA system, the monoclonal IgG antibodies were previously shown to activate the classical complement pathway synergistically, resembling polyclonal IgG antibodies, whereas IgA antibodies were unable to activate complement by either pathway. In the present study, IgA antibodies were found to inhibit significantly the activation of complement initiated by antigen-bound polyclonal or mixed monoclonal IgG antibodies, in relation to the amount of IgA antibodies applied and bound to antigen. IgA1 myeloma proteins devoid of antigen-binding activity were without effect. Inhibition was independent of the ability of the IgA antibodies to compete against the IgG antibodies in binding to antigen, and was demonstrable with physiological concentrations of antibodies. Similar results were obtained with polyclonal serum IgA having antigen-binding activity. However, the binding of C1q to antigen-complexed IgG was inhibited only by a monoclonal IgA antibody that could compete against one of the three monoclonal IgG antibodies that bound C1q synergistically. This observation implied that at least two mechanisms were involved in the inhibition of C3b fixation. Fabα fragments of monoclonal IgA antibodies, obtained by cleavage with IgA1 protease from *Haemophilus influenzae* type b, were found to have a similar inhibitory effect on C3b fixation to the intact IgA1 antibodies. This observation supports the hypothesis that IgA1 proteases contribute to the invasive pathogenicity of certain mucosal bacteria, by cleaving secretory IgA1 antibodies to antigen-binding Fabα fragments, which are not only defective in mucosal defense properties, but which also protect the organisms from other immune effector systems, such as complement activation.

1 Introduction

Antibodies of the IgG and IgM isotypes exert their protective functions largely by interacting, through structures located in their Fc regions, with powerful effector systems principally represented by C and phagocytosis. Circulating IgA antibodies by comparison, are generally unable to engage these systems [1, 2], and consequently appear to lack a major role in defense against infection. However, secretory IgA (S-IgA) antibodies, which are predominant at mucosal surfaces where the majority of infectious diseases arise, are credited with a major protective function at those sites, which is probably due to several properties. These include neutralization of viruses, toxins and enzymes [3–5], inhibition of adherence of organisms to mucosal surfaces [6, 7], and interaction with nonspecific factors such as lactoferrin, peroxidases, and mucin [8, 9]. Several of these properties are known to depend on the integrity of the S-IgA molecule and, in particular, on the presence of the Fc region with the attached secretory component (SC) [2]. The production by several significant human mucosal pathogens of proteases that uniquely cleave human IgA and S-IgA of subclass 1, yielding intact Fabα and Fcα [or $(Fc\alpha)_n$-SC] fragments, is therefore considered an important virulence attribute, that serves to diminish the effectiveness of S-IgA antibodies [2, 10–12]. A novel hypothesis concerning the role of IgA1 proteases in facilitating invasive infections, by such organisms as *Haemophilus influenzae* type b, *Neisseria meningitidis*, and *Streptococcus pneumoniae* (the principal causes of bacterial meningitis), predicts that Fabα fragments derived from S-IgA1 antibodies bound to the surface of the organism also interfere with other immune defense mechanisms [2, 13]. Our previous studies have shown that native human monoclonal IgA1 antibodies fail to activate C by either pathway, when bound to specific antigen [14]. The present report concerns investigations on the ability of these IgA1 antibodies and their Fabα fragments, obtained by cleavage with IgA1 protease, to inhibit complement activation mediated by IgG antibodies to the same antigen.

[I 7774]

* This work was supported by the Danish Medical Research Council, and by US-PHS grant AI 18745.

Correspondence: Michael W. Russell, Department of Microbiology, University of Alabama at Birmingham, UAB Station, Birmingham, AL 35294, USA

Abbreviations: **CCP:** Classical complement pathway **CF:** Complement fixation **SAT:** Staphylococcal α-toxin **SC:** Secretory component **S-IgA:** Secretory IgA

0014-2980/89/1212-2243$02.50/0

2 Materials and methods

2.1 Immunoglobulins

Human monoclonal IgA1 protein Fug (monomeric; κ light chain) and Tin (polymeric; λ light chain), and IgG1,κ proteins Fug, JJF and Kal, which all bind staphylococcal α-toxin (SAT) [15], were purified chromatographically to > 99.9% from myeloma sera, as described previously [14]. Polyclonal IgA and IgG having relatively high anti-SAT antibody activity were similarly purified from normal human serum. Concentrations of Ig and antibodies were determined by ELISA as described [14]. SAT coated on plastic in the conventional manner failed to bind anti-SAT antibodies adequately, but this was overcome satisfactorily by coating plates first with 2 μg/ml of avidin and then with 5 μg/ml of biotinylated SAT [14]. Purified proteins were also assayed by absorbance at 280 nm (α_{280} for 1 mg/ml = 1.35). ELISA to investigate mutual inhibition of binding between IgA and IgG mAb was performed on plates coated with 2 μg/ml of avidin and 2 μg/ml of biotinylated SAT, by first incubating paired plates overnight with serial twofold dilutions of IgA antibodies, starting at 500 ng/ml, and then for 4 h with 50 ng/ml of IgG antibodies. One plate of each pair was developed with peroxidase-conjugated anti-IgA (Dakopatts, Glostrup, Denmark), and the other with peroxidase-conjugated anti-IgG (Dakopatts), followed by o-phenylenediamine/H_2O_2 substrate. The converse experiment was also performed.

