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License to Clump: Secretory IgA Structure–Function Relationships Across Scales

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Abstract

Secretory antibodies are the only component of our adaptive immune system capable of attacking mucosal pathogens topologically outside of our bodies. All secretory antibody classes are (*a*) relatively resistant to harsh proteolytic environments and (*b*) polymeric. Recent elucidation of the structure of secretory IgA (SIgA) has begun to shed light on SIgA functions at the nanoscale. We can now begin to unravel the structure–function relationships of these molecules, for example, by understanding how the bent conformation of SIgA enables robust cross-linking between adjacent growing bacteria. Many mysteries remain, such as the structural basis of protease resistance and the role of noncanonical bacteria–IgA interactions. In this review, we explore the structure–function relationships of IgA from the nano- to the metascale, with a strong focus on how the seemingly banal "license to clump" can have potent effects on bacterial physiology and colonization.

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INTRODUCTION

Antibodies form a key part of defense against infection in jawed vertebrates. Their functions stem from the combinatorial linking of highly variable complementarity-determining regions (CDRs) on antigen-binding fragments (Fabs) with functional antibody constant regions (Fcs). The exons encoding for CDRs are somatically generated through recombination of V, D, and J gene segments during B cell development, and they can generate more than 10^{11} different CDR sequences (73). In contrast, Fcs are inherited and confer functions such as complement fixation, Fc receptor (FcR) binding, and multimerization (73) (**Figure 1**). However, different isotype heavy chains vary in their efficiencies and affinities for different effectors and, correspondingly, in their biological functions. As a result, this molecular Lego generates molecules with a huge range of binding specificities linked to tunable functions.

Of the different antibody constant regions, those encoding for secretory antibody isotypes are particularly intriguing. In mammals, this function is attributable mainly to immunoglobulin A (IgA), but the number of IgA variants is highly variable between species. Humans have IgA1 and IgA2, while mice have only one IgA isotype; rabbits, in contrast, have 15 different IgA isotypes, of largely unknown function (84). Teleost fish also produce secretory antibodies, but the main secretory isotype, referred to as IgT, seems to have evolved independently of IgA, indicating that secretory antibodies have evolved at least twice during the history of vertebrates (8, 108, 110).

A common feature of IgA isotypes is their ability to be assembled into polymeric IgA and to bind to the polymeric immunoglobulin receptor (pIgR) (68) (**Figure 1**). pIgR is a transmembrane receptor found on the basolateral surface of epithelial cells lining our mucus membranes, which transcytoses polymeric IgA across the cell to the apical surface. Proteases then cleave the receptor complex proximal to the membrane, leaving the extracellular domain of pIgR [referred to as secretory component (SC)] covalently bound, and releasing so-called secretory IgA (SIgA) (68). In

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CDR: complementarity-determining region

Antigen-binding fragment (Fab): one arm of an antibody, including the variable regions of both the heavy and light immunoglobulin chains

FcR: Fc receptor (generic term)

IgA: immunoglobulin A

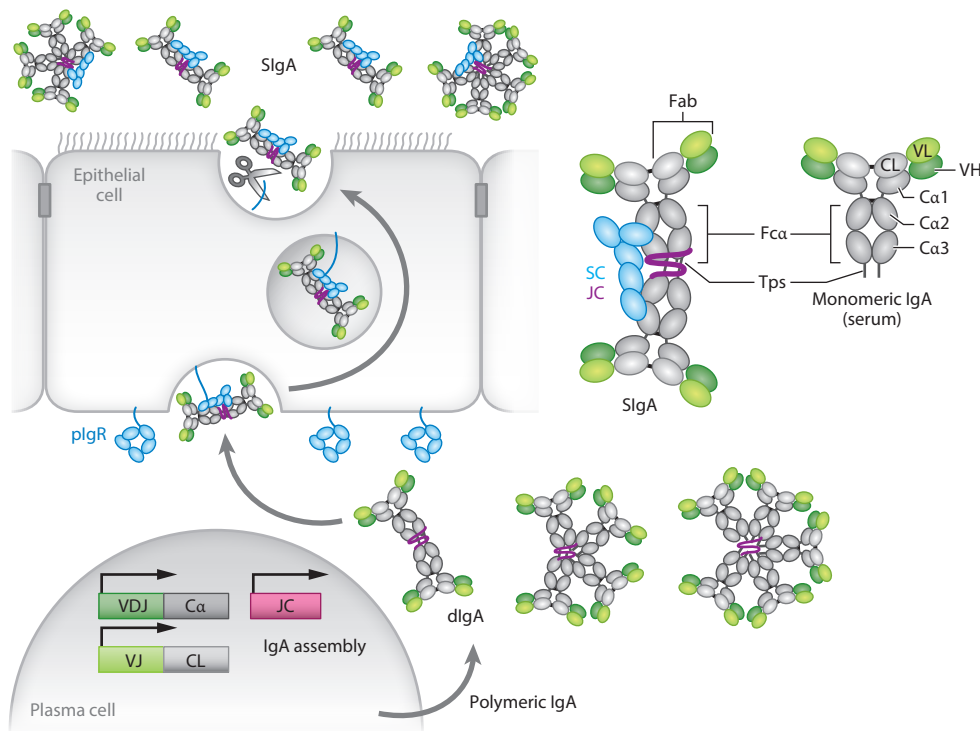**Secretory component (SC):** the cleaved extracellular domain of pIgR

Figure 1

The assembly, transcytosis, and function of IgA. This schematic summary shows secretory IgA (SIgA) and monomeric IgA domain organization, as well as dimeric, tetrameric, and pentameric IgA assembly in plasma cells and transcytosis to the mucosa. Plasma cells combine variable regions formed from V, D, and J segments with the IgA heavy-chain constant region (Cα) or the light-chain constant region (CL) and subsequently assemble polymeric IgAs using a joining chain (JC)-dependent process. Following assembly, polymeric IgAs are bound by the polymeric IgA receptor (pIgR), transcytosed through the epithelial barrier, and released into mucosal secretions as SIgA. Abbreviations: dIgA, dimeric IgA; SC, secretory component; Tps, tailpieces; VH, heavy-chain variable region; VL, light-chain variable region. Figure adapted from Reference 7 (CC BY 4.0).

humans, polymeric immunoglobulin M (IgM) can also be delivered to the mucosa by pIgR in an analogous manner (58). Therefore, the distribution of pIgR largely determines the distribution of secretory antibodies in the body. The receptor is strongly expressed on the absorptive epithelial cells of the gut, as well as in the bile ducts; nasal, oral, and respiratory epithelia; and urogenital epithelium (9, 10, 80). pIgR can also be dynamically regulated. For example, it is upregulated on the mammary epithelium during lactation, facilitating delivery of maternal SIgA to the neonatal gut (9, 99). There is also evidence that the presence of both inflammatory signals and dimeric IgA (dIgA) can upregulate both expression and transcytosis of pIgR (60, 62, 75, 79, 90).

As a result, SIgA reaches the external mucosal environments (e.g., the gut lumen) either by transcytosis or by passive immunization via colostrum and breast milk. The next questions are what these antibodies achieve in this environment and how IgA is specialized to carry out these tasks. Like other Ig isotypes, IgA can interact with host factors, including IgA-specific FcRs expressed mainly on myeloid cells (40, 77), and in some instances can apparently fix complement—a cascade of serum proteases resulting in proinflammatory mediator production and bacterial lysis (20). However, these functions are most closely associated with serum forms of IgA (20). In contrast, polymeric IgA is exported mainly via pIgR and released as SIgA at the mucosal surfaces.

pIgR: polymeric immunoglobulin receptor

SIgA: secretory IgA

IgM: immunoglobulin M

dIgA: dimeric IgA

In some compartments, especially the healthy gut lumen, both FcR-expressing immune cells and complement proteins are limited, implying that a major fraction of intestinal SIgA function may be independent of FcR binding and that this function may be linked to location. IgA can bind to bacteria either canonically (i.e., in a manner dependent on the sequence of the antibodies' CDRs) or noncanonically [e.g., via FcRs or via O- and N-glycans on the IgA hinge regions, joining chain (JC), and SC]. Recently elucidated structures of SIgA have shed light on the constraints of receptor and antigen binding, suggesting unexplored complexity in IgA-mediated protection (57, 58, 68, 95).

JC: joining chain

Body surfaces are the most common site of entry for pathogenic bacteria and viruses. Secretory antibodies are among the very few immune mechanisms that can act topologically outside the body to eliminate pathogens before they make contact with our cells. This ability has some advantages. For example, viruses, as well as some bacterial pathogens, cannot turn on active immunosuppressive programs without at least one round of productive replication (63). Secretory antibodies can also target pathogens at the point of colonization rather than invasion, thereby removing or controlling infectious reservoirs. Passive immunization of neonates via maternal SIgA in breast milk provides powerful protection from mucosal pathogens during this vulnerable stage of life, before the neonates' own antibody production matures (15).

