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Complement-coated antibody-transfer (CCAT); serum IgA1 antibodies intercept and transport C4 and C3 fragments and preserve IgG1 deployment (PGD)☆

Robert J. Boackle[a,b,*], Quang L. Nguyen[a], Renata S. Leite[a,b], Xiaofeng Yang[a], Jana Vesely[a]

[a] *Department of Stomatology, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425, USA*
[b] *Department of Microbiology and Immunology, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425, USA*

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Abstract

In periodontal disease, IgG1 and IgA1 antibodies produced in situ deposit on antigens in the affected tissues. Thus, there is an interest in the effect of co-deposited IgA1 antibodies on complement activation by IgG1-immune complexes. In the present study, we first analyzed the effect of IgA1-immune complexes on complement using human IgA1 antibodies to dansyl (with dansylated human serum albumin serving as the immobilized antigen). It was observed that these IgA1-immune complexes when incubated for prolonged times with 33% human serum as a source of complement received C4b and C3b deposition. As C4b and C3b deposited on the IgA1 antibodies and on the antigenic surface, the complement-coated IgA1 antibodies departed. These fluid-phase complement-coated IgA1 antibodies were transferred to antigen-coated microtiter-ELISA plates, where they became bound to the antigens. Thus, the complement-coated IgA1 antibodies retained their antigen-binding function, especially as a proportion of their covalently bound C3b progressively degraded to iC3b and C3d. Genetically engineered carbohydrate-deficient mutant human IgA1 antibodies were used to assess the role of carbohydrate in accepting the C4b and C3b depositions, and these studies indicated that the carbohydrate on the Fc-region of IgA1 played a positive role. Another interesting finding generated by this study was that when IgA1 was co-deposited with IgG1 antibodies, and serum complement was added, the IgG1 antibodies tended to remain on the antigenic surface. The co-deposited IgA1 antibodies not only controlled (reduced) the rate of the consumption of the first component of complement (C1) and of classical complement pathway activation by IgG1-immune complexes (and therein reduced the rate of complement-mediated dissolution of the IgG1-immune complexes), but also the co-deposited IgA1 antibodies simultaneously intercepted/accepted C4b and C3b, then departed, as complement began to cover the antigenic surfaces. The process in which complement-coated IgA1 antibodies transferred to non-complement-coated antigens is termed complement-coated antibody-transfer/transport (CCAT). In this way, IgA1 antibodies extended the efficiency of the complement system by insuring the specific IgA1 antibody-mediated transport of the captured biologically active complement fragments to those antigens stimulating the IgA1 antibody response but not yet neutralized (completely coated) with complement. Simultaneously by impeding the rate of C1 consumption and by intercepting C4b and C3b, IgA1 antibodies slowed C4b and C3b deposition on the antigenic surface and on the co-deposited IgG1 antibodies. Thus, in the presence of ongoing complement activation, the deposition of serum IgA1 antibodies enabled the co-deposited IgG1 antibodies to better maintain their ability to interact with antigens. We termed this latter phenomenon, preservation of IgG antibody deployment (PGD). In summary, co-deposited IgA1 antibodies maximized the efficiency of the complement system, transported their covalently bound complement fragments to specific antigens and sustained the effective deployment of IgG1 antibodies directed to those same antigens.
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Abbreviations: CCAT, Complement-coated antibody-transfer/transport; C1, the first component of complement; C4, the fourth complement component; C3, the third complement component; iC3b, inactivated C3b; PGD, preservation of IgG antibody deployment

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* Corresponding author. Tel.: +1 843 792 2551; fax: +1 843 792 6433.
E-mail address: boacklrj@musc.edu (R.J. Boackle).

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1. Introduction

In inflammatory periodontal disease, plasma cells in the affected tissues produce IgG and IgA antibodies specific for periodontal pathogens (Ogawa et al., 1989). The IgA1 and IgG1 isotypes predominate, especially at the early stages of the disease (Ogawa et al., 1989; Kilian et al., 1989; Kinane et al., 1999). In addition, these IgA1 and IgG1 antibodies interact with periodontal pathogens (Ogawa et al., 1989; Condorelli et al., 1998).

The present study was designed to ascertain the regulatory verses the supportive role of serum IgA1 antibodies on IgG1-mediated complement activation and deployment. Self-aggregated IgA fails to effectively compete against IgG antibody-coated cells for binding to the first component of complement (C1) (Boackle et al., 1974) due to a lack of interaction of IgA with C1q globular heads. Thus, it is logical that co-deposited IgA antibodies would reduce IgG-immune complex-mediated complement activation as evidenced by a lower deposition of the third complement component (C3) (Russell et al., 1989; Nikolova et al., 1994). However, both C3b and inactivated C3b (iC3b) have been shown to deposit on IgA-immune complexes, and more interestingly the carbohydrate on IgA is important for C3b deposition (Zhang and Lachmann, 1994, 1996). Likewise, intravenous non-specific IgA preparations inadvertently accept C4b and C3b depositions (Miletic et al., 1996). In view of the above findings, we sought additional reasons to explain the detection of low C3b deposition levels when IgG1 is co-immobilized with IgA1 on antigens.

Under certain conditions, immobilized IgA preparations have the potential to activate the Lectin Complement Pathway (Roos et al., 1991). Yet several relevant studies of the interactions of IgA with complement (Russell and Mansa, 1989; Burritt et al., 1976; Hiemstra et al., 1987; Hiemstra et al., 1988; Imai et al., 1988; Morton et al., 1993; Janoff et al., 1999) suggest an even deeper complexity to complement–IgA interactions. Unravelling this complexity is important because inadequate complement regulation, effectiveness or inappropriate utilization exacerbates a variety of diseases and conditions, including not only periodontal disease but also rheumatoid arthritis, asthma and HIV-1 infection (Schenkein et al., 1995; Hietala et al., 2004; Roberts et al., 2003; Hawlisch et al., 2004; Lachmann, 2002; Stoiber et al., 2003; Bajtay et al., 2004). Also, there is evidence within infected mucosal tissues for a role of the polymeric forms of serum IgA, which participate with complement and phagocytes in the killing of invasive mucosal pathogens (Janoff et al., 1999).