2.2 C-fixation (CF) assays

The CF ELISA of Baatrup et al. [16] was used, as described previously [14]. To examine inhibition of CF by IgA or Fabα fragments, plates coated with 5–10 μg/ml of avidin and 10–20 μg/ml of biotinylated SAT [14] (hereafter referred to as SAT-coated plates) were incubated overnight first with serial twofold dilutions of IgA or Fabα, starting at 10 μg/ml of monoclonal IgA1 or Fab, or at 40 μg/ml of polyclonal IgA. The plates were then treated with either polyclonal IgG at 10 μg/ml of anti-SAT antibody, or with an equal mixture of IgG1 anti-SAT mAb at 3.3–5 μg/ml each, for 4 h. As an alternative procedure, the IgA antibodies (or Fabα fragments) were serially diluted in a constant concentration of IgG antibody, and added to the plates overnight. Fresh (frozen at −70 °C) human serum diluted 1:25 in PBS, pH 7.4, with 0.15 mM Ca^{2+} and 0.5 mM Mg^{2+} [16] was then added as a source of C, and incubated for 20 min at 37 °C. Bound C3b was detected by developing with peroxidase-conjugated anti-C3c (Dakopatts), which gave essentially similar results to the use of an anti-C3d reagent [14]. These conditions were found to be suitable for the detection of the classical complement pathway (CCP) [14, 16], and the use of 10 mM Mg-ethylene glycol-bis(β-aminoethyl ether) tetraacetate in the complement serum diluent abrogated C3b fixation under these conditions.

To assay C1q binding, SAT-coated plates were treated first with serial dilutions of IgA antibodies, and then with 10 μg/ml of polyclonal IgG anti-SAT, or with a mixture of 10 μg/ml each of the three monoclonal IgG antibodies. Purified C1q (Cytotech, San Diego, CA) was then applied at a concentration of 2.5 μg/ml in 0.125 M borate, pH 8.3, 0.075 M NaCl, 0.05% Tween-20 [17], for 4 h at room temperature. Bound C1q was developed with anti-C1q (Dakopatts) conjugated to horseradish peroxidase (type VI; Sigma Chemical Corp., St. Louis, MO) by the two-step glutaraldehyde method [18].

2.3 IgA1 protease and cleavage of IgA

IgA1 protease was prepared from cultures of *H. influenzae* strain HK50 (ATCC 35891, American Type Culture Collection, Rockville, MD) as described [19]. IgA preparations (approximately 100 μg in 1 ml of PBS) were treated with 10 μl of the IgA1 protease overnight at 37 °C, and fractionated by HPLC on a 60 cm × 0.75 cm TSK-G3000SW column [19]. The fractions containing Fabα fragments were pooled, and tested by ELISA on SAT-coated plates, using peroxidase-conjugated anti-κ or anti-λ light chain reagents (Dakopatts), and with a mAb specific for the C_H3 domain of human IgA (clone BE2C1, kindly donated by Dakopatts) followed by peroxidase-conjugated anti-mouse IgG (Dakopatts). Protein concentration of the Fabα fragments was determined by absorbance at 280 nm. For control purposes, PBS alone was incubated with IgA1 protease and subjected to HPLC in the same way. Fractions corresponding to the elution volume of Fabα fragments were pooled.

2.4 Preparation of Fabγ

Polyclonal human IgG having anti-SAT antibody activity was treated with papain [20] and fractionated by FPLC on a Superose 12 HR16/50 column (Pharmacia LKB Biotechnology Inc., Uppsala, Sweden). The product was tested by ELISA on SAT-coated plates developed with peroxidase-conjugated anti-Fcγ (Dakopatts), and peroxidase-conjugated anti-κ plus anti-λ light chain reagents. Although the preparation undoubtedly contained Fc, only the Fab fragment was able to bind to antigen-coated plates, as shown in this test. Its protein concentration was determined by absorbance at 280 nm, and its anti-SAT content was estimated by reference to the proportion of specific antibody in the parent IgG preparation. A control preparation, consisting of buffer treated with papain and chromatographed in the same way, was also made.

3 Results

3.1 Inhibition of polyclonal IgG-mediated CF

Purified normal human IgG at 10 μg/ml of anti-SAT antibody applied to SAT-coated plates activated the classical complement pathway (CCP) as assessed by fixation of C3b [14]. When either of two IgA1 anti-SAT mAb was titrated into this, CCP activation was inhibited in relation to the dose of IgA1 antibody applied and the amount of IgA bound (Fig. 1). Binding of IgG antibodies was not noticeably affected, since the concentration of IgG antibody applied effectively saturated the binding assay. The polymeric IgA1-Tin revealed a greater inhibitory effect than the monomeric IgA1-Fug, and > 50% inhibition was achieved by 1 μg/ml of IgA1-Tin. The addition of both IgA1 mAb together to polyclonal IgG resulted in a small additive