Recent progress in understanding how IgA is specialized to maximize its effectiveness is key to applying the functions of SIgA to disease prophylaxis worldwide. In this review, we attempt to bridge the gap between structure and function across scales to highlight what we already understand and where we need to be heading in IgA research.

ANGSTROM-SCALE STRUCTURE–FUNCTION INTERACTIONS: THE SHAPE OF THE TOOL DICTATES ITS FUNCTIONS

SIgA has the remarkable ability to bridge host interactions with a diverse array of molecules, including antigens and other microbial and host proteins and carbohydrates. These nanoscale molecular interactions are supported by intricate protein structures that make SIgA an ideal molecular multitool for complex mucosal environments. As noted above, mammalian IgA and IgM heavy chains (and orthologous proteins found in other vertebrates) can be assembled into polymeric immunoglobulins, which typically contain between two and five immunoglobulin monomers and one JC (**Figure 1**). However, the number of antibodies in each polymeric immunoglobulin and their potential to assemble with the JC are variable, and some species, including teleost fish, have lost the JC from their genomes (21, 32, 33, 53). In mammals, IgA destined for mucosal secretions is typically assembled in tissue-resident plasma cells that combine two IgA monomers with one JC to release dIgA. Trimeric, tetrameric, and pentameric forms of IgA can also be produced, albeit in far lower abundance (21). All of these forms are bound and transcytosed by pIgR and released as SIgA.

High-resolution molecular structures of polymeric IgAs and SIgAs remained unknown until 2020, when a handful of structures determined independently by three groups revealed many long-hidden secrets and opened the door to new questions (7, 57, 102). The molecular details and assembly of these structures (and related secretory IgM structures) have been reviewed elsewhere (68). Here, we review basic elements of these structures and correlate them with SIgA's biological functions.

The cryo-electron microscopy (cryo-EM) structure of dIgA, the predominant SIgA precursor, was recently reported (7). The dIgA structure revealed two IgA monomers bent and tilted relative to each other, creating distinct convex and concave sides (**Figure 2a,b**). This apparently rigid geometry is stabilized by the JC, which is located near the center of the complex. The JC has
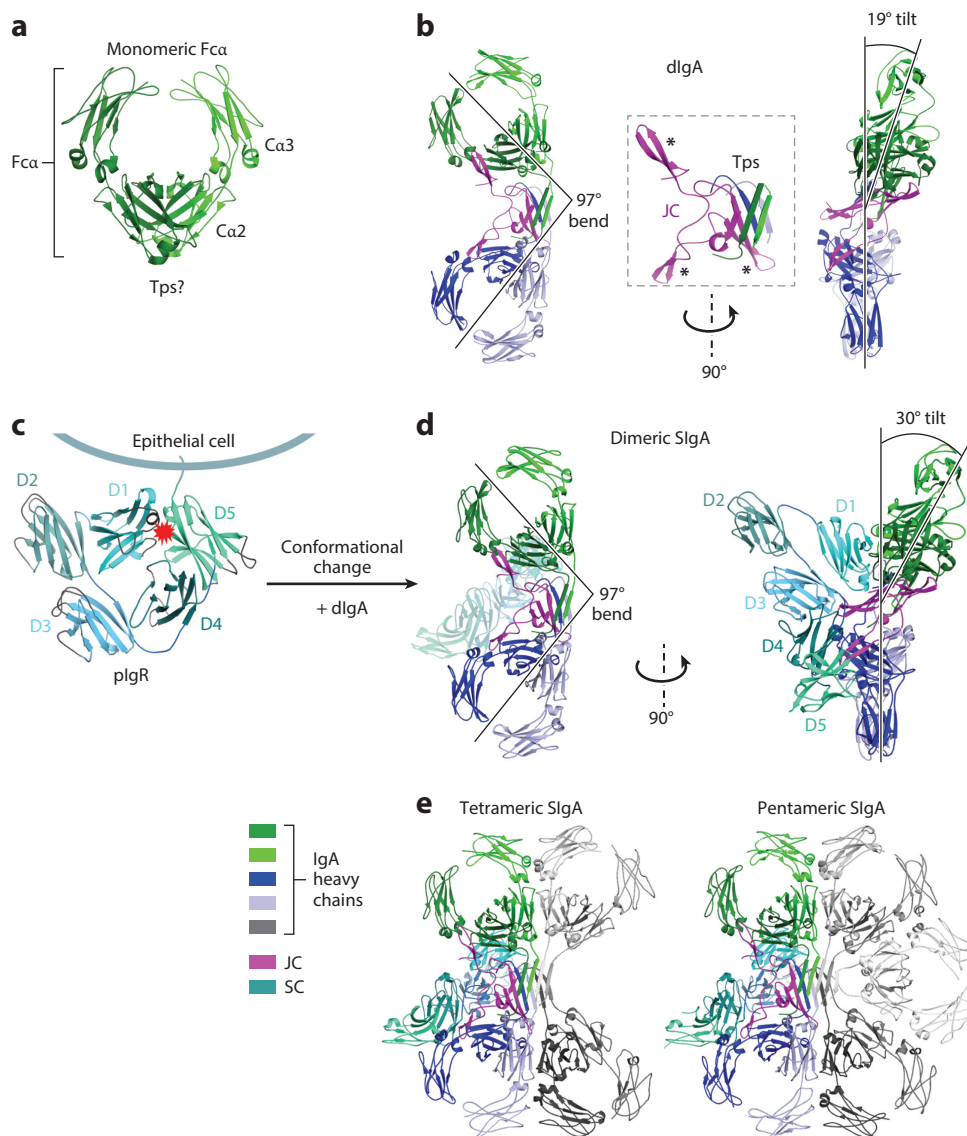
Figure 2

Structures representing different forms of IgA and the polymeric immunoglobulin receptor (pIgR).
(*a*) Crystal structure of monomeric Fcα (PDB code 1OW0). Two of the four Cα2 and Cα3 domains are
labeled; the structure(s) of the unliganded tailpieces (Tps) is unknown. (*b*) Cryo-electron microscopy
(cryo-EM) structure of mouse dimeric IgA (dIgA) (PDB code 7JG1). Fabs are disordered. The dIgA is shown
in two orientations, with the angles of bend and tilt between the two Fcαs indicated. (*Inset*) Enlarged image
of the joining chain (JC) and Tps. Asterisks indicate the β-hairpins, or wings. (*c*) Crystal structure of
unliganded secretory component (SC) domains (D1–D5). The red star denotes the D1–D4–D5 interface,
which breaks upon dIgA binding. (*d*) Cryo-EM structure of mouse secretory IgA (SIgA) (PDB code 7JG2),
shown in two orientations. Fabs are disordered. (*e*) Cryo-EM structures of tetrameric and pentameric human
SIgA lacking Fabs (PDB codes 6UE8 and 6UEA). Components shared with dimeric SIgA are colored as in
the other panels, and additional Fcαs and Tps are shaded in gray. The legend at bottom left applies to all
panels. Portions of figure adapted from Reference 7 (CC BY 4.0).

long been shrouded in mystery, with its evolutionary origin and functions uncertain and, until the publication of recently solved structures, its fold unpredicted (14). The dIgA (and SIgA) structures solved part of the mystery, revealing that the JC folds together with four IgA C-terminal extensions called tailpieces (Tps), one from each IgA heavy chain. The Tps and JC adopt β-strands that fold into a β-sandwich-like domain, which glues the two IgAs together. Additionally, the JC has several appendages [termed β-hairpins (57) or wings (7)] that extend away from the center of the antibody and contact both Fcs. These appendages effectively act like hands, grasping each antibody and likely holding it in the observed bent conformation and conferring elements of asymmetry to the complex—elements that, as discussed below, are likely to affect effector functions (7) (**Figure 2b**).

The dIgA structure is presumed to represent dIgA secreted by plasma cells, which is subsequently bound by pIgR on the basolateral surface of the epithelium. Structural studies revealed that the unliganded pIgR ectodomain, or SC, adopts a compact conformation in which five immunoglobulin-like domains (D1–D5) are arranged in a closed conformation that opens upon dIgA binding (**Figure 2c**). The SIgA structures revealed that the pIgR ectodomain is asymmetrically bound to the front of the dIgA complex, where D1–D5 protrude from the center of the molecule. Despite its polarized location, SC contacts all four heavy chains and the JC (**Figure 2d**). Studies have implicated both the JC and the SC D1 as necessary for SIgA formation, and SIgA structures reveal extensive interactions between D1, the third IgA constant domain (Cα3), multiple Tps, and the JC. D1 binding to these elements is predicted to trigger conformational change among D1–D5 (37, 95). SIgA structures demonstrate that this conformational change results in a new interface between D1 and D3 that bridges those domains with D4 and D5, which contact the constant domain in the second IgA monomer (68). Overall, SIgA maintains the same angle of bend between the two IgA monomers observed in dIgA; the overall angle of tilt changes by ∼10° but is not associated with prominent structural changes in the JC or heavy chains (7). Furthermore, published structures of dimeric forms of human and mouse SIgA are superimposable (68).

