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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 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 Fuc Man	Glycoprotein Glycoprotein 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, Neu5,9Ac_2	Sialoconjugates
Paramyxoviruses	Mucosal tissues	Neu5Ac	Sialoconjugates
Coronaviruses	Mucosal tissues	Neu5, 9Ac_2	Sialoconjugates
Reo- and rota-viruses	Intestinal tract	Sialic acid	Sialoconjugates
Respiratory syncytial virus	Respiratory 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-