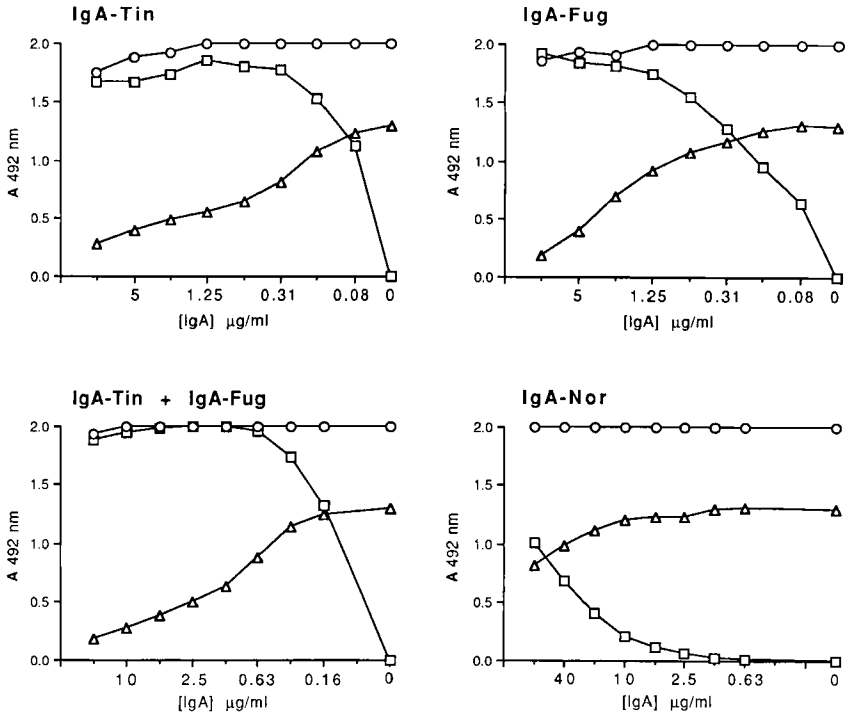

Figure 1. IgA antibody-mediated inhibition of the CCP initiated by polyclonal IgG antibody to SAT. SAT-coated plates were incubated overnight with monoclonal IgA-Tin, IgA-Fug, or both together, or with normal polyclonal IgA (IgA-Nor), each serially diluted in 10 µg/ml of polyclonal IgG anti-SAT. Bound IgA (□) and IgG (○) were developed in replicate plates by means of peroxidase-conjugated anti-IgA or anti-IgG, respectively. CCP (△) was activated by adding fresh normal serum diluted 1 : 25 in PBS with Ca^{2+} and Mg^{2+} for 20 min at 37 °C, and bound C3b was revealed by developing with peroxidase-conjugated anti-C3c. Each point shows the mean (± SE, which is too small to show) of triplicate values less background given in control wells not treated with IgG anti-SAT antibody.

inhibitory effect, compared with either IgA1 protein alone (Fig. 1). IgA1 myeloma proteins Kah (polymeric) and Mor (monomeric), which do not bind to SAT, did not show any inhibitory effect. Polyclonal IgA having anti-SAT activity also exerted a small inhibitory effect on IgG-mediated CCP activation, in relation to the amount of IgA antibody bound (Fig. 1). It should be noted that out of the total polyclonal IgA applied only a very small proportion was specific antibody.

3.2 Competition between IgA and IgG mAb in CF

IgA1-Fug was derived from the same serum as IgG1-Fug, and they shared the same idiotype [14]. Consequently, they probably have the same epitope specificity. This was demonstrated by mutual inhibition of binding to SAT in ELISA (Fig. 2), which showed reduction of IgG1-Fug binding in proportion to IgA1-Fug binding. The converse experiment revealed that IgA1-Fug binding was inhibited by IgG1-Fug (not shown). In contrast, the binding of IgG1-Fug was not inhibited by IgA1-Tin (Fig. 2), and the binding of other IgG1 proteins JJF and Kal was not inhibited by either IgA1-Fug or IgA1-Tin and *vice versa* (not shown).

Although each IgG1 mAb alone was not able to activate the CCP, mixtures of any two or preferably all three together showed synergistic CCP activation [14]. Both IgA1-Tin and IgA1-Fug were able to inhibit CCP activation mediated by a mixture of all three IgG1 mAb (Fig. 3), and the effect was greater with polymeric IgA1-Tin than with monomeric IgA1-Fug, even though IgA1-Tin could not compete against any of the IgG mAb in binding to SAT. The experiment illustrated in Fig. 3 was performed by pre-incubating the SAT-coated plates with the IgA1 antibodies, and then adding the mixture of IgG1 antibodies. Similar though slightly less pronounced results were obtained when

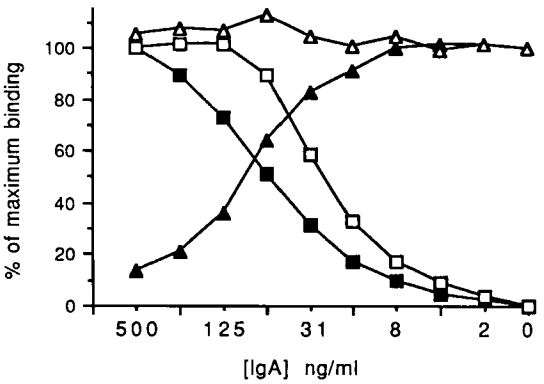

Figure 2. Competitive inhibition of the binding of IgG-Fug by IgA-Fug or IgA-Tin anti-SAT antibodies. SAT-coated plates were incubated overnight with serially diluted IgA-Fug or IgA-Tin, and then for 4 h with 50 ng/ml of IgG-Fug. Replicate plates were then developed with peroxidase-conjugated anti-IgA or anti-IgG. Each point shows the mean of duplicate values calculated as the percentage of maximum binding, for IgA antibodies at the highest dose applied, and for IgG antibodies in the absence of IgA. (■), IgA-Fug bound; (▲), IgG-Fug bound after IgA-Fug; (□), IgA-Tin bound; (△), IgG-Fug bound after IgA-Tin.

the IgA1 antibodies were titrated into a constant amount of mixed IgG1 antibodies.