In several of the experiments presented here, IgG1-immune complexes were used to activate the classical complement pathway and quickly generate the deposition of sufficient levels of the fourth complement component (C4) and C3 (Schumaker et al., 1986). The C3b molecules (including those generated via the C3b-amplification loop) deposit both on the antigen and on the Fab (C_H1gamma) region (Shohet et al., 1993) of the antigen-bound IgG1. The favored attachment of complement fragments to the Fab region of IgG1antibodies may alter the affinity for antigen (Horgan et al., 1992), rather than the intrinsic potential of the Fc region of IgG1 to interact with C1q (Ziccardi, 1986). Once sufficient levels of C4bC2a and C3b deposit on the antigen and on IgG1, the IgG1-immune complexes become disrupted, the $C1qr_2s_2$ association becomes loosened and C1-inhibitor readily inactivates the activated $C1r_2$ and $C1s_2$ enzymes. The action of C1-inhibitor prevents unnecessary, wasteful consumption of native C4 and C2 (Ziccardi, 1988). In addition to inactivating and removing $C1r_2$ and $C1s_2$, C1-inhibitor is capable of dislodging the entire activated $C1qr_2s_2$ complex, when the avidity between immobilized immunoglobulins and C1 is low (Chen and Boackle, 1998; Chen et al., 1998); as when relatively low levels of IgG1 are deposited on antigens or when IgA1 antibodies are co-deposited with IgG1 antibodies on antigens. Thus, it is most relevant to approximate physiological serum conditions in order to maintain the natural degree of control by C1-inhibitor when testing for the role of IgA in complement functions.

In the present studies, we used genetically engineered human IgA1 and IgG1 chimeric antibodies with identical variable regions (identical amino acid sequences in the variable domains) for the dansyl antigen (Chuang and Morrison, 1997). We report here for the first time the discovery of two new functions for human serum IgA1 antibodies, complement-coated antibody-transfer/transport (CCAT) and the preservation of IgG1 antibody deployment (PGD).

2. Methods

2.1. Buffers

The buffers used were BBS^{2+} pH 7.4, an isotonic barbital buffered saline consisting of 150 mM sodium chloride, 4.5 mM sodium barbital, 0.15 mM calcium chloride (Ca^{2+}) and 1.0 mM magnesium chloride (Mg^{2+}); and EGTA-Mg^{2+} buffer pH 7.4, containing 8.0 mM ethyleneglycol-bis-tetraacetic acid (EGTA), 150 mM sodium chloride, 4.5 mM sodium barbital and 2.0 mM magnesium chloride.

2.2. Human serum complement

Fresh normal human serum (NHS) served as a source of human complement and was prepared as previously described (Boackle et al., 1993). Human subjects donating blood provided signed a human consent form approved by the Institutional Review Board of the Medical University of South Carolina. Serum was used neat or diluted no more than 1:3 in BBS^{2+} to provide near physiological levels of C1-inhibitor function at the onset of the reactions.

2.3. Antibody and antigen preparations

Dansylated human serum albumin (DNS-HSA) was prepared by coupling dansyl-chloride (Sigma) to ultra-pure

human serum albumin, HSA (Boehringer Mannheim Biochemicals, IN) via methods previously used to prepare dansylated bovine serum albumin (Chuang and Morrison, 1997).

Well characterized chimeric antibodies to dansyl (Chuang and Morrison, 1997) including IgG1 and IgA1 (wild type) as well as mutant IgA1 antibodies deficient in carbohydrate were kindly provided by S.L. Morrison and K. Chintalacharuvu. The genetically engineered IgG1 antibody had human constant regions for IgG1 (i.e., human C_H1gamma1, C_H2gamma1 and C_H3gamma1). Likewise, the IgA1 antibody to dansyl had human constant regions (C_H1alpha1, C_H2alpha1 and C_H3alpha1 including the secreted tailpiece). The antibodies (both IgG1 and IgA1) had identical light chains. The variable region (domain) of the heavy chain and the variable region of the light chain were of mouse origin (the genetic information was originally obtained from a mouse hybridoma to the dansyl antigen). In addition to wild-type IgA1 antibodies to dansyl that were just described, three different carbohydrate deficient mutant IgA1 antibodies to dansyl were utilized in our studies. These mutants of IgA1 antibodies had carbohydrate deficiencies at selected sites on the Fc-region, and were derived by preventing the possibility of the attachment of N-linked carbohydrate at specific sites. The carbohydrate deficient mutant termed IgA1/C_H2 was point-mutated at asparagine 263 (this asparagine was point mutated to glutamine), the mutant termed IgA1/C_H3 was point-mutated at asparagine 459 (substituted to glutamine) in the tailpiece region and the mutant termed IgA1/C_H2/C_H3 had amino acid point mutations at both asparigine 263 and asparagine 459. Thus, the human sections of each of the above chimeric IgA1 and IgG1 antibodies included the entire constant heavy region and the entire constant light (kappa) chain region. However, all of the genetically engineered IgG1 and IgA1 antibodies had identical mouse variable regions (identical amino acid sequences in the entire variable heavy and variable light chain domains) for the dansyl antigen (Chuang and Morrison, 1997). The ratios of monomeric to polymeric forms for each IgA1 antibody preparation, including the chimeric IgA1 mutant antibodies have been described in detail as well as their relative affinities for dansyl (Chuang and Morrison, 1997). Polymeric and monomeric forms of the chimeric IgA1 antibodies were used together in our experiments.

