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Short Review

# Specific antibody activity, glycan heterogeneity and polyreactivity contribute to the protective activity of S-IgA at mucosal surfaces

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ABSTRACT

An explanation of the principles and mechanisms involved in peaceful co-existence between animals and the huge, diverse, and ever-changing microbiota that resides on their mucosal surfaces represents a challenging puzzle that is fundamental in everyday survival. In addition to mechanical barriers and a variety of innate defense factors, mucosal immunoglobulins (Igs) provide protection by two complementary mechanisms: specific antibody activity and innate, Ig glycan-mediated binding, both of which serve to contain the mucosal microbiota in its physiological niche. Thus, the interaction of bacterial ligands with IgA glycans constitutes a discrete mechanism that is independent of antibody specificity and operates primarily in the intestinal tract. This mucosal site is by far the most heavily colonized with an enormously diverse bacterial population, as well as the most abundant production site for antibodies, predominantly of the IgA isotype, in the entire immune system. In embodying both adaptive and innate immune mechanisms within a single molecule, S-IgA maintains comprehensive protection of mucosal surfaces with economy of structure and function.



## 1. Role of secretory IgA (S-IgA) in mucosal immunity

Large surface areas of mucosal membranes (~200–400 $m^2$) are in constant contact with a highly diverse microbiota [1–6] estimated to comprise ~15,000–36,000 species and 1800 genera [7,8] and exceeding the total number of nucleated cells by an order of magnitude [1,2,5,9] ($10^{13}$ nucleated cells *vs.* ~$10^{14}$ bacterial cells). More than 99.9% of all commensal bacteria are found in the gastrointestinal tract, particularly in the large intestine [5,10]. Through evolution, the selective pressure arising from environmental antigens of microbial and food origin has resulted in a strategic, functionally advantageous distribution of cells involved in antigen uptake and processing, and the initiation of immune responses in mucosal tissues [9,11–13]. The mucosal immune system contains this antigenic onslaught without compromising the integrity of the mucosal barrier [11] or exhausting the immune system, in part through the induction of mucosal (oral) tolerance [14,15]. In addition to mechanical barriers and humoral effectors of innate immunity [6,11,16], mucosal antibodies and mucosal T cells provide antigen-specific protection [12,17].

The characteristic distribution of antibodies in blood and external secretions, including the intestinal fluid, reflects the functional adaptation of various Ig isotypes to different immune compartments. Given that mucosal membranes are the most important site of antigen encounter, it should not be surprising that most antibody production takes place in mucosal tissues, particularly the intestine, rather than in the bone marrow, spleen, and lymph nodes [12,18–21], and that the daily synthesis of IgA far exceeds that of IgG, IgM, IgD and IgE combined [19–22]. Importantly for mucosal protection, more than two-thirds of total IgA production ends up in the external secretions [19,21]. Quantitative studies of the origin of mucosal antibodies, particularly in the intestinal tract, demonstrate that >95% is of local origin and only trace amounts are derived from the circulation [19,22,23].

The mucosal microbiota, epithelial cells, and the mucosal immune system constitute a stable and interdependent "tripod" that maintains mucosal homeostasis by complex mechanisms [3,4,6,24–28]. For example, epithelial cells display surface receptors that are selectively exploited by bacteria adhering to their apical surfaces [1,2,28–30], and express the basolateral membrane receptor (polymeric Ig receptor; pIgR) that transports locally produced polymeric (p) IgA into the external secretions [23]. Bacteria

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**Table 1**
Examples of glycans as adhesion sites and receptors for selected bacteria and viruses that colonize, or infect, mucosal surfaces (adapted from [1,26,29,60–78,132]).