3.3 Inhibition of CF by Fab fragments

When IgA1-Tin and IgA1-Fug were treated with IgA1 protease, fractionated by molecular sieve HPLC, and tested in ELISA, the Fabα fragments retained binding to SAT as revealed by means of anti-light chain reagents, but were devoid of detectable Fc regions as determined by means of an anti-C_H3 reagent (Table 1). In CF-ELISA, the Fabα fragments showed almost the same capacity to inhibit

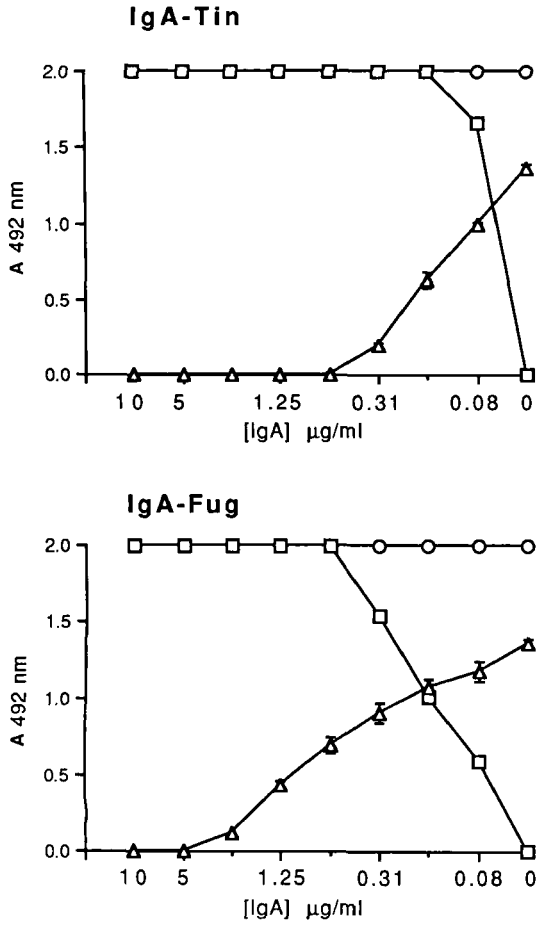

Figure 3. IgA antibody-mediated inhibition of the CCP initiated by mixed IgG mAb to SAT. SAT-coated plates were incubated overnight with serial dilutions of monoclonal IgA-Tin or IgA-Fug, and then for 4 h with a mixture of 3.3 µg/ml each of monoclonal IgG-Fug, IgG-JJF and IgG-Kal. Bound IgA (□) and IgG (○) were developed in replicate plates by means of peroxidase-conjugated anti-IgA or anti-IgG, respectively. The CCP was activated by adding fresh normal serum diluted 1 : 25 in PBS with Ca^{2+} and Mg^{2+} for 20 min at 37 °C, and bound C3b (△) was revealed by developing with peroxidase-conjugated anti-C3c. Each point shows the mean (± SE, which is usually too small to show) of triplicate values less background given in control wells not treated with IgG anti-SAT antibody.

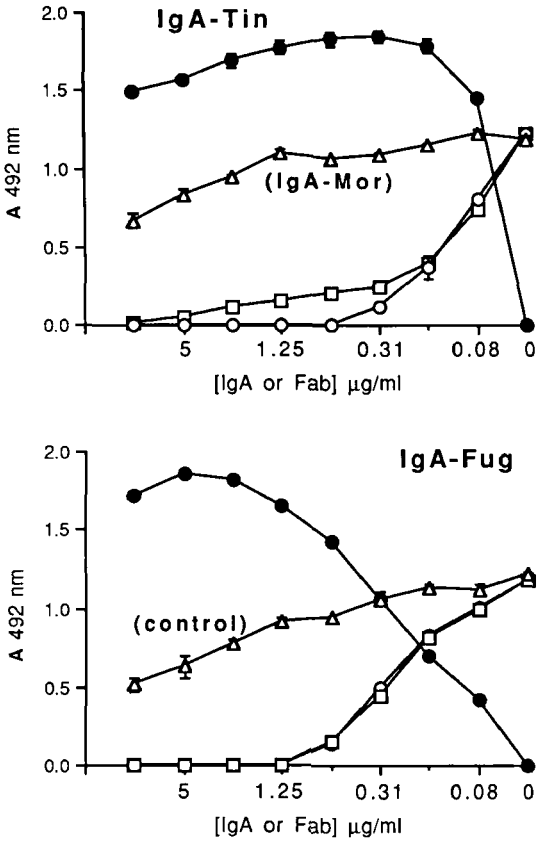

Figure 4. Inhibition of the CCP by Fabα fragments of IgA antibodies to SAT. SAT-coated plates were incubated overnight with serial dilutions of Fabα from IgA-Tin or IgA-Fug, or with intact parent IgA antibodies, and then for 4 h with a mixture of 5 µg/ml each of monoclonal IgG-Fug, IgG-JJF and IgG-Kal. Bound Fabα (●) was developed in replicate plates by means of peroxidase-conjugated anti-κ plus anti-λ light chain reagent. The CCP was activated by adding fresh normal serum diluted 1 : 25 in PBS with Ca^{2+} and Mg^{2+} for 20 min at 37 °C, and bound C3b was revealed by developing with peroxidase-conjugated anti-C3c. (○) Inhibition of CCP activation by Fabα fragments; (□) inhibition of CCP activation by intact IgA antibodies; (△) inhibition of CCP activation by Fabα from IgA-Mor which does not bind to SAT (upper panel), or by similarly diluted enzyme control (lower panel). Each point shows the mean of duplicate values (± SE) less background given in control wells not treated with IgG anti-SAT antibody.

CCP activation as the intact IgA1 antibodies, when examined on a basis of comparable protein concentration (Fig. 4). For control purposes, Fabα fragments similarly prepared from IgA1-Mor which does not bind to SAT, and the enzyme blank preparation were also tested in CF-ELISA. Both of these showed a similar small degree of CCP inhibition at high dose (Fig. 4), suggesting that substances derived from the IgA1 protease preparation interfered with C activation. However, at dilutions equivalent to concentrations of anti-SAT Fabα fragments that showed complete inhibition of CCP activation (~ 1 µg/ml), the effects of the non-specific Fabα-Mor fragment or enzyme blank were negligible.