2.4. Microtiter-kinetic-ELISA

A microtiter-kinetic-ELISA (Chen and Boackle, 1998; Chen et al., 1998) was employed for testing the level of C4b deposition after incubating antigen-immobilized IgG1 and IgA1 antibodies with human serum. In this procedure, pre-titrated doses of antigen (DNS-HSA) in carbonate buffer pH 9.6 were immobilized in triplicate on microtiter ELISA plates (Immobilon I, Costar, Cambridge, MA) for 18 h at 4 °C, washed then blocked with 2% ultra-pure HSA in 0.15 M phosphate-buffered saline, pH 7.4 (PBS), and washed again. Then, chimeric IgG1 antibodies with or without specified doses of chimeric IgA1 antibodies were added to the immobilized antigen and incubated for 60 min at 37 °C. After the unbound proteins were washed with PBS, undiluted human serum (or when specified, a 1:3 dilution of human serum) was added to the wells as a source of complement and incubated at 37 °C for either 30 min or for the specified times. After thoroughly washing with 0.05% Tween-20 in phosphate-buffered saline (TPBS), the plates were probed for the level of C4b deposition using specific sheep anti-C4-HRP (The Binding Site, San Diego, CA), which was diluted in TPBS containing 0.2% ultra-pure HSA. The substrate was prepared by mixing 3% hydrogen peroxide and 2,2-azinobis (3-ethylbenz-thiazoline-6-sulfonic acid), ABTS (Sigma, St. Louis, MO) in 0.1 M citric acid buffer, pH 4.0. After the wells were washed with TPBS, the substrate was added and the plates were immediately read at 415 nm in a V_{max} Kinetic Microplate Reader (Molecular Devices, Menlo Park, CA).

In other experiments (shown in Fig. 5a and b), selected premixed ratios of human IgG1 to human IgA1 myeloma preparations were directly immobilized overnight at 4 °C. Immobilization of immunoglobulins (most notably IgA1 myeloma preparations) directly to plastic may lead to atypical complement-activating conformations (Russell and Mansa, 1989), which can activate complement when incubated with diluted serum. Less than 10% serum reduces the critical rate-limiting level C1-inhibitor allowing exacerbated complement activation (Boackle et al., 1993; Dillman et al., 1995; Chen and Boackle, 1998; Chen et al., 1998). The purified human IgG1 was from Sigma and the myeloma IgA1 was a gift from Dr. A.C. Wang.

In addition, specified premixed ratios of IgA1 and IgG1 chimeric antibodies were incubated with immobilized antigen (DNS-HSA, 0.3 μg/well) overnight at 4 °C (shown in Fig. 5c and d). In both cases, a 1:3 dilution of fresh human serum was added for 30 min at 37 °C, washed then the wells were probed for deposition of C3 (C3b and iC3b), using sheep-antibody to human C3c determinants (Calbiochem), followed by rabbit anti-sheep IgG-HRP; or for C4b deposition with sheep anti-human C4b (Calbiochem) followed by rabbit anti-sheep IgG-HRP (Calbiochem). The residual IgG1 chimeric antibodies (Fig. 5d) on antigens were directly probed using specific goat anti-gamma chain-HRP (The Binding Site).

2.5. Complement transfer and detection of bound C3d determinants

Microtiter-kinetic-ELISA was used to determine the levels human serum C3d determinants (C3b, iC3b and/or C3d) deposited on immobilized wild-type IgG1- and IgA1-immune complexes (i.e., remaining immobilized on the first microtiter plate) as compared to the released complement-coated (cc) IgG1 and cc-IgA1 that were transferred to a second microtiter plate, containing only immobilized DNS-HSA antigen. In the former samples, immune complexes were formed by the addition of 1.0 μg of IgG1 chimeric antibody or of IgA1 chimeric antibody to immobilized (HSA-blocked)

DNS-HSA (0.3 μg/well) for 1 h at 37 °C. After the wells were washed, a 1:3 dilution of fresh human serum in BBS^{2+} was added and incubated for 30 min at 37 °C. Subsequently, the microtiter plates were placed on ice and 20 μl of cold 0.1 M EDTA (disodium ethylenediamine-tetraacetate), pH 7.4 was added to the wells for 10 min to prevent further complement activation. The fluid phase contents were then transferred to a second ELISA microtiter plate with pre-immobilized (HSA-blocked) DNS-HSA, and the cc-IgG1 and cc-IgA1 were allowed to bind for 18 h at 4 °C. After the unbound proteins were washed with PBS containing 0.05% Tween-20, both the original plate and the antigen-coated plate receiving the antibody transfer were probed for C3d determinants using sheep anti-human C3d antibody (Abcam, INC, Cambridge, MA), followed by rabbit anti-sheep IgG-HRP (The Binding Site).

3. Results

Using immobilized antigens (dansylated human serum albumin) and genetically engineered human chimeric antibodies each with identical mouse variable regions (V_H and V_L) to dansyl, we compared IgG1-immune complexes to IgA1-immune complexes to establish their independent interactions with complement. Physiologically relevant serum dilutions (i.e., undiluted serum or no less than 33% serum) were used to provide adequate C1-inhibitor function, the normal rate-limiting factor controlling the initiation of the classical complement pathway (Boackle et al., 1993; Dillman et al., 1995; Chen and Boackle, 1998; Chen et al., 1998) and to supply alpha-2-macroglobulin that with C1-inhibitor maintains normal control over the initiation of the lectin complement pathway (Ambrus et al., 2003), recently implicated with immobilized IgA preparations (Roos et al., 2001).