| | Epithelial cell | | |
|---|---|---|---|
| | Target tissue | Glycan structure | Form |
| Bacterium | | | |
| *Escherichiae* with Type 1 fimbriae | Intestine<br>Urinary tract | Man5GlcNAcGlcNAc | Glycoprotein |
| P | Intestine | Gal(α1,4)Gal | Glycoprotein |
| S | Intestine | NeuAc(α2,3), Gal(β1,3), GalNAc-*O*-linked | Glycoprotein |
| *Helicobacter pylori* | Stomach | NeuAc(α2-3)Gal | Glycolipid |
| *Pseudomonas aeruginosa* | Intestine | Galβ3GlcAc<br>Fuc<br>Man | Glycoprotein<br>Glycoprotein<br>Glycoprotein |
| | Respiratory tract | GalNAcβ1-4Gal | Glycoprotein |
| *Shigella dysenteriae* | Intestine | AsialoGM1 ganglioside | Sialoconjugate |
| *Neisseria gonorrhoeae* | Genital | Gal(β1,4) GalNAc | Glycoprotein |
| *Bordetella pertussis* | Respiratory | Gal(β1,4)Glc ceramide | Ceramide |
| *Haemophilus influenzae* | Respiratory tract | GlcNAcβ3Gal | Glycoprotein |
| *Streptococcus pneumoniae* | Respiratory tract | NeuAc(α2-3)-GalβGlcNAc | Glycoprotein |
| Virus | | | |
| Influenza A, B, C | Mucosal tissues | Neu5Ac, Neu5,9$Ac_2$ | Sialoconjugates |
| Paramyxoviruses | Mucosal tissues | Neu5Ac | Sialoconjugates |
| Coronaviruses | Mucosal tissues | Neu5, 9$Ac_2$ | Sialoconjugates |
| Reo- and rota-viruses | Intestinal tract | Sialic acid | Sialoconjugates |
| Respiratory syncytial virus | Respiratory<br>Mucosal tissues | Glycosamine glycans | Glycoproteins |
| HIV | Epithelial cells | Galactosylceramide | |

Man: mannose, Fuc: fucose, Gal: galactose, GlcNAc: *N*-acetyl glucosamine, GalNAc: *N*-acetyl galactosamine, NeuAc: sialic acid.

endogenous to the intestinal tract, oral cavity, and probably also the respiratory and genital tracts, are coated *in vivo* with S-IgA [9,13,17,31–39] that limits their epithelial adherence and penetration, thereby confining them to the mucosal surfaces. Numerous models have demonstrated the role of antibodies, especially S-IgA, in protecting the intestinal and other mucosal tracts. This has most convincingly been demonstrated *in vivo* in germ-free, colostrum-deprived newborn piglets [40–42], which, unlike humans, mice, rats, or rabbits, are born without transplacentally acquired Ig. In the absence of maternal as well as endogenous antibodies, milk-deprived piglets die of septicemia (usually *E. coli*) within 1–2 days after birth, whereas milk-fed animals survive [40]. Furthermore, piglets fed milk or serum, survive oral challenge with *E. coli*, whereas control animals deprived of Ig, irrespective of its source, succumb to the infection. In mice in which pIgR is copiously expressed on hepatocytes (not the case in humans, pigs, or dogs) and pIgA from the circulation is selectively transported into the bile and thence into the gut lumen [23,43], pathogen-specific pIgA hybridoma antibodies derived from "backpack tumors" [44–47] protect mice against oral challenge with *Salmonella enterica* serovar Typhimurium, *Vibrio cholerae*, or rotavirus [44,45,47–49]. In contrast IgG hybridoma antibodies of the same specificity are not protective, due to the lack of receptor-mediated transport of IgG into the intestine.

### 1.1. Mechanisms of S-IgA-mediated protection

Numerous such experiments clearly demonstrate protection *in vivo* dependent on the presence of antigen-specific IgA antibodies that interfere with pathogen adherence to or penetration through the mucosal barrier, or neutralize biologically active antigens such as viruses or toxins [41,47,48,50–54]. Likewise many *in vitro* studies of specific antibody-mediated inhibition of bacterial adherence to epithelial cells corroborate these findings [30,55–57]. However, agglutination and inhibition of the adherence of *E. coli* with Type 1 fimbriae to colonic epithelial cells that express a corresponding receptor can be mediated by IgA independently of specific antibody [30,58,59]. S-IgA and IgA myeloma proteins of both subclasses agglutinate *E. coli*, and mannose (Man) inhibits this agglutination. Furthermore, adherence of *E. coli* to human epithelial colonic cells can be inhibited by S-IgA as well as by IgA2 myeloma proteins. Analysis of the carbohydrate composition and complete primary structure of the oligosaccharide side-chains reveal that the most active pIgA2 myeloma protein contain several Man-rich *N*-linked glycan chains [30]. Thus, Man-dependent adherence of *E. coli* to epithelial cell receptors mediated by Type 1 fimbriae is competitively inhibited by similar glycans on S-IgA and IgA2 myeloma proteins acting as decoy receptors. Consequently, we have proposed that IgA proteins exhibit protective functions through antibody-dependent specific immunity as well as glycan-dependent innate immunity [30]. This concept was confirmed *in vitro* for other microbial ligand-glycan receptors [1,26,29,60–78]. In addition to *E. coli*, many other bacteria such as *Helicobacter pylori*, *Streptococcus pneumoniae*, *Clostridium difficile*, *Shigella flexneri*, *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae*, and some viruses (Table 1) interact with epithelial receptors via their glycan moiety.