Since Fab fragments of IgG lack the C1q binding site found in the Fcγ region, these also might be expected to inhibit CCP activation mediated by intact IgG antibodies. When Fabγ fragments of polyclonal IgG having antibody activity

Table 1. Absence of the Fcα region in Fabα fragments of IgA antibodies bound to SAT-coated plates

Protein	Conc. (µg/ml)	Intact IgA		Fabα fragment	
		Anti-κ/λ	Anti-Fcα	Anti-κ/λ	Anti-Fcα
IgA1,κ Fug	1.0	1.452[a)]	1.903	1.143	0
IgA1,λ Tin	1.0	1.768	1.831	1.451	0

a) Mean absorbance at 492 nm (less background) in triplicate wells developed with peroxidase-conjugated anti-κ plus anti-λ light chain reagents or anti-Fcα reagent, as shown.

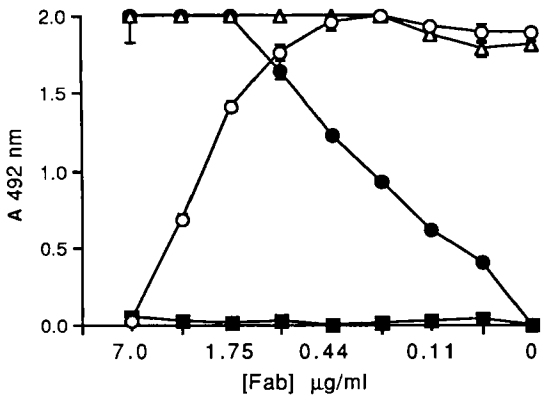

Figure 5. Inhibition of the CCP by Fabγ fragments of IgG polyclonal antibody to SAT. SAT-coated plates were incubated overnight with serial dilutions of Fabγ having antibody activity to SAT, or with similarly diluted enzyme blank, and then for 4 h with 10 μg/ml of polyclonal IgG anti-SAT. Bound Fabγ (●) and Fcγ (■) were developed, in replicate plates not exposed to intact IgG, by means of peroxidase-conjugated anti-κ plus anti-λ light chain, or anti-Fcγ reagents, respectively. The CCP was activated by adding fresh normal serum diluted 1 : 25 in PBS with Ca^{2+} and Mg^{2+} for 20 min at 37 °C, and bound C3b was revealed by developing with peroxidase-conjugated anti-C3c. (○) Inhibition of CCP activation by Fabγ fragments; (△) inhibition of CCP activation by similarly diluted enzyme blank control. Each point shows the mean of triplicate values (± SE) less background given in control wells not treated with IgG anti-SAT antibody.

against SAT were tested in CF-ELISA for inhibition of polyclonal IgG antibody-mediated CCP activation, C3b fixation was inhibited in relation to the amount of Fabγ bound (Fig. 5). A similarly diluted enzyme control preparation had no effect on the CCP. These results were similar to those obtained with Fabα fragments, except that Fabγ fragments appeared to be less inhibitory, relative to the dose applied and the amount bound, than Fabα fragments.

3.4 Inhibition of C1q binding

It seemed possible that inhibition of IgG-mediated CCP activation by IgA antibodies might be due to disruption of the matrix of Fcγ regions that constitutes the receptor for C1q [21]. A C1q binding ELISA showed that C1q was bound by polyclonal or mixed IgG mAb to SAT in a dose-dependent manner, but not by IgA antibodies (Fig. 6A). However, neither IgA mAb nor polyclonal IgA having anti-SAT activity significantly inhibited the binding of C1q to polyclonal IgG antibodies (Fig. 6B). In contrast, IgA-Fug, which could compete against IgG-Fug for binding to SAT (see above), was able to inhibit C1q binding mediated by an equal mixture of IgG-JJF, IgG-Kal, and IgG-Fug applied to SAT-coated plates (Fig. 6C). Neither IgA-Tin, which does not compete against any of the IgG mAb, nor IgA1-Car, which lacks anti-SAT activity, was able to inhibit C1q binding.