3.1. IgG1-immune complexes

Under the above conditions, we observed a rapid initial C4b deposition on IgG1-immune complexes, followed by a significant decrease in the detection of bound C4b determinants (Fig. 1). The initial deposition of C4b is consistent with the relatively strong complement-activating ability of IgG1-immune complexes on the classical complement pathway via C1 activation. The time-dependant shift to a decrease in the detection of bound C4b was due to several concomitant factors. These included C4b catabolism, masking of C4b by C3b binding to C4b, and most relevant to our present discussion, to a discontinuation of C1-mediated C4b deposition, because of the irreversible departure of the C1 activator (IgG1 antibodies) from the complement-coated antigenic surfaces (due to complement-mediated dissolution of the IgG1-immune complexes).

3.2. IgA1-immune complexes and CCAT

Unlike IgG1, the IgA1-immune complexes induced very low levels of C4b deposition initially, but over a 30-min

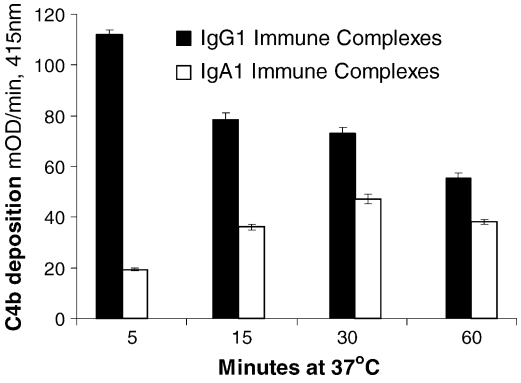

Fig. 1. Time-responses are shown of C4b deposition on IgG1-immune complexes or IgA1-immune complexes using undiluted fresh human serum as a source of complement. Antibodies, IgA1 or IgG1 (1.0 μg) were applied to the immobilized antigen DNS-HSA (300 ng/well) for 1 h at 37 °C. After washing, human serum was added and incubated for 5, 15, 30 or 60 min at 37 °C. The deposited C4b was detected using sheep anti-human C4-HRP in a microtiter kinetic-ELISA. Data are the mean ± S.D. of triplicate determinations of two independent experiments.

period, a gradual increase in C4b deposition was observed, consistent with the explanation that IgA1 antibodies were readily accepting C4b (Fig. 1). The generated C4b was the result of relatively weak IgA1-immune complex mediated complement activation together with a low-level background complement activation, which became more amplified as the complement control proteins (e.g., C1-inhibitor) became consumed with time. In other words, as the complement control proteins became consumed while controlling background complement activation by the immobilized dansylated albumin (antigen), the IgA1-immune complexes appeared to acquire a greater ability to induce or extend complement activation (possibly involving the lectin, classical and/or alternative complement pathways). However, the precise mechanisms (pathways involved) were not discerned in this study. Nonetheless, it appeared that complement-coated IgA1 antibodies (cc-IgA1) retained their ability to reversibly bind to antigens during the initial period of incubation. However, after 30 min of incubation with serum, a continual decrease in C4b detection began to occur. We determined that one of the reasons for this decrease was the departure of the cc-IgA1 from the antigen, especially as C4b and C3b began to accumulate on the antigenic surface. To test our cc-Ig-departure-transfer/transport hypothesis, we collected the fluid from the complement-treated immune complexes (containing the released complement-coated antibodies). Using conditions that restricted further complement activation, we placed the fluids from each of the two complement treated samples (the antigen-immobilized IgG1 and the antigen-immobilized IgA1) into new microtiter wells coated with DNS-HSA antigens, and probed for deposited complement-coated antibodies. We observed that cc-IgA1 antibodies were more effective than cc-IgG1 at transferring to the fresh non-complement-coated antigenic surfaces (CCAT) and also that a proportion

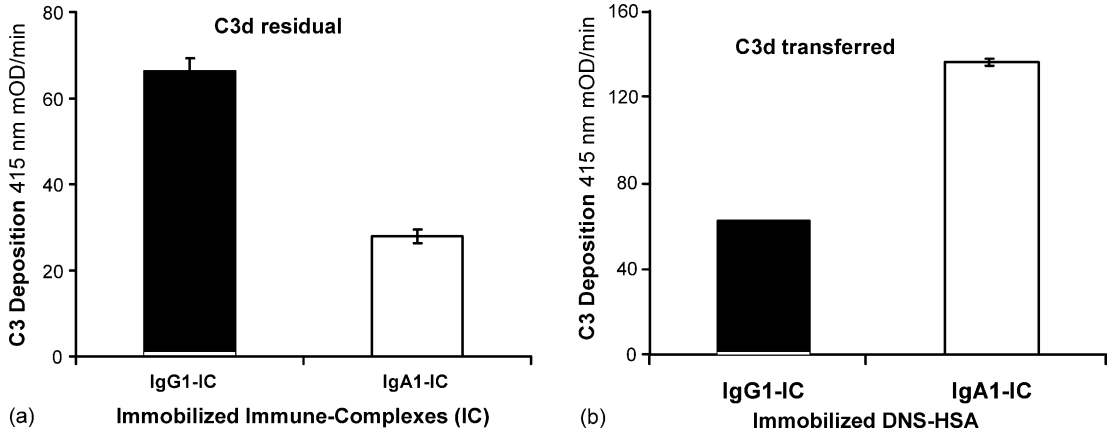

Fig. 2. Detection of C3 fragments that are covalently bound to immune complexes (a) residually-bound C3 fragments remaining on antigens and antibodies, i.e., immobilized IgG1-immune complexes as compared to residually-bound C3 fragments remaining immobilized on IgA1-immune complexes after 30 min incubation with neat fresh human serum; (b) released fluid phase/transferred complement-coated IgG1 (cc-IgG1) and complement-coated IgA1 (cc-IgA1) from those first microtiter plates becoming bound to immobilized antigen DNS-HSA on new microtiter plates. Background deposition was subtracted. Similar results were observed when probing for iC3b neo-determinants using specific anti-iC3b monoclonal antibodies.

of the C3b molecules that were covalently bound to IgA1, progressively degraded to iC3b and C3d (Fig. 2a and b).