Although most SIgA in humans, and likely in other mammals, is dimeric, higher-order polymers exist and have been visualized by cryo-EM. Structures representing tetrameric and pentameric SIgA (but lacking Fabs) are analogous to dimeric forms of SIgA but with two or three additional monomers stacked on the convex side. This arrangement is mediated by both contacts between adjacent Fcα chains (Fcαs) and additional Tp interactions, which essentially add β-strands to the central β-sandwich. The tetrameric SIgA is characterized by a gap between the third and fourth Fcαs, whereas a fifth Fcα fills this gap in the pentamer (57). Together, SIgA structures have implications for canonical and noncanonical interactions between IgA and antigens, and between host and microbial proteins, including FcRs.

CANONICAL MOLECULAR INTERACTIONS INVOLVING IgA

Although Fabs were either disordered or not included in SIgA structures visualized by cryo-EM, computational modeling suggests that the bent and tilted relationship between the two IgA monomers in dimeric forms of SIgA will influence the possible positions that Fabs can occupy (**Figure 3a**). This means that the orientation of one Fab relative to other Fabs (or relative to other parts of the structure, such as FcR binding sites) is distinct from that of other classes of antibodies and is likely to influence antigen binding and, thus, the antibody's functional outcomes.

Modeling suggests that Fabs are most likely to occupy positions closest to the concave side of SIgA, away from FcR binding sites and SC (**Figure 3a**). Exactly how this geometry might influence antigen engagement remains experimentally unexplored. However, it is tempting to speculate that this arrangement might provide some advantage for binding to common mucosal antigens, for example, those spaced optimally to bind to both sets of Fabs or perhaps to flexible
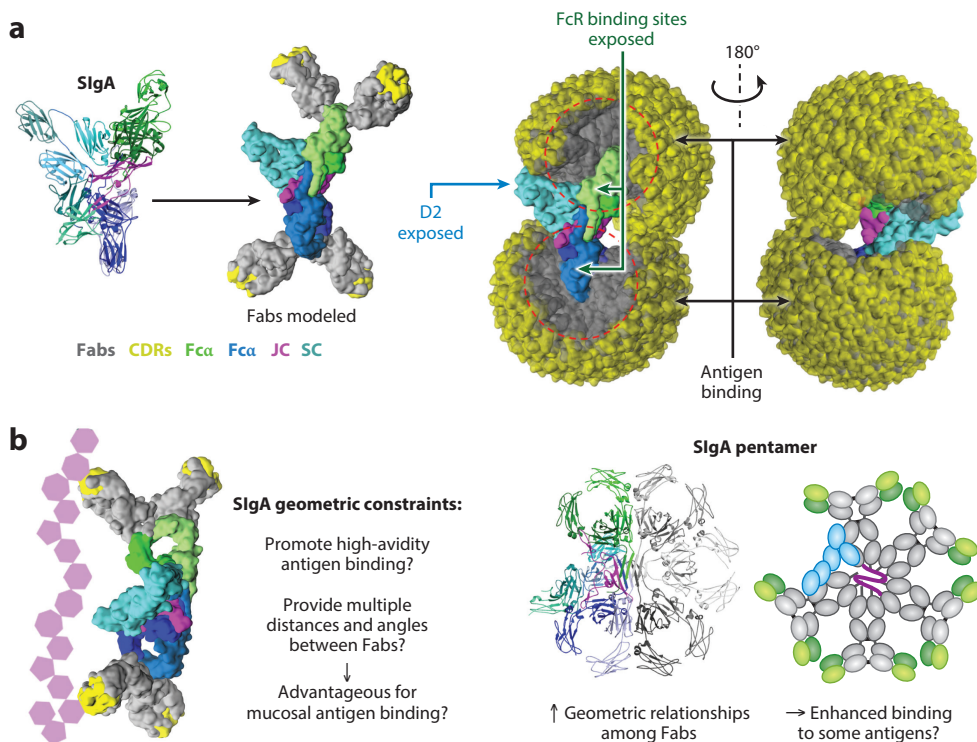
Figure 3

Structural implications for canonical secretory IgA (SIgA) interactions with antigens. (*a*) SIgA structure and associated computational modeling of Fabs and complementarity-determining region (CDR) positions (PDB code 7GJ2). Modeling (7) allowed each Fab to adopt ~8,000 positions and eliminated steric clashes, thereby providing an envelope of potential space in which CDRs (*yellow*) might bind antigen. However, in vivo, other factors such as hinge length and flexibility are expected to provide additional constraints. In addition to showing possible positions of Fabs and associated antigen binding, modeling has revealed the accessibility of secretory component (SC) D2 (*blue arrow*) and the convex side of the two Fcαs (*dashed red circles*), which contain two Fc receptor (FcR) binding sites (*dark green arrows*). (*b*) SIgA structure with modeled Fabs engaging a schematic antigen and the structure and schematic representation of pentameric SIgA, which collectively depict possible ways that the structures of SIgA may provide advantages for mucosal antigen binding. Abbreviation: JC, joining chain. Portions of figure adapted from Reference 7 (CC BY 4.0).

antigens such as carbohydrates; it might also influence the strength of antigen cross-linking (**Figure 3b**). In the context of mammals, the existence of multiple IgA isotypes in some species is likely to add further complexity to how this geometry engages antigen. For example, human IgA1 has extended linkers and O-linked glycosylation bridging the Fc and the Fab compared with human IgA2, which has shorter linkers that are likely to leave the Fab and Fc in contact and limit the total volume of space sampled.

Polymeric state is also likely to play a role in how SIgA engages antigen. Although most SIgA in humans is dimeric, as noted above, higher-order SIgA exists. While modeling of Fab positions has not been reported for tetrameric and pentameric SIgA structures, it is clear that higher-order SIgA has the potential to permit a greater number of interactions with antigen. The results of this greater potential for increased Fab–antigen interactions may be diverse. For example, when eight or ten Fabs from one SIgA bind antigen, the higher-order polymer may provide enhanced

binding avidity compared with its dimeric counterpart. When only four Fabs bind antigen, the higher-order SIgA may provide enhanced geometry (e.g., different angles between sets of Fabs) compared with its dimeric counterpart. Future studies will be needed to address these unknowns (**Figure 3***b*).

NONCANONICAL MOLECULAR INTERACTIONS INVOLVING IgA

Secretory Component

While discussions of SIgA often focus on canonical interactions with antigen, the antibody supports numerous noncanonical interactions that are also critical for its function. Perhaps the most recognized are IgA interactions with pIgR. pIgR is critical for delivering SIgA to mucosal secretions; yet, functionally, why its ectodomain (SC) remains attached to SIgA is less clear, especially in light of new structural data indicating that it does not markedly alter the shape of dIgA or the potential positions that Fabs might adopt (7). SC has been reported to protect SIgA from degradation and to play a role in binding to host and bacterial factors (51).

Indeed, recent SIgA structures reveal that SC forms extensive interfaces with the IgA heavy chains and the JC, where it may protect areas that are especially vulnerable to proteolysis (7, 57). However, only ~16% of the SIgA core (not including Fabs) is reportedly covered by SC, leaving most of the SIgA surface exposed and potentially susceptible to proteases (7) (**Figure 4***a*). SC might provide indirect protection from degradation by influencing SIgA location, an outcome that might also contribute to other functions. Indeed, SC is located on one face of SIgA and exhibits significant accessible surface area, which may position it to interact with host or microbial factors. The second domain of SC, D2, is particularly accessible because it is located distally from SIgA's center, where it contacts only other SC domains and is decorated in carbohydrates that, along with other accessible carbohydrates, could mediate SIgA interactions with host and microbial factors including mucus, IL-8, *Escherichia coli* fimbrial lectins, and even microbial toxins (7, 51, 76) (**Figure 4***a*). Furthermore, biochemical experiments have demonstrated that removal of human SC D2 does not prevent binding to dIgA; additionally, D2 is absent from SC in birds, reptiles, and amphibians, suggesting that its functions extend beyond a structural role and are likely to differ among vertebrate species (96).

