Humans live in symbiosis with $10^{14}$ commensal bacteria among which $>99\%$ resides in their gastrointestinal tract. The molecular bases pertaining to the interaction between mucosal secretory IgA (SIgA) and bacteria residing in the intestine are not known. Previous studies have demonstrated that commensals are naturally coated by SIgA in the gut lumen. Thus, understanding how natural SIgA interacts with commensal bacteria can provide new clues on its multiple functions at mucosal surfaces. Using fluorescently labeled, nonspecific SIgA or secretory component (SC), we visualized by confocal microscopy the interaction with various commensal bacteria, including *Lactobacillus, Bifidobacteria, Escherichia coli*, and *Bacteroides* strains. These experiments revealed that the interaction between SIgA and commensal bacteria involves Fab- and Fc-independent structural motifs, featuring SC as a crucial partner. Removal of glycans present on free SC or bound in SIgA resulted in a drastic drop in the interaction with Gram-positive bacteria, indicating the essential role of carbohydrates in the process. In contrast, poor binding of Gram-positive bacteria by control IgG was observed. The interaction with Gram-negative bacteria was preserved whatever the molecular form of protein partner used, suggesting the involvement of different binding motifs. Purified SIgA and SC from either mouse hybridoma cells or human colostrum exhibited identical patterns of recognition for Gram-positive bacteria, emphasizing conserved plasticity between species. Thus, sugar-mediated binding of commensals by SIgA highlights the currently underappreciated role of glycans in mediating the interaction between a highly diverse microbiota and the mucosal immune system.

Human mucosal surfaces comprising the mouth, respiratory, digestive, and urogenital tracts represent $\sim 400$ m$^2$, i.e. 200 times more than the global skin area. The human gastrointestinal tract is peacefully colonized by a large ecosystem estimated to belong to $> 1800$ genera, which represents $10^{14}$ bacteria, exceeding by more than 10 times the body cells (1, 2). Overall, the intestinal immune system has the dual task to protect the sterile core of the organism against invasion and dissemination of pathogens and maintain a peaceful relationship with commensal microorganisms. To preserve mucosal homeostasis, a complex communication needs to be established between a narrow triptch: the microbiota, the epithelial cells, and the mucosal immune system.

Secretory IgA (SIgA)$^2$ produced by plasma cells in the lamina propria represents the major immunoglobulin found at mucosal surfaces. The protective role of SIgA has been well established in the context of infection where the antibody (Ab) acts as a first line of defense through bacterial coating, thus largely preventing attachment to epithelial surfaces and resulting in a process referred to as immune exclusion (3). In contrast, to maintain an abundant and well balanced gut microbiota, such a clearance mechanism must be limited to a level guaranteeing homeostasis. Evidence is accumulating that emphasizes a complex cross-talk between the epithelium and microbiota that triggers SIgA secretion in the gut lumen of neonates already (4, 5). In contrast, SIgA production is reduced at barely detectable level in germ-free animals, whereas normal values of IgA can be reached within a few weeks following intestinal recolonization with various microbiotas (6–8). Recently, data demonstrated the induction of strain-specific SIgA secretion following reintroduction of *Enterobacter cloacae* in the gut of specific pathogen-free mice, indicating a direct impact of this microorganism on the subjacent immune cells (9). Furthermore, SIgA has been described to promote biofilm formation at the gut surface, underlying a straight relationship linking mucosal Abs and the gut microorganisms (10, 11). However, the molecular mode of action of SIgA in regulating microbiota colonization remains enigmatic. One can speculate that interaction between SIgA and commensals plays a role in modulating the colonization by the microbiota in steady-state conditions. Moreover, *in vivo* coating of commensal bacteria by SIgA has been described in analysis of human feces (12, 13). Because abundant intestinal secretion of natural SIgA with unknown specificity has also been described, we speculated that the latter can be involved in binding to commensals (8, 14–16).