3.3. Carbohydrate mutant IgA1 antibodies and complement deposition

In other experiments, we sought evidence that the carbohydrate on IgA1, particularly on the Fc-region of antigen-bound IgA1 antibodies might be a favorable acceptor site for C4b and for C3b. Unlike IgG1, the Fc-region of IgA1 does not have the potential to interact with (nor become covered by) C1q globular heads. Thus, the attachment/placement of C4b and C3b molecules onto the carbohydrate of the Fc region and or hinge region of IgA1 antibodies was clearly a possibility. Such covalent deposits together with the accumulation of C4b and C3b on the antigenic surface could form spatial hindrances that could favor the separation and departure of the complement-coated IgA1 antibodies (cc-IgA1) from complement-coated antigens. To examine this possibility, we utilized mutant IgA1 antibodies with amino acid substitutions yielding carbohydrate-deficiencies within their Fc region and compared them to wild type IgA1 antibodies. Using EGTA-Mg^{2+} to chelate Ca^{2+} and therein block the initiation of the classical and lectin complement pathways, we confirmed the findings of Chuang and Morrison (1997), that the total level of C3b deposition dramatically diminished on the carbohydrate-deficient mutant-IgA1-immune complexes as compared to wild-type IgA1-immune complexes. We concluded that the carbohydrate on the Fc-region was a suitable site for C4b deposition onto IgA1 and for the subsequent formation of functional C3-convertase enzymes (e.g., C4b2a and perhaps C3bBb). Interestingly, when we provided Ca^{2+} and Mg^{2+}, to allow the full initiation of the classical and lectin complement pathways, the final level of C3 deposition on the carbohydrate deficient mutant IgA1-immune complexes was similar to the wild-type IgA1-immune complexes (Fig. 3). In further studying this phenomenon, we observed that the selected absence of carbohydrate on the CH2-region of IgA1, allowed that particular IgA1-mutant antibody when immobilized on antigenic surfaces to acquire the ability to incur significant C4b deposition. [At this time we have preliminary data (to be published as a separate manuscript) that this mutant IgA1 may have activated C1 resulting in C4b deposition.] Importantly in BBS^{2+}, none of the cc-IgA1-carbohydrate deficient mutants were as readily released from the antigenic surfaces as the wild type cc-IgA1 mutants, which had a fully glycosylated Fc-region. This lack of departure of the C3d-coated IgA1-mutants from the DNS-HSA antigenic surface is one of the reasons for the detection of elevated C3d. Thus, deposition of C3 fragments on the carbohydrate-deficient IgA1-mutants occurred but in the absence of the Fc-carbohydrate, this deposition failed to promote the effective release of the cc-IgA1 mutants from the antigenic surface. From these results, we hypothesized that the carbohydrate, on the Fc-region of wild type IgA1 antibodies played important roles in accepting the deposition of C4b and C3b molecules and perhaps in allowing C4b2a and C3bBb activity, which subsequently amplified the level of C3b deposition on the Fc region and on the antigenic surface and increased the degree of physical hindrance with the complement-coated antigenic surface, leading to a favored cc-IgA1 (wild-type) antibody release from that complement-coated antigenic site. In Fig. 4, a drawing is provided to portray the release-transfer concept.

3.4. Direct co-immobilization of IgA1 and IgG1 on ELISA plates

We also examined the effects on complement deposition of mixtures of IgA1 and IgG1 that were directly immobilized on microtiter plates. Firstly, a determination of the optimal