Human SC is known to mediate interactions with *Streptococcus pneumoniae* SIgA–binding protein (SpsA; also known as CbpA or PspC), a pneumococcal adhesin (38). Recent cryo-EM structures of SIgA bound to a SpsA domain revealed binding to SC D3 and D4 via exposed residues that are not conserved in other mammalian SC sequences (102, 105, 109) (**Figure 4***b*). The specific outcomes of this interaction remain under investigation; however, SpsA binding to human pIgR prior to SIgA release from the epithelial cell membrane may promote *S. pneumoniae* invasion and systemic infection via a pIgR retrograde transcytosis pathway. Furthermore, *S. pneumoniae* binding to SIgA released into mucosal secretions may also function to sequester SIgA functions and/or facilitate degradation by *S. pneumoniae* IgA proteases (13, 29, 38). On the other hand, *S. pneumoniae* binding to free SC or SIgA has the potential to exclude bacteria and thereby protect the integrity of the host epithelial cell barrier (50). Taken together, pIgR and SC can be considered critical not only for SIgA transport to the mucosa but also as a shield and platform to mediate interactions with known, and perhaps unknown, binding partners—some of which may be beneficial and some detrimental to the host.

Other Fc Receptors

In addition to pIgR, SIgA is subject to interactions with several host and microbial FcRs, including the human IgA Fcα receptor 1 (FcαRI), also known as CD89 (20). FcαRI is expressed on
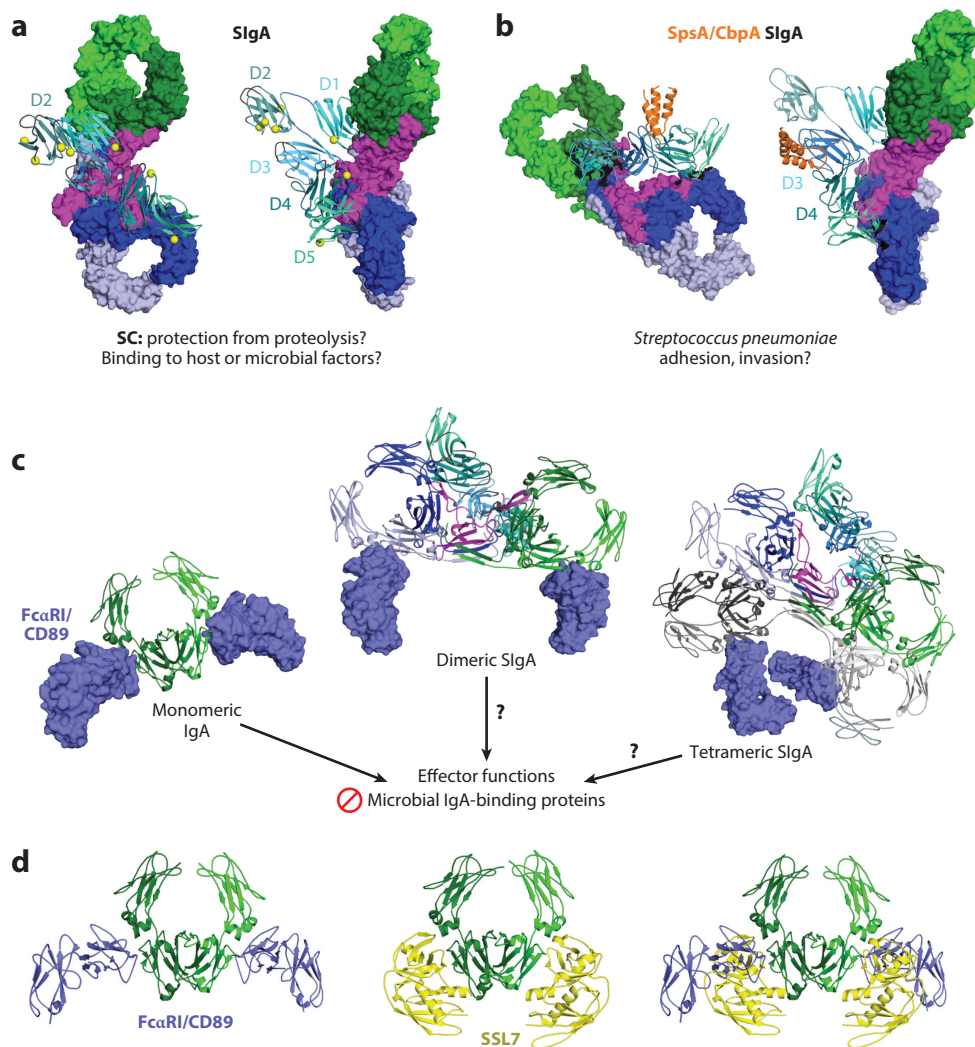
Figure 4

Structural implications for noncanonical secretory IgA (SIgA) interactions. (*a*) Potential functions of
secretory component (SC). Cryo-electron microscopy (cryo-EM) structure of mouse SIgA (PDB code
7JG2). The dimeric IgA (dIgA) component is rendered as a molecular surface and SC as a cartoon, with
potential N-linked glycosylation sites shown as yellow spheres to illustrate how SC may protect IgA and/or
promote interactions with host and microbial factors. (*b*) Microbial interactions with SC. Cryo-EM structure
of human SIgA in complex with the N-terminal domain of *Streptococcus pneumoniae* surface protein
SpsA/CbpA (*orange*), which binds SC D3 and D4 (PDB code 6LXW). (*c*) Fc receptor (FcR) interactions with
IgA. The crystal structure of FcαRI–Fcα (PDB code 1OW0) is depicted along with models (not determined
structures) for dimeric and tetrameric SIgA binding to FcαRI, to illustrate how the relative orientations of
FcR binding sites differ among IgAs. In pentameric SIgA (not shown), none of the FcR binding sites are
accessible. In the context of SIgA-coated antigens (not shown), SIgA–FcαRI interactions may promote
downstream effector functions, which, in turn, may be inhibited through interactions with microbial proteins
that bind similar sites. (*d*) Structures of the FcαRI–Fcα complex and the *Staphylococcus aureus* SSL7–Fcα
complex (PDB code 2QEJ) are shown as cartoon representations, along with a structural alignment showing
FcαRI and SSL7's overlapping binding sites. Portions of figure adapted from Reference 7 (CC BY 4.0).

the surface of many myeloid lineage cells, where it binds IgA through a site located at the IgA C_H2–C_H3 interface. This site overlaps with the pIgR binding site and was visualized in an Fcα–FcαRI cocrystal structure more than 20 years ago (21, 40) (**Figure 4c**). FcαRI–IgA–antigen engagement can promote receptor clustering and associated signaling through the FcR γ chain, which can result in a diverse array of outcomes, ranging from phagocytosis to cytokine release, depending on the cell type expressing FcαRI and the polymeric state of the IgA (21). While FcαRI can bind monomeric, polymeric, and secretory forms of IgA, it reportedly binds SIgA with moderately lower affinity in vitro, and on cells, its binding to SIgA reportedly requires lectin Mac-1 (CD11b/CD18) (77, 101).

Structural studies have provided clues as to how FcαRI function may be tailored to the type of IgA it engages. The crystal structure of soluble FcαRI bound to monomeric Fcα revealed two FcαRI binding sites, one on each heavy chain (40), resulting in a symmetrical complex (**Figure 4c**). In both dIgA and dimeric forms of SIgA, two of the four FcαRI accessible sites are occluded by the JC (and SC), leaving two FcR binding sites (one on each of the two Fcs) sterically accessible, a finding recently confirmed by a cryo-EM structure of human SIgA in complex with FcαRI (61). These sites are located on the convex edge of dimeric SIgA, away from regions predicted to be occupied by Fabs, and appear likely to provide an unobstructed approach to FcRs (7) (**Figure 3**). Notably, despite FcαRI activity being associated largely with monomeric IgA, dimeric SIgA structures reveal accessible FcαRI sites, suggesting that SIgA interactions with FcRs have proved functionally advantageous throughout evolution. Two FcαRI binding sites are also accessible on tetrameric forms of SIgA, although their locations relative to each other are different from those of dimeric SIgA (**Figure 4c**). Notably, in pentameric SIgA, all sites are blocked either by the JC or by contacts between adjacent Fcs (57).

Taken together, these observations indicate that all forms of IgA (e.g., monomeric, dimeric, tetrameric, and their secretory forms), except pentamers, contain two accessible FcαRI binding sites; in monomeric IgA, the two sites are located on one Fc and separated by ∼39 Å, whereas in the dimeric and tetrameric forms, the sites are located on two different Fcs and separated by ∼95 Å and 58 Å, respectively (**Figure 4c**). Thus, each monomeric/polymeric form of IgA may provide a unique set of FcαRI binding sites that are oriented differently relative to bound antigen and relative to copies of FcαRI on the cell membrane. These differences may affect the clustering of multiple FcαRI molecules on a cell surface, the distances between their signaling domains, and, thus, signaling and effector functions.