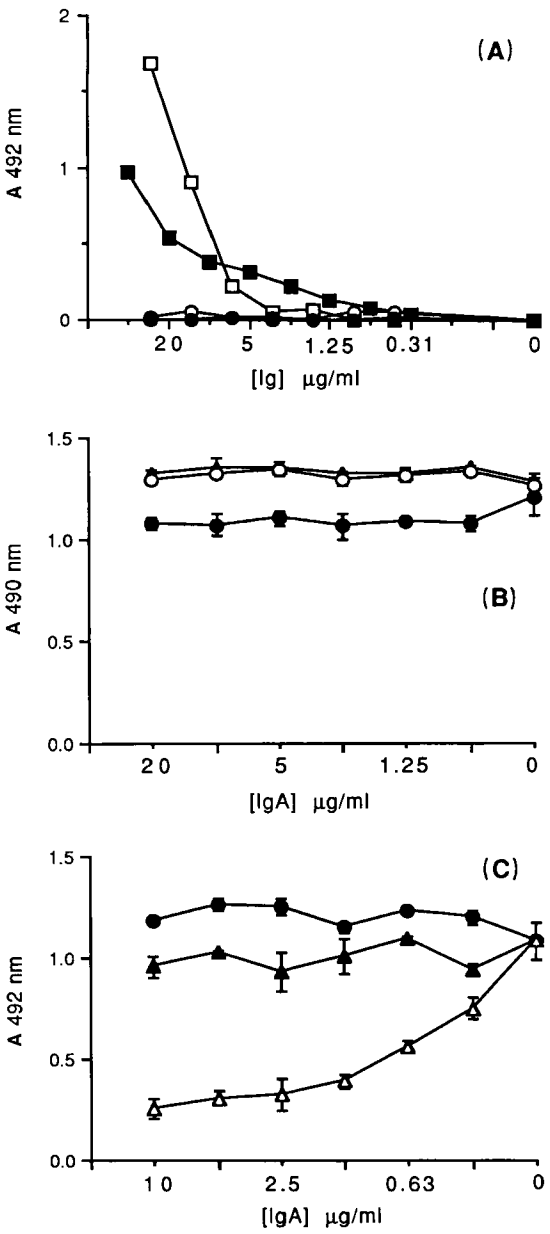

Figure 6. (A) Binding of C1q by IgG but not by IgA antibodies bound to SAT-coated plates. (□) polyclonal IgG; (■) mixed monoclonal IgG; (○) polyclonal IgA; (●) IgA-Tin. (B) Lack of inhibition by IgA anti-SAT antibodies of C1q binding to polyclonal IgG anti-SAT antibodies. (○) polyclonal IgA; (●) IgA-Tin; (▲) IgA-Car, which does not recognize SAT. (C) Inhibition by IgA anti-SAT antibodies of C1q binding to a mixture of three IgG anti-SAT mAb. (●) IgA-Tin; (△) IgA-Fug; (▲) IgA-Car, which does not recognize SAT. SAT-coated plates were incubated overnight with serial dilutions of IgG or IgA (A), or with serial dilutions of IgA followed by a constant concentration of IgG (B, C). C1q (2.5 μg/ml) was applied for 4 h, and bound C1q was developed with peroxidase-conjugated anti-C1q. Each point shows the mean of duplicate or triplicate values (± SE), less appropriate background.

4 Discussion

IgG initiates the CCP by binding C1q through receptors in the C_H2 domain, which have recently been identified with three highly conserved amino acid residues [22] that are not present in IgA. As activation of C1 requires multivalent binding of the globular heads of C1q, the spatial arrangement of IgG molecules and their Fc regions is held to be important for this interaction [21]. This presumbly accounts

for the observation that mixtures of two or more individual IgG1 mAb bound to SAT are necessary for effective CCP activation [14], and for C1q binding, as seen in these studies. The insertion of IgA into the matrix of IgG molecules on an antigen surface might therefore be expected to disrupt the multivalent array of Fcγ that constitutes the receptor for C1q, and so lead to inhibition of CCP activation. However, polyclonal and two IgA mAb were found to inhibit the activation and deposition of C3b onto solid-phase IgG antibody-antigen complexes, without necessarily inhibiting C1q binding, which was shown only by a monoclonal IgA that was able to compete against monoclonal IgG in binding to antigen. Presumably, polyclonal IgA antibodies should have the same effect against polyclonal IgG antibodies, but we were unable to obtain a high enough concentration of polyclonal IgA antibodies to test this prediction. These findings imply that there must be at least two mechanisms whereby IgA antibodies can inhibit IgG-mediated C activation: by competitive displacement of IgG antibodies, resulting in diminished C1q binding; and by other mechanisms not dependent on competition for binding to antigen. Possibly the insertion of IgA among IgG molecules in an antigen-antibody complex permits C1q to bind to IgG in such a manner (for example, univalently) that it does not become activated.

Regardless of the mechanism involved, significant inhibition of CCP activation mediated by 10 μg/ml of polyclonal IgG antibody was achieved by 1 μg/ml of IgA mAb, which is well within physiological range. Although polymeric IgA-Tin was more inhibitory than monomeric IgA-Fug, it is unlikely that its polymeric form was responsible for this because their respective Fabα fragments, which are univalent, displayed the same difference in inhibitory activity. This finding also demonstrates that the Fc region of IgA is not necessary for inhibition of C activation.

In humans, serum IgA is produced in quantities comparable with IgG [1, 24, 25], yet its biological function has remained largely obscure. Soon after its initial discovery, IgA was fund to lack the C-activating and opsonic properties associated with IgG and IgM antibodies [1, 2, 26]. Although aggregated, chemically cross-linked, or denatured human serum and secretory IgA [27–29], as well as native rat or mouse IgA antibodies [30–33], have been shown to activate the alternative C pathway, native human IgA antibodies bound to antigen do not activate either C pathway [14, 23, 34–36]. Our experiments, utilizing unique competing and noncompeting human IgA and IgG mAb to SAT, elucidate the observations that IgA antibodies interfere with C-mediated bacteriolysis [37], hemolysis [38], and Arthus reactions [39]. In other systems, IgA antibodies or myeloma proteins have been shown to inhibit cutaneous anaphylaxis [39, 40], various forms of cell-mediated cytotoxicity [41–43], and chemotaxis [44, 45] or phagocytosis [46, 47] by neutrophils. On the other hand, under certain conditions, IgA may enhance the chemotactic effect of other attractants [48], or promote C-independent phagocytosis [49–53]. However, since bound C3b functions as an opsonin [54], and C5a has chemotactic properties for phagocytes [55], it may be expected that IgA antibodies would also inhibit C-dependent phagocytosis and related phenomena. Thus, our results support the concept that IgA is anti-inflammatory, and while this may be undesirable in the context of infectious diseases or neoplasia, IgA may also function to protect the internal milieu from the adverse effects of other immune effector mechanisms [24].