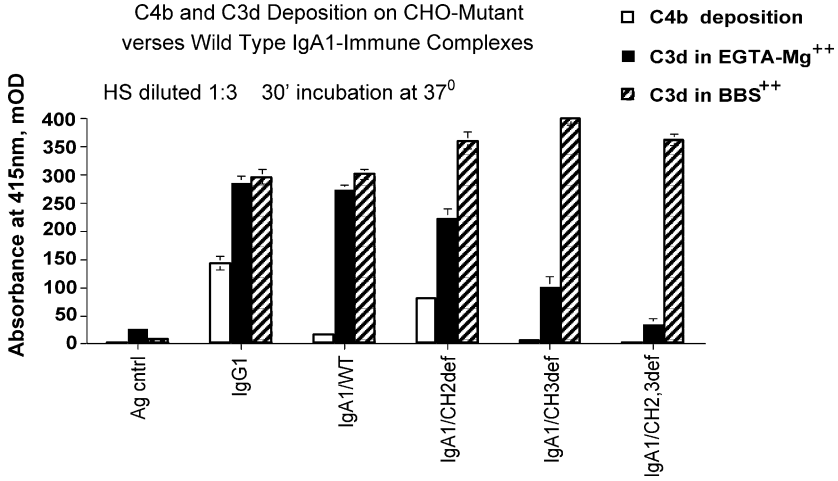

Fig. 3. Detection of C4b and C3d deposition on wild-type IgA1, mutant IgA1 and control IgG1-immune complexes. To the microtiter ELISA plates, 100 μl of DNS/HSA (Ag) was immobilized at 300 ng/well for 18 h at 4 °C. The wells were blocked for 60 min with 1% ultrapure-HSA. The IgA1 wild-type antibodies, carbohydrate deficient IgA1 antibodies or IgG1 antibodies (to dansyl) were added to the immobilized DNS-HSA and incubated for 1 h at 37 °C. Fresh normal human serum served as a source of complement and was diluted to 33% in BBS^{2+} or in EGTA-Mg^{2+} buffer, and incubated with each well for 30 min at 37 °C. After the unbound proteins were washed, the wells were probed for C4b deposition using sheep anti-human C4-HRP, or for C3d deposition using sheep anti-human C3d antibody followed by rabbit anti sheep IgG-HRP. In this figure, the wild-type IgA1 antibody is abbreviated IgA1/WT, the IgA1 mutant antibody with a deficiency in carbohydrate in the CH2 region at amino acid 263 (asparagine mutated to glutamine) is simply labelled IgA1/CH2def. The IgA1 mutant with the deficiency in carbohydrate in the tailpiece at amino acid 459 (asparagine mutated to glutamine) is labelled IgA1/CH3def. The IgA1 mutant with the deficiency in carbohydrate at both 263 and at 459 is labelled IgA1/CH2def/CH3def.

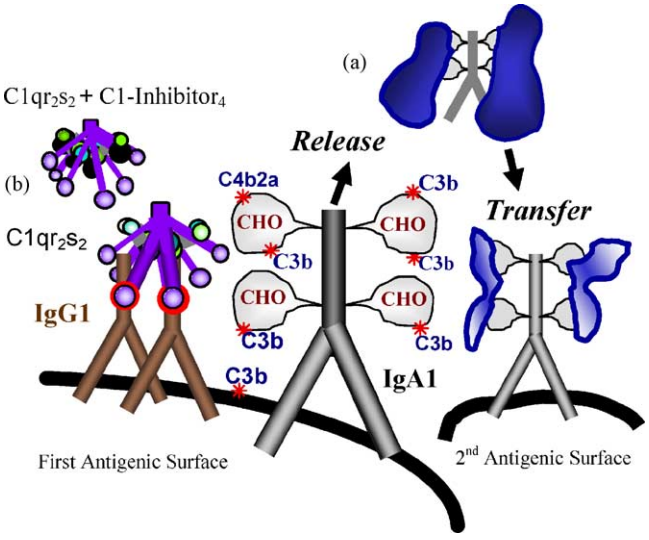

Fig. 4. Diagram of the (a) complement-coated antibody transfer/transport (CCAT) theory and (b) the IgA1-mediated preservation of IgG antibody deployment (PGD) theory. (a) After intercepting/accepting C4b and C3b, the reversibly bound complement-coated IgA1 antibodies were favorably released from the complement-coated antigenic surface yet maintained their antigen-binding function, as the C3b-IgA1 progressively degraded (e.g., to iC3b-IgA1 and C3d-IgA1), facilitating cc-IgA1 transfer to non-complement-coated antigenic sites. (b) Co-deposited IgA1 antibodies (adjacent to IgG1 antibodies) lowered the overall avidity of the IgG1 antibodies with C1q and thereby facilitated the action of C1-inhibitor in removing the entire activated C1 macromolecular complex, prolonging the ability of IgG1 to remain bound to the antigenic surface while exposing the Fc-region of the IgG1 within the immune complex (facilitating potential interactions with Fc_{gamma} receptors).

IgG1 dose that provided the most sensitive changes in complement deposition was made. To that optimal dose of IgG1, increasing levels of IgA1 were added, and then the mixtures were directly immobilized to ELISA plates. One reason, we used direct (irreversible) immobilization was to prevent the departure of the immunoglobulins after complement deposition. In addition, 33% fresh human serum was used as a source of complement, in order to provide near physiological levels of control by C1-inhibitor at the onset of the reaction to control the level of complement activation. Under these conditions, the immobilized human IgG1 (positive controls) were able to activate C1, causing classical complement pathway activation resulting in consumption of C1-inhibitor. The IgA1 myeloma preparations, when co-immobilized with IgG1 generated more complement deposition than IgG alone (Fig. 5a and b). It should be noted that the irreversible attachment of the IgA1 to the plastic surface prevented the departure of the cc-IgA1. Likewise, complement activation became less well controlled by the serum complement inhibitors due to the persistent presence of the complement-coated irreversibly immobilized IgA1 myeloma preparations. This finding suggested the possibility that due to the consumption of functional C1-inhibitor (and perhaps C3b-complement regulators, such as Factor H) by the ongoing IgG1-mediated classical pathway activation (i.e., within the confines of the microtiter wells), the co-immobilized IgA1 myeloma preparations were enabled or allowed to co-initiate complement activation and simultaneously serve as an excellent deposition site for C4b and C3b and for C4b2a and C3bBb.