Rarely have published experiments involving FcαRI distinguished between different polymeric forms of IgA, yet mixtures containing monomeric, dimeric, tetrameric, and pentameric forms represent three possible orientations of two sites as well as a nonbinder. More broadly, these observations suggest a mechanism for how different polymeric states of SIgA might promote different outcomes through FcαRI engagement. The location of the FcαRI-expressing cells is also an important factor. For example, SIgA and its antigen complex might encounter FcαRI via a myeloid lineage cell (e.g., dendritic cell) sampling mucosal antigens or following epithelial uptake by a microfold cell or another specialized epithelial cell by receptors such as Dectin-1 and the transferrin receptor in the gut (21). In contrast, in lung, phagocyte populations patrolling the mucosal surface can directly interact with SIgA (41, 92).

The significance of host FcR binding to IgA is illustrated by the fact that multiple bacterial pathogens known to colonize the nasopharynx and respiratory tract, including group A and B streptococci and *Staphylococcus aureus*, have evolved structurally diverse proteins, sometimes called decoy receptors, that bind sites overlapping with the FcαRI binding site. Among these proteins are *S. aureus* SSL7 and *Streptococcus pyogenes* M4 (also known as Arp4) and M22 (also known as

Sir22) (52, 107). The structure of SSL7 in complex with monomeric Fcα reveals two copies of SSL7 binding in a similar orientation to FcαRI (85) (**Figure 4d**). Bacterial IgA-binding proteins are thought to enhance virulence by blocking downstream effector functions such as phagocyte-mediated killing (52). As noted above for FcαRI, localization will influence the outcomes, yet due to a lack of FcαRI in mouse models, it remains challenging to dissect the role of these proteins in mucosal colonization versus invasive disease. *S. pyogenes* and *S. aureus* expressing these receptors are likely to become coated in noncanonically bound SIgAs. Bound copies of SIgA may protect bacteria on mucosal surfaces from phagocytosis and killing by phagocytes, or they may inhibit the production of effective inflammatory response after tissue/blood invasion (52, 82, 107). Notably, a recently reported cryo-EM structure of human SIgA bound to *S. pyogenes* M4 indicates that the stoichiometry of binding and the potential orientation of bound SIgA on a bacterium surface are likely to differ among bacterial IgA-binding proteins and host FcRs (61). The functional implications of these differences remain unexplored, but they are likely important for understanding how SIgA bridges the host–microbe interface.

Despite gaps in our understanding of the function of host cell FcRs, the coevolution of bacterial proteins that block these sites and the preservation of two sites on SIgA suggest that IgA–FcR interactions are an important feature of SIgA responses. Immunoglobulin G (IgG)–FcR interactions have clear functional roles in protective systemic immunity, and an Occam's razor approach suggests that IgA–FcR interactions perform similar roles in mucosal tissues. However, confusing aspects remain. Except for pIgR, known or probable IgA FcRs [including Fcα/mR and several members of the FcR-like family (21)] are expressed mainly by tissue-resident cells or in the lung, such that they operate in an environment where there is also abundant IgG. An important aspect of future research will be to understand how IgG and IgA functions interact in these environments. Moreover, it is curious that CD89 is not functional in mice, and it remains unclear whether this function has been taken over by another, as-yet-unidentified receptor or whether IgA–FcR interactions became unfavorable during a phase of mouse evolution.

Microbial Protease Susceptibility

The association with SC protects SIgA from mammalian digestive proteases, via mechanisms that remain to be completely elucidated (7, 95). However, SIgA is susceptible to attack by microbial proteases, including well-studied proteases that target human IgA1 and are expressed by *S. pneumoniae* and *Haemophilus influenzae* (54). IgA1 proteases are structurally diverse but culminate in the release of Fab fragments from the Fc. This process decouples antigen binding and Fc-based effector mechanisms, thereby limiting phagocytosis or agglutination via masking of pathogen surface antigens with Fabs (48, 87, 91). In contrast to the bacterial proteins described above, most IgA1 proteases bind the IgA Cα3 domain near the Fabs, allowing cleavage of the extended IgA1 linker (**Figure 5a,b**). The *S. pneumoniae* IgA1 protease is a giant metalloproteinase that binds both copies of IgA C_H3 simultaneously, enforcing a one-to-one stoichiometry. Protease binding mediates an active-site conformational change that facilitates IgA cleavage and can be blocked by monoclonal antibodies (87). A structural analysis demonstrated that the IgA1 protease binding site on SIgA1 is accessible, supporting the function of these proteases in the mucosa and their activity as a critical part of host–microbe interaction (103).

Linker length has long been recognized as a factor influencing IgA function and susceptibility to proteases, and IgA1 proteases are not active against IgA2 because of its compact hinge region (91). Interestingly, O-glycans associated with the hIgA1 hinge can lose galactosylation, generating epitopes for IgG-mediated recognition. These IgG–IgA1 complexes are deposited in the kidney, causing glomerulonephritis and kidney failure (IgA nephropathy) (18, 97). Speculatively, it appears
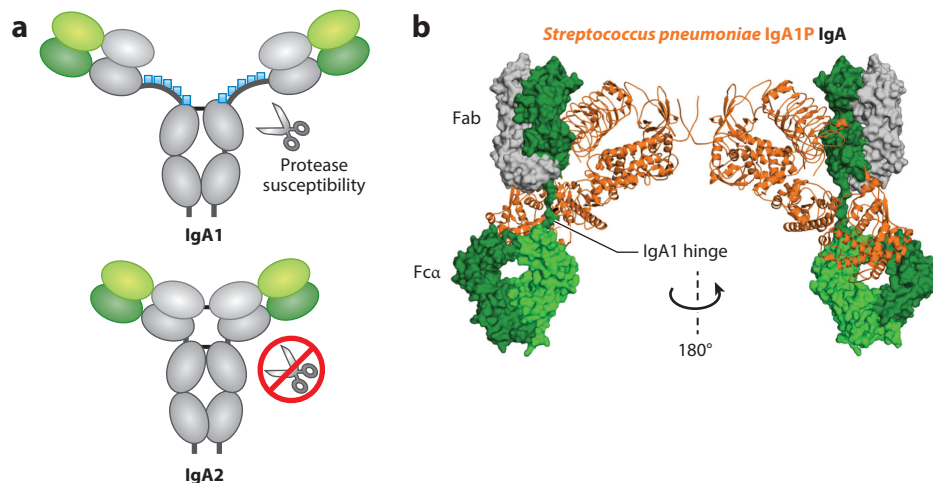
IgG:
immunoglobulin G

Figure 5

Bacterial protease interactions with IgA. (*a*) Schematic showing differences between human IgA1 and IgA2. The human IgA hinge is elongated and decorated with O-linked glycans (*blue squares*), whereas the IgA2 hinge is shorter, allowing for more contacts between domains. (*b*) The structure of the *Streptococcus pneumoniae* IgA1 protease (IgA1P; *orange*) bound to Fcα and one Fab (*green*), shown in two orientations (PDB code 6XJA). The IgA1 hinge is bound in the IgA1P active site.

that acquisition of the IgA1 isotype in humans is a trade-off between better mucosal protection on one side and risk of autoantigen generation and microbial protease susceptibility on the other.

HOW DO IgA STRUCTURE AND CANONICAL BINDING TRANSLATE INTO EFFECTS ON BACTERIAL POPULATIONS?

Nanoscale Molecular Interactions Translate into Mesoscale Consequences

The polymeric nature of SIgA lends itself to clumping targets. Each SIgA binds to multiple antigens, tethering them together in a manner that is necessarily constrained by SIgA structure. In canonical antigen binding via Fabs, each dimeric form of SIgA can bind four antigens. Assuming sufficiently high affinity, the binding of four antigens by a single SIgA is still possible only if the available antigens are correctly spaced to match the possible locations of the Fabs (7). If this is the case, for example, in abundant repetitive bacterial surface glycans, then SIgA binding is expected to tether the four antigens close together over long timescales (43). The consequences of keeping four identical antigens in close proximity depend on the nature of the antigens themselves.

IgA can also aggregate at the cellular scale. In this case, at least one Fab of the complex needs to be bound to each of two different cells. Multiple cross-links can drive cross-linking into larger clumps. The ability of IgA to clump at multiple scales dictates its range of functions extending beyond, and likely independent of, Fc-mediated cellular interactions.

Canonical Interactions of Secretory IgA with Bacterial Surface Antigens: Clumping Antigens onto Individual Cells

Cell biologists have long exploited the multivalent property of antibodies to bring, and keep, antigens close together (3, 46). The fact that antibodies keep targets spatially close has helped define paradigms in eukaryotic cell trafficking and signaling. Moreover, although the relative importance

of all possible consequences of aggregating antigen on bacteria has not been fleshed out, several groups have mapped IgA binding and apparently detect alterations in bacterial functions or gene expression (17, 34, 59, 89).