The novel finding that Fabα fragments generated by IgA1 protease cleavage of human IgA1 antibodies also inhibited IgG-mediated CCP activation suggests that bacterial IgA1 proteases may have at least two effects related to the virulence of mucosal pathogens. Removal of the $(Fc\alpha)_n$-SC part of S-IgA diminishes its protective properties, including its ability to inhibit the adherence of microorganisms to mucosal surfaces [56]. In addition, the residual Fabα fragments, which retain antigen-binding activity [57], may still bind to the bacterial surface and either block the binding of intact antibodies of the same or different isotype, or interfere with their secondary effector functions. The present results demonstrate the latter possibility. Fabγ fragments from IgG antibody also inhibited CCP activation, but this effect was smaller than that of IgA antibodies or their Fabα fragments, and may even be due to a different mechanism, because C3b-binding sites on IgG have been located in the Fab region [58]. However, this is probably of little biological significance at mucosal surfaces, where IgG is less abundant than S-IgA. Furthermore, S-IgA is especially resistant to most proteases other than the uniquely specific IgA1 proteases [1], whereas IgG is readily cleaved to Fabγ, Fcγ, and even smaller fragments by many mammalian and bacterial proteases of broad specificity. The exquisite specificity of IgA1 proteases limits their effect to the generation of intact Fabα and Fcα fragments. Furthermore, these enzymes are not inhibited by mammalian protease inhibitors other than specific antibodies [10, 12], and, unlike other less specific proteases, their action is not dissipated by competition between different protein substrates. Although our experiments were performed with serum-type monomeric and polymeric IgA1, the secretory form of IgA1 prevalent at mucosal surfaces is also susceptible to cleavage by IgA1 proteases [10, 12], and the Fabα fragments generated are the same in either case. Evidence of cleavage of S-IgA at mucosal surfaces *in vivo* is provided by the finding of typical cleavage fragments in secretions of individuals colonized by IgA1 protease-producing bacteria [59]. These considerations have led to a hypothesis regarding the significance of IgA1 proteases in an invasive disease, bacterial meningitis, which is principally caused by IgA1 protease-producing *H. influenzae* type b, *N. meningitidis* and *S. pneumoniae* [13]. According to this hypothesis, binding of Fabα antibody fragments by these organisms at mucosal surfaces protects them from other immune defense mechanisms and, together with other virulence factors, enables them to penetrate mucosal tissues. The present results show that one major anti-bacterial defense mechanism, IgG-mediated C activation, which is important for resistance to *N. meningitidis* [60] and *H. influenzae* [61], and probably enhances opsonophagocytosis of *S. pneumoniae* [62], is inhibited by both intact IgA antibodies and their Fabα fragments.

We thank Bendt Mansa for providing the human myeloma sera, Elin Hanberg and Pamela White for technical assistance, Jiri Mestecky and John Volanakis for valuable discussions and critical assessment of the manuscript, and Dorthe Eggertsen and Maria Bethune for secretarial assistance.

Received July 4, 1989.