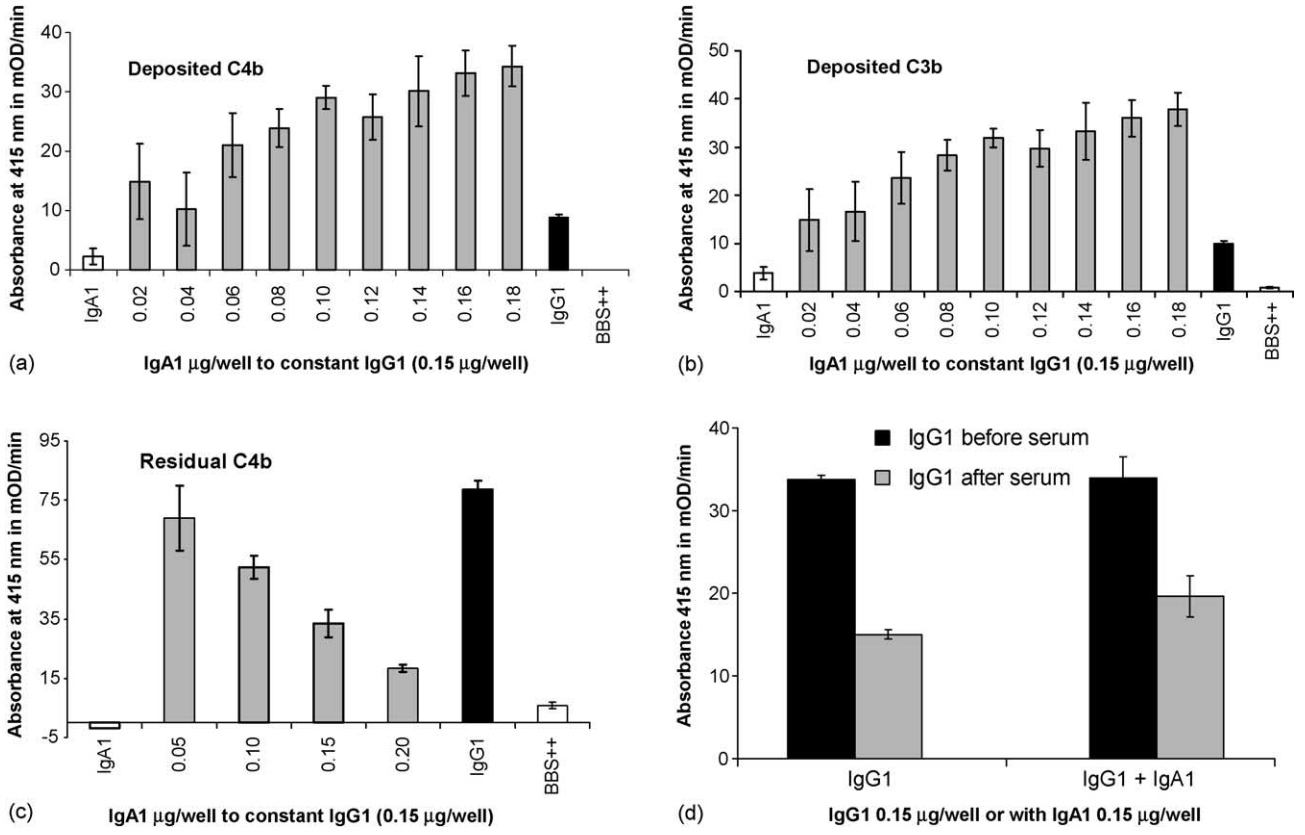

Fig. 5. Using deposited IgA1 and/or IgG1 experiments were designed to detect the deposition of complement after 30 min of incubation at 37 °C using 33% fresh human serum as a source of complement. In (a), the levels of C4b deposition and in (b), the levels of C3b deposition were determined using kinetic ELISA. In these two figures (a and b), a mixture of specified ratios of myeloma IgA1 to myeloma IgG1 were first irreversibly immobilized directly to the microtiter plate overnight then blocked with 2% highly purified human albumin, before the addition of 33% serum as a source of complement. [The use of similar ratios of normal human serum IgA1 (rather than myeloma IgA1) with myeloma human IgG1 under the same experimental conditions caused a similar trend in C4b deposition]. In (c), dansylated human serum albumin (DNS-HSA) was first immobilized, but in this case antigen-antibody complexes were formed, that is, IgG1 antibody and IgA1 antibody to dansyl were used at the specified ratios. Thus, after incubation with 33% serum as a source of complement, the detectable levels of deposited human C4b included those C4b molecules deposited on the residually bound IgA1 and/or IgG1 antibodies. Notably, the detectable levels of C4b were susceptible to the level of departure of the C4-coated antibodies from the antigenic surface. In (d), the stabilizing effect of co-deposited IgA1 antibodies in maintaining the binding of the IgG1 antibodies to the immobilized antigen (DNS-HSA) was examined. Dansylated human serum albumin was immobilized overnight then blocked with 2% human albumin. Subsequently, IgG1 antibodies to dansyl or IgG1 plus IgA1 antibodies to dansyl were added. On the left, the level of IgG1 antibody to dansyl before the addition of serum complement is plotted next to the level of residually detected IgG1 antibody after incubation with complement (33% fresh human serum for 30 min at 37°). On the right, the effect of the co-deposited IgA1 antibodies on the detectable level of IgG1 antibodies before the addition of serum complement is plotted next to the level of residually detected IgG1 antibodies after incubation with complement. The important finding is that co-deposited IgA1 antibodies helped to maintain the binding of the IgG1 antibodies to the DNS-HSA antigenic surface.

3.5. Deposition of individual chimeric antibodies on antigens

Unlike the case in Fig. 5a where IgA1 alone (in the absence of co-deposited IgG1) was directly immobilized to plastic in ELISA and demonstrated little C4b deposition, when undiluted serum was added and incubated at 37 °C with IgA1 chimeric antibodies that were deposited (alone, with no IgG1 antibodies) on immobilized antigens (DNS-HSA), C4b deposition was detected (Fig. 1). One possibility that may explain this C4b deposition is a conformational change in the IgA1 antibodies upon interacting with their immobilized antigens. However, caution must be exercised in developing precise theoretical explanations (i.e., type of complement pathways being activated) due to the fact that the DNS-antigen alone tended to cause a low level (background) C1 fixation (activation) that with extended times could result in consumption of C1-inhibitor within the microtiter well. The continual consumption of C1-inhibitor would result in a more poorly controlled complement system, creating conditions for significant classical and lectin pathway complement activation by relatively weak complement activators.