Thus, it has become obvious that the *N*- and *O*-glycans of S-IgA provide a link between innate glycan-mediated and adaptive specific antibody-dependent protection (Fig. 1). This concept, of paramount importance in IgA-mediated mucosal defense, prompts additional considerations. First, it has been shown that bacteria indigenous to the oral cavity and intestinal tract are coated *in vivo* with IgA [9,17,31–39,79–81]. However, it is not known whether this coating depends on specific antibody-antigen or glycan-mediated interactions. Considering the enormous numbers of bacteria ($\sim 10^{12}$/g of intestinal content) [10], their diversity (~15,000–36,000 species of 1800 genera) [7], and the large number of potential antigenic determinants on many bacterial structures, it is unlikely that such coating is based *exclusively* on specific recognition by S-IgA antibodies. Secondly, in the large intestine IgA2-producing cells are dominant in contrast to other mucosal tissues [82,83], and antibodies to antigens (*e.g.*, endotoxin) of Gram-

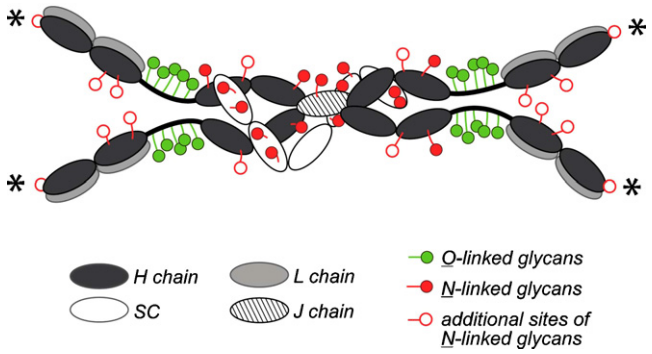


**Fig. 1.** Model of a human dimeric S-IgA molecule with assigned *adaptive* (*specific*-antibody) activity and *innate* (glycan-dependent) activity [88]. Asterisk—possible *N*-glycosylation sites within the CDR3 segment of the $V_H$ region of α chains [97,98,128].

negative bacteria are associated predominantly with the S-IgA2 subclass [84–86]. Thirdly, in addition to glycans on the H chain of IgA [87–91], secretory component (SC), the extracellular segment of pIgR, is extremely rich in glycans comprising 7 *N*-linked chains [88,92–94] that also act as highly effective inhibitors of adherence for some bacterial species (*e.g.*, *Shigellae*, *S. pneumoniae* [64,65,67–70]). Finally, prevention of the adherence of enormously diverse and variable mucosal microbiota is likely to be at least partially independent of specific antibody activity, reflecting the immediate need for protection against a broad spectrum of daily encountered microorganisms. Thus, in concert with the postulated Fab-mediated "polyreactivity" of S-IgA antibodies [95–100], glycan-mediated interactions are likely to further enforce protective functions of S-IgA.

Skeptics of these concepts may argue that mucosal defenses in IgA-deficient individuals should be significantly compromised. Indeed, the majority of such patients display a higher incidence of respiratory and intestinal infections [101–103]. Currently, IgA deficiency is defined as <50 mg IgA/100 ml of serum, regardless of S-IgA which is not taken into account although it is usually also diminished. Because complete absence of IgA in sera or secretions of IgA-deficient patients is extremely rare, it is possible that even low levels of S-IgA may provide some level of protection. Furthermore, in most IgA-deficient individuals S-IgA is replaced by S-IgM [102–110]. Thus, in IgA-deficient individuals, 65–75% of total Ig-containing cells in the intestines produce IgM, in sharp contrast to normal individuals (in the large intestine, ~90% of cells produce IgA, ~6% IgM, and ~4% IgG) [18,109]. Most importantly in this context, IgM and IgA display many common structural features, including: ability to form polymers; presence of J chain [111,112]; ability to bind pIgR (thereby forming SC-containing secretory IgM) [23,65,106,113]; homologies between primary structures of Cμ3, Cμ4 and Cα2, Cα3 domains and the C-terminal "tail-piece" [111,114,115]; and VH gene segment representation [116,117], indicating their close evolutionary and functional similarity [114]. This structural homology also extends to the glycan moieties: IgM and IgA molecules display a similar number and domain location of *N*-linked glycan side-chains, and both contain Man-rich chains [114,118–122]. Therefore, despite differences in the number of Ag-binding sites (up to 10 for IgM, and 4–8 for IgA dimers and tetramers), and ability to activate complement, it is clear that IgA and IgM are structurally, functionally, and evolutionarily closely related isotypes.