Some of the first observed impacts of IgA on bacterial behaviors were on flagellar-based swimming (17, 34, 59). These early studies suggest that physical mechanisms limit swimming: Even when IgA binds surface glycolipids rather than flagella, this can sterically block flagellar rotation or could lead to flagellar entanglement. More recent studies imply that IgA may affect the expression of flagellar machinery, limiting bacterial motility (89). Therefore, IgA binding to both flagellar and nonflagellar surface antigens seems to be able to influence bacterial motility and, by implication, fitness within the host.

Insight into the possible mechanisms explaining loss of motility comes from the recent observation that dIgA binding to bacterial surface glycolipids reduces bacterial outer membrane fluidity (43). In this study, a dIgA recognizing the *Salmonella* O-antigen—the surface-exposed, polymeric glycan moiety of lipopolysaccharide (LPS)—was sufficient to limit and decrease the rate of LPS diffusion in the outer membrane. The decrease in membrane fluidity required higher-order interactions, as binding by the monomeric Fab fragment did not change outer membrane fluidity (43). Therefore, dIgA reduces lateral mobility by clustering antigen on the bacterial surface. It is likely that clustering proteinaceous antigens will have a similar effect on membrane dynamics (16, 65, 86). Moreover, IgA-mediated clustering of proteins with signaling properties may alter bacterial gene expression and associated phenotypes (89).

Recent studies in which monoclonal SIgA was adoptively transferred into mice have suggested that transcriptional changes occur in response to canonical SIgA binding to *E. coli* or *Bacteroides thetaiotaomicron* (49, 82, 83, 89). Altered *B. thetaiotaomicron* gene expression was observed upon canonical SIgA binding to the glycan of lipooligosaccharide (83) or to a fructan-degrading surface enzyme (49). An IgA recognizing the *E. coli* outer membrane porin *OmpC* also led to recovery of *E. coli* with transcriptional downregulation of this porin in vivo (89). The mechanism linking canonical IgA binding to transcriptional regulation remains unclear, and future studies will need to distinguish direct transcriptional regulation from selection of transcriptional states in the gut or indirect effects such as altered inflammatory signaling in the host (**Figure 6**). Nevertheless, on the basis of these data, it is tempting to speculate that bacterial cells may be able to sense and respond to aggregation of surface antigen.

Canonical Interactions of Secretory IgA with Bacterial Surface Antigens: Clumping Cells into Aggregates

That IgA can aggregate cells has long been at the center of a function referred to as immune exclusion, which is the ability of SIgA to prevent the interaction of mucosal pathogens with host tissues. The multivalent nature of IgA allows it to cross-link antigens between two antigenically identical cells. While it is clear that antibody affinity and the accessibility of antigen on the cell surface are major determinants of this process, we often overlook the fact that antigenically identical bacteria need to come into close contact (within 30 nm of one another) for cross-linking to be physically possible (5, 71). In the large intestine, opportunistic enteropathogens typically exist in densities below 10^5 cells per gram, while the microbiota is present at densities above 10^{11} cells per gram. Therefore, a pathogen is often outnumbered a million to one by the microbiota, and the chance of two identical pathogenic bacteria colliding is very low (71). As a result, classical agglutination (i.e., the process of aggregating bacteria via random collisions of identical cells) is largely precluded during early stages of pathogen invasion. However, mucosal pathogens tend to grow and expand rapidly during early infection. At the point of septation, two antigenically identical daughter cells
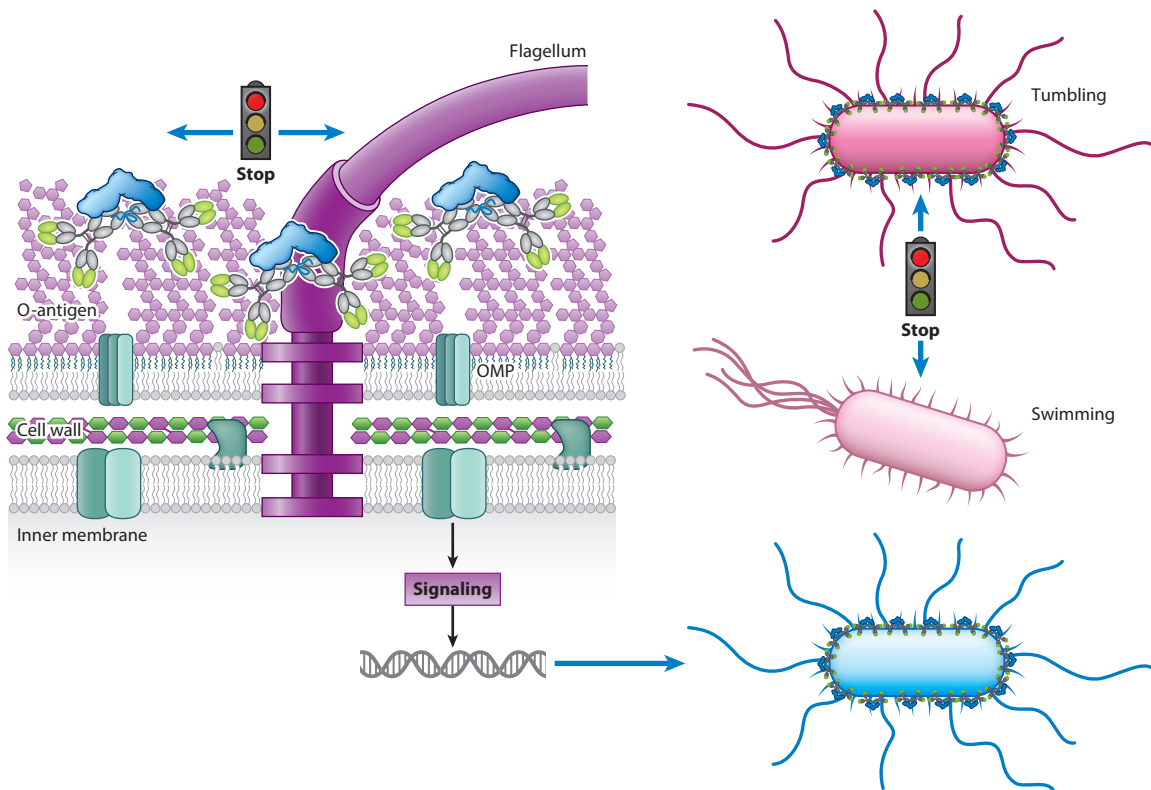
LPS:
lipopolysaccharide

Figure 6

Binding of dimeric IgA or secretory IgA to *Salmonella* lipopolysaccharide abolishes outer membrane fluidity by locking glycolipids around abundant outer membrane porins (OMPs). This process prevents lateral diffusion within the outer membrane (43), which in turn may prevent relocalization of peritrichous flagella, that is, chemotaxis-driven switching between tumbling (*dark pink*) and swimming (*light pink*). Alternatively, loss of outer membrane fluidity may induce outer membrane stress, resulting in activation of stress-responsive transcriptional programs (switching from the dark pink phenotype to the blue phenotype). Figure adapted from images generated with BioRender.com.

are necessarily in very close contact and can be cross-linked by IgA (71). This process, referred to as enchained growth, generates aggregates of bacteria with a pronounced clonal structure (i.e., enchained growth generates population structure in the gut) (5, 71). When enchained growth is the main mechanism driving SIgA-mediated aggregation, aggregate size depends on the relative growth or clearance rate of the bacterium targeted and the half-life of IgA cross-links (5, 71).

In murine nontyphoidal *Salmonella* infections (6), the main site of bacterial growth is the lumen of the terminal ileum, cecum, and ascending colon. A high-affinity anti-*Salmonella* SIgA response can be induced by oral vaccination with either live (44) or whole-cell inactivated (72) oral vaccines. *Salmonella*-binding SIgA generates aggregates in the cecum lumen that are prevented from approaching the gut wall, generating protection (71). Population structure generated by enchained growth also physically isolates clones that could otherwise exchange genetic material by contact-dependent processes, limiting the spread of plasmids among gut enteropathogens (22, 71). Additionally, the gut environment is actively flowing, and feces formation is effectively a sampling process from the relatively fluid content of the upper large intestine. When combined with flow and sampling, SIgA-enchained aggregates will tend to be removed en bloc, that is, more
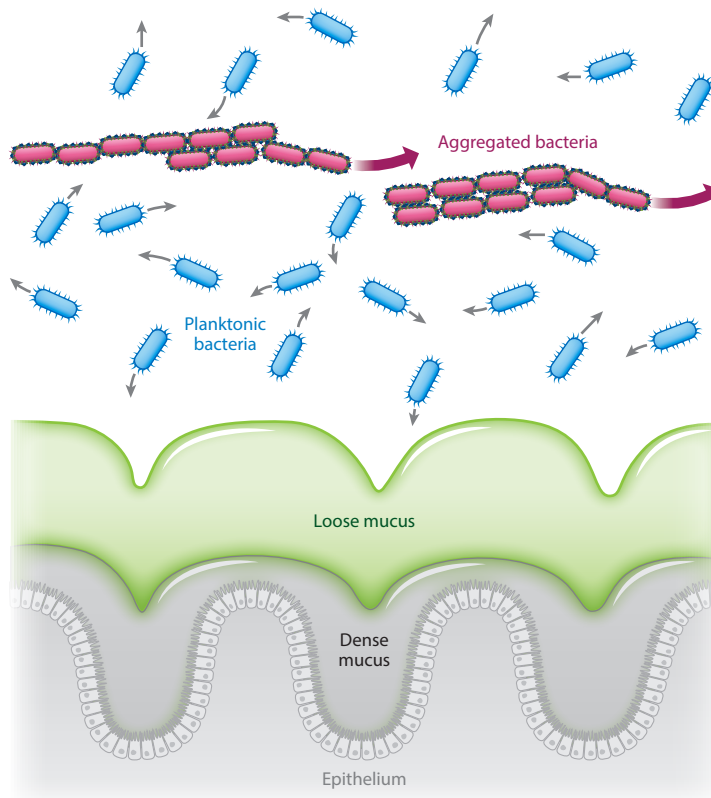
Figure 7