3.6. Co-deposition of chimeric antibodies

However, when IgG1 and IgA1 chimeric antibodies were co-deposited onto immobilized antigens and incubated with serum complement, the restrictive effects of co-deposited IgA1 on C1 fixation by antigen-bound IgG1 antibodies, boosted the function of the C1-inhibitor activity in this

system. This important function together with the ready departure from the antigenic surface of IgA1 antibodies, after accepting (becoming coated with) complement fragments, resulted in lower levels of C4b detected on the antigen-coated wells (Fig. 5c).

3.7. Preservation of IgG antibody deployment

Interestingly, the co-deposition of IgA1 antibodies alongside IgG1 antibodies on antigens tended to preserve or stabilize the antigen-binding function of IgG1 (Fig. 5d). This stabilization was due in part to the IgA1 mediated boosting of the function of the C1-inhibitor mentioned above; and to the interception of C4b and C3b by co-deposited IgA1 antibodies, which protected (the C_H1 of) the adjacent IgG1 antibodies and simultaneously sheltered the antigenic surface from rapid complement deposition. Preserving the antigen-binding capacity of IgG1 antibodies by co-deposited intact IgA1 antibodies could have important biological significance by allowing specific IgG1 antibodies to retain their unimpeded antigen-binding function as they reversibly move from one antigenic surface to another antigenic surface, efficiently initiating the complement cascade at those locations. This interpretation may explain why in vivo, human serum IgA anti-erythrocyte antibodies act synergistically with IgG anti-erythrocyte antibodies in generating complement-mediated hemolysis (Sokol et al., 1997).

4. Discussion

We identified two novel biological functions for human IgA1 antibodies. The first discovery was complement-coated antibody-transfer/transport. Via this mechanism the IgA1 antibodies, most notably those deposited on antigens served as acceptors and interceptors of C4b and C3b.

Even in the absence of IgG1-immune complexes, C4b and C3b fragments were generated as a consequence of the intrinsic complement activating properties of DNS-antigenic surface, and later by the complement-activating properties of the complement-coated IgA1-immune complexes, especially as the complement control proteins, such as C1 inhibitor and Factor H, became consumed in that confined area, allowing C4b2a and C3bBb formation (and function) while attached to the Fc-region of the IgA1.

Upon becoming equipped with these biologically active complement fragments, the IgA1 antibodies departed from antigenic surfaces, as the antigenic surfaces eventually became coated with complement, and then the complement-coated IgA1 antibodies (cc-IgA1) specifically targeted (transferred to) non-complement-coated antigens.

A direct benefit provided by these complement-transporting IgA1 antibodies would be to target those specific antigens (not yet coated with complement) that stimulated the (original) in situ IgA1 response. Thus, biologically active complement fragments C3b, iC3b and C3d are brought via these IgA1 antibodies to those antigens, obviating the need for the initiation of complement activation at the succeeding targeted sites. The formation of immune complexes between complement-coated IgA1 antibodies and specific antigens would immediately promote enhance immune adherence followed by enhanced phagocytosis via the complement receptors (i.e., CR1, CR3 and CR4) located on phagocytic cells.

Based on the experiments with the IgA1 carbohydrate-mutants, the carbohydrate on the Fc-region of IgA1 appeared to be an important deposition site for complement (C4b and C3b) allowing the cc-IgA1 to maintain the ability to bind to non-complement-coated antigens. Similarly, the arming of phagocytes or of antigen presenting cells (e.g., dendritic cells, monocytes/macrophages, B cells and other host cells expressing complement receptors) with complement-coated IgA1 antibodies would allow the Fab regions of those cell-bound cc-IgA1 antibodies to retain antigen-binding function. In periodontal disease, epithelial cells in the inflamed gingival tissues have complement receptor 1 (CR1). In addition these cells begin to express MHC II (Matsuyama et al., 2005). Thus, in areas of periodontal inflammation, such cells may utilize the IgA1-complement-mediated mechanism to attach, ingest and process specific antigens recognized by IgA1 antibodies in the area, thereby boosting the specific T helper cell response to those antigens.

The receptor for C4b and C3b (CR1) is found ubiquitously on most host cell surfaces including erythrocytes and is critical for proper antigen clearance (Walport and Lachmann, 1988). From this perspective, coating of host cells with complement-coated IgA1 antibodies may be an important host defence mechanism. Via their CR1, normal host cells may utilize complement-coated IgA1 antibodies to capture specific antigens at the surface of tissues/organs therein preventing a deep penetration of those antigens. In chronic severe periodontal disease, the destruction of complement proteins, complement regulators and IgA1 antibodies by the proteases (Frandsen et al., 1995; Takahashi et al., 1997) released from periodontal pathogens or by host neutrophils, would disrupt CCAT and proper antigen clearance mechanisms. Interference with the proper clearance of microbial products with mitogenic properties may partially account for the peculiar specificity of certain salivary antibody responses (Suber et al., 1984).

The second newly discovered biological function for IgA1 antibodies was termed preservation of IgG antibody deployment. It would be advantageous to prolong the antigen binding function of those IgG1 antibodies directed to antigens that induced the original in situ antibody response in the infected (mucosal) tissues. For example, the prolonged presence of IgG1 antibodies on antigens would promote Fcγ-receptor mediated biological reactions that together with complement deposition on the antigen would greatly enhance immune adherence and phagocytosis as well as facilitate ADCC and NK activities. In this manner, PGD (working with CCAT) and may account for the positive roles of specific serum IgA antibodies in cancer cell elimination (van Egmond et al., 2001)

and in protecting against HIV-1 infection (Mazzoli et al., 1999).

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