## 2. IgA-associated glycans display remarkable heterogeneity

Structural studies of human polyclonal S-IgA and monoclonal (myeloma) IgA1 and IgA2 proteins reveal considerable heterogeneity with respect to number, sites of attachment, composition, and primary structure of their glycan side-chains [30,71–77,87–94,115,122–127], which is likely to be of enormous biological importance. Because different microorganisms interact with epithelial cells through diverse glycan receptors, heterogeneity of IgA-associated glycans affords a variety of structures that can effectively inhibit these interactions.

Glycan moieties in S-IgA molecules are associated with H chains, J chain, and SC [88,90–94,124], but Man-rich *N*-linked glycans that inhibit the binding of Type 1 fimbriae to epithelial receptors occur only on the H chains [30,122]. However, other bacteria may interact with *N*- or *O*-linked glycans on H chains or SC (Table 1, section B). Although the majority of *N*-linked glycans are found in the Fc region of the α chains [88–89,114,115,124], there is great heterogeneity in the number and composition of individual glycan chains [30,122] and additional *N*-linked glycan chains may also be present in the Fd fragment (*N*-terminal half of the α chain comprising VH and CH1 domains), within the third complementarity-determining region (CDR3) [97,98,128]. The authors of these novel and functionally important studies propose that a high rate of somatic mutation in the CDR3 taking place within intestinal IgA-producing cells [97,98,116,117,128,129] generates a glycosylation-signaling sequence that alters the specificity of intestinal IgA antibodies. Thus, antigen-binding by Fab segments of S-IgA is determined by both specific antibody activity and glycan-dependent interactions.

The heterogeneity of *N*- and *O*-linked side-chains, with respect to their number, composition, and types of glycosidic bonds is further extended because many of them are incomplete, truncated forms [30,78,88,122]. Most importantly, and in sharp contrast to the combinatorial possibilities of amino acids, glycans can generate a remarkably higher number of structures, due to the variety of glycosidic bonds. Thus, a sequence of 6 (out of 20) amino acids can theoretically generate $6.4 \times 10^7$ distinct hexapeptides, while there are potentially $1.44 \times 10^{15}$ different hexasaccharides [130].

Specific antibody diversity is generated in an antigen-independent fashion during the differentiation of B lymphocytes by a number of mechanisms including recombination of multiple VJ (for L chains) and VDJ (for H chains) gene segments, combinatorial diversity of L and H chains, somatic hypermutation, gene conversion, and others [131]. The result of these genetic events is the generation of B lymphocytes with surface membrane Ig molecules that accommodate an enormous number of potential antigens, leading, *after* antigen-specific recognition, to B cell proliferation, differentiation, and the ultimate secretion of large amounts of antigen-specific antibodies. It is conceivable that analogous mechanisms operate in the generation of innate, glycan-mediated mechanisms of protection. Through random generation of enormously diverse glycan structures on mucosal glycoproteins, including S-IgA, S-IgM, SC, mucin, and lactoferrin, glycan configurations are generated that complement the equal heterogeneity of microbial adhesins. The protective effectiveness of these mechanisms may be further enhanced by subsequent somatic mutations within V regions of H and L chains, including the generation of glycosylation signals that lead to alterations of antibody specificities.

Parallel structural and functional exploration of the principles of adaptive (specific antibody) and innate (glycan) S-IgA-mediated immunity is likely to generate novel approaches to the design of broadly protective compounds that work by selectively interfering with the adherence and penetration of pathogens, or that contain the commensal microbiota residing at mucosal surfaces.

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