Secretory IgA–mediated aggregation increases efficiency of bacterial clearance. Large aggregates of *Salmonella* Typhimurium (*pink*) are excluded from the epithelium (*light gray*) and from both dense mucus (*dark gray*) and loose mucus (*green*). As aggregates can no longer swim against the flow of gut content, they are more efficiently removed by flow in comparison to planktonic bacteria (*blue*), which are free swimming, generating a relative fitness disadvantage for IgA-targeted bacteria. Figure adapted from images generated with BioRender.com.

efficiently than planktonic bacteria (71) (**Figure 7**). Enchained growth–mediated aggregation by IgA therefore produces measurably higher rates of clonal extinction for SIgA-targeted bacteria than are observed in unvaccinated mice (71).

Any process driving clonal extinction is going to be a major selective pressure. Correspondingly, we expect high-affinity canonical SIgA responses to select for immune escape (i.e., to select for variants that are poorly aggregated by SIgA in the gut) (23, 42, 71). Moreover, the specific antigens targeted by SIgA need to be exposed to the bacterial surface, and they are often the targets of phage receptors as well (23). Bacteria therefore have a double pressure to be able to rapidly vary their surface structures, and correspondingly, wherever we have looked for IgA-driven surface variation, we have typically found it (35, 36, 100). In *Salmonella*, the protective IgA response targets mainly the O-antigen of LPS (30). This polymeric glycan carpets the *Salmonella* surface, largely hiding any conserved smaller surface proteins such as outer membrane porins. Several systems exist to modify the glycan repeat units of LPS prior to polymerization, including O-acetylation, glucosylation, and methylation of the sugars (100). In *Salmonella* Typhimurium, the abequose O-acetyl transferase gene, *oafA*, contains a 7-base-pair repeat, driving microsatellite instability
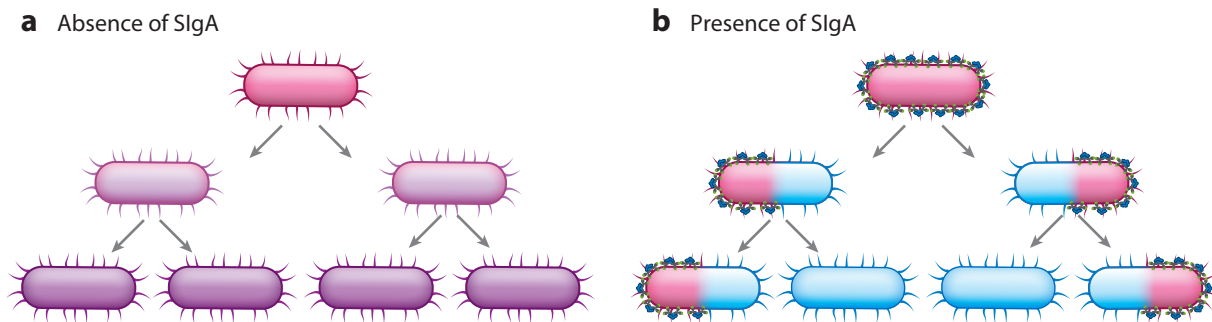
a Absence of SIgA   **b** Presence of SIgA

Figure 8

Nanoscale O-antigen clustering increases the speed of secretory IgA (SIgA) escape. Schematics depict *Salmonella* Typhimurium growth (*a*) in the absence of SIgA and (*b*) in the presence of SIgA. SIgA-clustered O-antigen can no longer diffuse in the outer membrane, such that after phase variation, "old" SIgA-bound O-antigen remains clustered at the oldest poles. This clustering results in faster generation of SIgA-free sister cells than would be expected by dilution alone. Figure adapted from images generated with BioRender.com.

(39, 93); as a result, there is a high probability of selecting for mutants carrying the deletion (23). Additionally, glucosyl transferase operons are commonly carried on phages, and several are found in the genomes of commonly used *Salmonella* Typhimurium strains in the context of degraded phage genomes, indicating a benefit to the cells (55). The expression of these operons is regulated by dam-dependent methylation, allowing rapid phase variation between glucosylated and nonglucosylated forms of the O-antigen (12, 19, 56).

At this point, clumping activity of IgA across scales again becomes relevant. We have already discussed how SIgA cross-links *Salmonella* Typhimurium cells via enchained growth. Now we must imagine the situation in which one cell within an aggregate undergoes O-antigen structural variation, due to either mutation or phase variation. At the point when this process occurs, all new O-antigen synthesized will have the "new" structure and will be poorly recognized by vaccine-induced SIgA. However, all of the "old" reactive O-antigen is still there, and still bound by SIgA, such that this antigenic variation is not immediately beneficial. We expect that, as this cell doubles and divides, the old SIgA–O-antigen complexes would be diluted by half with each cell division (**Figure 8**). Therefore, it would take several divisions until the SIgA cross-links are too few and the bacterial clump disbands.

However, investigations of this phenomenon at the single-cell level revealed that this is not quite what happens. Indeed, because IgA clusters O-antigens at the nano- or microscale, limiting outer membrane diffusion (**Figure 6**), the old O-antigen becomes concentrated at the oldest poles (**Figure 8**). This means that some daughter cells inherit more old IgA–O-antigen complexes than do others (43). Daughter cells originating from the poles of the original mother cell actually inherit ~60% of the complexes. This accumulation of IgA–O-antigen complexes in a subset of cells causes sister cells to lose the ability to cross-link to others in the aggregate sooner than expected and, ultimately, leads to faster immune evasion by the bacterial collective. The observation that IgA segregates surface antigen is likely a general principle of IgA–bacteria interactions, as phase variation of antigenic targets, whether glycan or protein, is common in bacteria (100).

Another way for enteropathogens to avoid SIgA-mediated enchained growth is to display, or upregulate, abundant surface antigens that are only weakly linked to the bacterial surface (66, 106) (**Figure 9**). In fact, one can think of the SIgA cross-links as a series of coupled springs stretching from one cell membrane to the other. This spring system can break not only at the SIgA–antigen
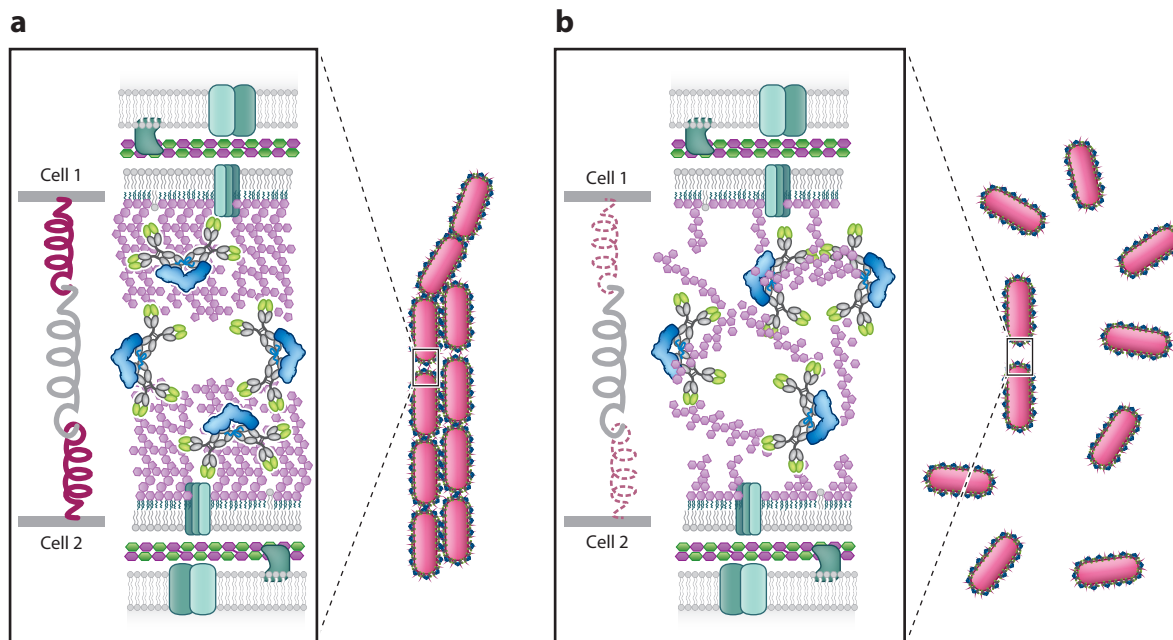
Figure 9