5 References

1 Heremans, J. F., in Sela, M. (Ed.), *The Antigens*, vol. 2, Academic Press Inc., New York 1974, p. 365.
2 Kilian, M., Mestecky, J. and Russell, M. W., *Microbiol. Rev.* 1988. *52:* 296.
3 Ogra, P. L., Cumella, J. C. and Welliver, R. C., in Bienenstock, J. (Ed.), *Immunology of the Lung and Upper Respiratory Tract*, McGraw-Hill Book Co., New York 1984, p. 242.
4 Holmgren, J., Anderson, Å., Wallenström, G. and Ouchterlony, Ö., *Infect. Immun.* 1972. *5:* 662.
5 Smith, D. J., Taubman, M. A. and Ebersole, J. F., *Clin. Exp. Immunol.* 1985. *61:* 416.
6 Abraham, S. N. and Beachey, E. H., in Gallin, J. F. and Fauci, A. S. (Eds.), *Advances in Host Defense Mechanisms*, vol. 4, Raven Press, New York 1985, p. 63.
7 Liljemark, W. F., Bloomquist, C. G. and Ofstehage, J., *Infect. Immun.* 1979. *26:* 1104.
8 Moldoveanu, Z., Tenovuo, J., Pruitt, K. M., Mansson-Rahemtulla, B. and Mestecky, J., *Ann. N.Y. Acad. Sci.* 1983. *409:* 848.
9 Magnusson, K.-E. and Stjernström, I., *Immunology* 1982. *45:* 239.
10 Kilian, M. and Reinholdt, J., in Easmon, C. S. F. and Jeljaszewics, J. (Eds.), *Medical Microbiology*, vol. 5, Academic Press, London 1986, p. 173.
11 Mulks, M. H., in Holder, I. A. (Ed.), *Bacterial Enzymes and Virulence*, CRC Press, Boca Raton 1985, p. 81.
12 Plaut, A. G., *Annu. Rev. Microbiol.* 1983. *37:* 603.
13 Kilian, M. and Reinholdt, J., *Adv. Exp. Med. Biol.* 1987. *216B:* 1261.
14 Russell, M. W. and Mansa, B., *Scand. J. Immunol.* 1989. *30:* 175.
15 Mansa, B., Kjems, E. and Lind, I., *Progr. Immunobiol. Scand.* 1970. *4:* 60.
16 Baatrup, G., Svehag, S.-E. and Jensenius, J. C., *Scand. J. Immunol.* 1986. *23:* 397.
17 Smith, G. W., Myles, M. J. and Simpson, I. J., *J. Immunol. Methods* 1984. *67:* 167.
18 Avrameas, S. and Ternynck, T., *Immunochemistry* 1971. *8:* 1175.
19 Mestecky, J. and Kilian, M., *Methods Enzymol.* 1985. *116:* 37.
20 Stanworth, D. R. and Turner, M. W., in Weir, D. M. (Ed.), *Handbook of Experimental Immunology*, vol. 1, 3rd Edit., Blackwell Scientific Publications, Oxford 1978, p. 6.1.
21 Borsos, T. and Rapp, H. J., *Science* 1965. *150:* 505.
22 Duncan, A. R. and Winter, G., *Nature* 1988. *332:* 738.
23 Waldo, F. B. and Cochran, A. M., *J. Immunol.* 1989. *142:* 3841.
24 Mestecky, J., Russell, M. W., Jackson, S. and Brown, T. A., *Clin. Immunol. Immunopathol.* 1986. *40:* 105.
25 Conley, M. E. and Delacroix, D. L., *Ann. Int. Med.* 1987. *106:* 892.
26 Ishizaka, T., Ishizaka, K., Borsos, T. and Rapp, H., *J. Immunol.* 1966. *97:* 716.
27 Götze, O. and Müller-Eberhard, H. J., *J. Exp. Med.* 1971. *134:* 90s.
28 Boackle, R. J., Pruitt, K. M. and Mestecky, J., *Immunochemistry* 1974. *11:* 543.
29 Hiemstra, P. S., Gorter, A., Stuurman, M. E., Van Es, L. A. and Daha, M. R., *Eur. J. Immunol.* 1987. *17:* 321.
30 Miller, G. W., *J. Immunol.* 1976. *117:* 1374.
31 Pfaffenbach, G., Lamm, M. E. and Gigli, I., *J. Exp. Med.* 1982. *155:* 231.
32 Rits, M., Hiemstra, P. S., Van Es, L. A., Bazin, H., Vaerman, J. P. and Daha, M. R., *Mol. Immunol.* 1987. *24:* 1047.
33 Rits, M., Kints, J. P., Bazin, H. and Vaerman, J. P., *Scand. J. Immunol.* 1987. *25:* 359.
34 Colten, H. R. and Bienenstock, J., *Adv. Exp. Med. Biol.* 1974. *45:* 305.
35 Römer, W., Rother, U. and Rollcke, D., *Immunobiology* 1980. *157:* 41.
36 Imai, H., Chen, A., Wyatt, R. J. and Rifai, A., *Clin. Exp. Immunol.* 1988. *73:* 479.
37 Griffiss, J. McL., *J. Immunol.* 1975. *114:* 1779.
38 Russell-Jones, G. J., Ey, P. L. and Reynolds, B. L., *Mol. Immunol.* 1980. *17:* 1173.
39 Russell-Jones, G. J., Ey, P. L. and Reynolds, B. L., *Int. Arch. Allergy Appl. Immunol.* 1981. *66:* 316.
40 Ishizaka, K., Ishizaka, T. and Hornbrook, M. M., *J. Allergy* 1963. *34:* 395.
41 O'Niell, P. A. and Romsdahl, M. M., *Immunol. Commun.* 1974. *3:* 427.
42 Mathew, G. D., Qualtiere, L. F., Neel, H. B. and Pearson, G. R., *Int. J. Cancer* 1981. *27:* 175.
43 Komiyama, K., Crago, S. S., Itoh, K., Moro, I. and Mestecky, J., *Cell. Immunol.* 1986. *101:* 143.
44 Van Epps, D. E. and Williams, R. C., *J. Exp. Med.* 1976. *144:* 1227.
45 Ito, S., Mikawa, H., Shinomiya, K. and Yoshida, T., *Clin. Exp. Immunol.* 1979. *37:* 436.
46 Van Epps, D. E., Reed, K. and Williams, R. C., *Cell. Immunol.* 1978. *36:* 363.
47 Wilton, J. M. A., *Clin. Exp. Immunol.* 1978. *34:* 423.
48 Arashi, M., Sibille, Y., Merrill, W. W., Rits, M., Bazin H. and Vaerman, J. P., *Infect. Immun.* 1989. *57:* 452.
49 Lowell, G. H., Smith, L. F., Griffiss, J. McL. and Brandt, B., *J. Exp. Med.* 1980. *152:* 452.
50 Fanger, M. W., Goldstine, S. N. and Shen, L., *Mol. Immunol.* 1983. *20:* 1019.
51 Yeaman, G. R. and Kerr, M. A., *Clin. Exp. Immunol.* 1987. *68:* 700.
52 Gorter, A., Hiemstra, P. S., Leijh, P. C. J., Van der Sluys, M. E., Van den Barselaar, M. T., Van Es, L. A. and Daha, M. R., *Immunology* 1987. *61:* 303.
53 Pier, G. B., Thomas, D., Small, G., Siadak, A. and Zweerink, H., *Infect. Immun.* 1989. *57:* 174.
54 Joiner, K. A., Brown, E. J. and Frank, M. M., *Annu. Rev. Immunol.* 1984. *2:* 461.
55 Snyderman, R. and Pike, M. C., *Annu. Rev. Immunol.* 1984. *2:* 257.
56 Reinholdt, J. and Kilian, M., *J. Dent. Res.* 1987. *66:* 492.
57 Mansa, B. and Kilian, M., *Infect. Immun.* 1986. *52:* 171.
58 Gadd, K. J. and Reid, K. B. M., *Biochem. J.* 1981. *195:* 471.
59 Kilian, M., Reinholdt, J., Mortensen, S. B. and Sørensen, C. H., *Bull. Eur. Physiopathol. Resp.* 1983. *19:* 99.
60 Nicholson, A. and Lepow, I. H., *Science* 1979. *205:* 298.
61 Schneerson, R., Rodrigues, L. P., Parke, J. C. and Robbins, J. B., *J. Immunol.* 1971. *107:* 1081.
62 Hosea, S. W., Brown, E. J. and Frank, M. M., *J. Infect. Dis.* *k*1980. *142:* 903.