Loosely coupled bacterial surface antigen can prevent secretory IgA (SIgA)-mediated enchained growth. Bacteria–SIgA–bacteria cross-linking can be thought of as a series of coupled springs that break at the weakest link. (*a*) The O-antigens of lipopolysaccharide are strongly anchored in the bacterial outer membrane via a hexa-acyl lipid A. (*b*) Bacterial capsules, or release of surface antigens, can weaken bacterial cross-linking at the cellular level by failing to strongly link the two bacterial outer membranes. Figure adapted from images generated with BioRender.com.

bond but at any of the linkage points, including the antigen–cell membrane bond (**Figure 9**). Many bacterial capsules are sufficiently large to shield other surface antigens and can readily be shed from the bacterial surface, suggesting that they could inhibit SIgA aggregation. Another alternative is to shed of large amounts of outer membrane vesicles, generating "decoy" antigen that saturates relevant IgA specificities in the gut lumen (47, 98).

Intriguingly, recent studies suggest that IgA can also promote the maintenance of certain microbes in the gut microbiota. One study showed that SIgA-targeting *Bacteroides fragilis* is necessary for colonization of the dense colonic mucus by this strain, and the presence of specific SIgA allowed a bound strain to invade this niche (24). There are also conflicting reports of the influence of SIgA on colonization of pathobiont strains of *Helicobacter*, which typically invade the mucus of the stomach and small intestine, and at least some reports have hinted that SIgA promotes colonization (2, 94).

The mechanism by which IgA enhances bacterial fitness remains unclear, but a plausible explanation relates to the location of aggregate formation. If enchained growth occurs within dense mucus and the physical structure of the aggregate allows it to continuously invade newly produced mucus layers, rather than being sloughed off as the mucus is replaced, this would be an obvious advantage (69). Alternative explanations may also involve a division of labor within SIgA-enchained aggregates. Microbial ecologists have demonstrated that cells growing in aggregates are better at degrading polymers than cells growing planktonically (26–28). Instead of aggregated cells competing for nutrients, cooperation in fiber degradation between spatially linked cells can increase the growth rates of cells within the collective. Such division of labor is often observed in biofilms.

Given that IgA is capable of aggregating cells, it is possible that SIgA binding could provide a benefit to fiber degraders and is in line with the observation that IgA enhances *Bacterioides* expression of polymer degradation enzymes (49, 83).

IgA Functions: The Next Frontier

SIgA might provide benefit for the mucosal subpopulations independently of aggregation, by altering cell signaling, metabolism, or stress resistance. Another potential benefit of SIgA binding, which has not been extensively investigated, is interaction between SIgA and bacteriophage sensitivity. As these two moieties tend to target the same surface structures, it is possible that SIgA can inhibit specific bacteriophage binding. Alternatively, loss of outer membrane fluidity may alter sensitivity to bacteriophages or other noxious stimuli. Given that the relative contributions of these effects will depend on the antigen targeted, the antibody affinities, and the lifestyle and metabolism of the targeted bacterial species, extensive research is needed to untangle these complex responses.

Most mechanistic research on canonical SIgA interactions has focused on bacteria and eukaryotic viruses in the intestine. There is also an increasing body of literature on IgA–fungal interactions in the gut. *Candida* species commonly colonize the mammalian intestine, and SIgA targeting hyphal-enriched cell surface adhesins inhibits hyphae formation and therefore pathogen-like behavior (25, 78).

The respiratory and reproductive tracts are very different from the gut, with different functions and correspondingly different types of colonization and fluid dynamics. The dominant mechanisms of SIgA function are therefore likely to differ between body sites. For example, cilia-mediated mucus flow in the respiratory mucosa is a highly complex process that may concentrate bacteria in faster-flowing regions, potentially increasing the efficiency of classical agglutination at low overall bacterial densities in the lung (45). Moreover, phagocytic cells are found on the mucosal surfaces of the respiratory tract (1), such that FcR-mediated functions may play a more dominant role than in the gut. In particular, SIgA concentrations are higher in the upper than the lower respiratory tract, consistent with a role as a gatekeeper to colonization by opportunistic pathogens (13, 54, 88).

HOW DOES NONCANONICAL BINDING TRANSLATE INTO EFFECTS ON BACTERIAL POPULATIONS?

SIgA can also influence the microbial ecology of mucosal bacteria via noncanonical interactions, that is, binding that is not dependent on the antibody CDRs. Interactions between bacteria and the N-glycans of SC, either free or bound to dIgA, have also been described for a range of bacterial species (67). The relevance of these interactions was recently demonstrated by use of an ovalbumin-specific recombinant IgA to examine the effects on colonization with the abundant commensal bacterium *B. thetaiotaomicron* (74). The authors of this study observed upregulation of a polysaccharide-utilization locus that would be consistent with increased foraging of glycans (74). Murine SIgA is heavily decorated with both N- and O-glycans along the convex face, making it an abundant source of polysaccharides that *B. thetaiotaomicron* is capable of degrading (11). Total SIgA levels vary over the circadian cycle, and the cycling pattern of some commensal bacterial species appears to depend on this IgA rhythmicity (81). It seems likely that these mechanisms can be attributable at least in part to noncanonical trophic interactions. Notably, in most cases it is not clear whether SIgA glycan binding is only that (i.e., a method to coat the bacterial surface with a host protein for the purpose of protection) or whether it is part of the glycan foraging process.

Overall, the complex positive and negative selective pressures of SIgA on bacterial populations can be neatly summarized as "sculpting" the intestinal microbiota (104). However, oversimplifying

and extrapolating observations from one bacterial strain to the next and one body site or host to another are highly dangerous in this context. Rather, we need detailed information on the mode of binding, the function and variability of the bound antigen, and the mucosal lifestyle of the targeted species to understand the consequences of SIgA binding. The complexity of bacteria–IgA interactions is particularly well demonstrated by human selective IgA deficiency. Note that this deficiency cannot be accurately modeled by IgA knockout mice. First, it is an acquired syndrome, with unknown etiology, in which loss of IgA appears during childhood or early adulthood (4). In contrast, IgA knockout mice genetically lack IgA production from birth. Second, the human pIgR readily exports IgM at mucosal surfaces (58, 68), such that some functions of SIgA can be taken over by secretory IgM in humans. In mice, there is little to no secretory IgM detected in the intact, healthy gut lumen, despite elevated serum IgM in IgA knockout mice. Therefore, most useful information has come from clinical studies (31, 64, 70). Interestingly, secretory IgM seems to only partially compensate for SIgA function, and intestinal dysbiosis cannot be completely avoided in these patients (31, 70). What it is about the structure of secretory IgM that prevents it from completely mimicking the functions of SIgA remains a mystery.

CONCLUSION

The elucidation of the structure of both human and mouse SIgA complexes has raised new questions about IgA structure–function relationships (7, 57, 58). How is the convex shape of SIgA able to link between adjacent cells, when binding with all four sites to one cell appears to be thermodynamically much more stable? How does the polymeric state of IgA and SIgA influence canonical interactions with antigen? What are the functional outcomes of noncanonical interactions such as binding to host and microbial FcRs? How does enchained growth benefit mucus colonization for a subclass of bacteria? What are the relative contributions of the biophysical effects of aggregation and the gene expression–dependent effects of SIgA binding to changes in colonization dynamics? How can we reconcile aggregate formation by SIgA with increased mucosal sampling of IgA-associated antigens—are there size restrictions on this process, and what are the receptors involved? Are there unknown bacterial or host interaction partners for the protruding domains of SC? Are these involved in the function, localization, or stabilization of SIgA?

With improved recombinant protein tools that allow modification of the SIgA structure, as well as *Bcr* knock-in mice, it should become possible to experimentally address many of these questions in the near future. Furthermore, the potential of recombinant SIgA as a therapeutic agent has yet to be fully explored, and understanding how SIgA function can be rationally manipulated will be key to successfully realizing this goal.

DISCLOSURE STATEMENT

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Contents

Errata

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