SIgA is mostly composed of dimeric IgA made of two monomers linked together with J chain and secretory component

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$^2$The abbreviations used are: SIgA, secretory IgA; Ab, antibody; BL, *Bifidobacterium lactis*; Bt, *B. thetaiotaomicron* DSM 2079; Cy3, indocarbocyanine-3; D2241, *E. coli* strain D2241; dg suffix, deglycosylated; hSC, human SC; LPR, *Lactobacillus rhamnosus*; LSCM, laser scanning confocal microscopy; mSC, mouse SC; Nissle, *E. coli* strain Nissle 1917; pIgA, polymeric IgA; SC, secretory component; SCol, colostrum-derived SC; SIgAcol, colostrum-derived SIgA; ST11, *Lactobacillus paracasei*.
**EXPERIMENTAL PROCEDURES**

**Microorganisms and Growth Conditions—Lactobacillus paracasei** ST11 (NCC2461 provided by Nestlé Research Center, Lausanne, Switzerland) and *Lactobacillus rhamnosus* LPR (NCC4007 provided by Nestlé Research Center, Lausanne, Switzerland) were grown in Man-Rogosa-Sharpe broth at 37 °C for 48 h. Bacteria were washed three times in phosphate-buffered saline (PBS) by successive centrifugations at 2000×g for 48 h. Bacteria were harvested twice a week, filtered through 0.22-μm membranes, and separated by size-exclusion chromatography to recover pIgA from other molecular forms and undesired ingredients (35). Fractions containing pIgA forms were pooled, concentrated using a Pellicon XL filter unit (100-kDa cut-off; Millipore) coupled to a Labscale system (Millipore), and finally stored at 4 °C. IgGC20 was purified by affinity chromatography using protein G-Sepharose 4 fast flow beads (GE Healthcare) according to the manufacturer’s instruction. Prior to storage at 4 °C, the sample buffer was exchanged against PBS by filtration over an Amicon Ultra 100K cartridge (Millipore). Mouse SC (mSC) was produced and purified as described (36). The purified protein was stored in PBS at 4 °C until use. The bicinechonic acid protein assay kit (Pierce) was used for protein measurement. Fluorescent molecules (pIgA, IgG, mSC) were obtained using the FluoroLink mAb indocarbocyanine-3 (Cy3) labeling kit (Amersham Biosciences) according to the procedure provided by the manufacturer. SlgA-Cy3 were obtained by combining pIgA-Cy3 molecules with mSC (5/1 (w/w)) in PBS according to the conditions defined in Crottet and Corbély (37). The effective reassociation between pIgA and mSC proteins was checked by SDS-PAGE under nonreducing conditions using sera specific for mouse α chain, J chain, or SC (38).

**Preparation of SlgA and SC from Colostrum**—Aliquots of individual frozen human colostral samples were centrifuged at 13,000×g for 20 min at 4 °C to remove lipids and cells (39). The lower layer containing proteins was recovered and sterile-filtered. Delipidized colostrum was applied on a Superdex 200 column (100×2.6 cm; GE Healthcare) equilibrated in PBS. Colostrum-derived SlgA (SlgAcol) and SC (Sccol) were indentified, respectively, in the first and the third eluting peaks and comigrated with leftovers of unresolved IgG and lactoferrin. Pooled fractions were concentrated using Amicon Ultra 30K cartridge to reach a final volume of 10 ml. Contaminating IgG was removed using batch incubation with 2 ml of protein G-Sepharose 4 fast flow beads (GE Healthcare) equilibrated in PBS; SlgA and SC were collected as unbound material. The buffer of the SlgA or SC solutions was exchanged for 20 mM sodium phosphate buffer (pH 7.3) using filtration through Amicon Ultra 30K cartridge. To get rid of residual lactoferrin, the SlgA and SC materials were then fractionated, respectively, onto 2 ml of Q-Sepharose fast flow beads (GE Healthcare) and SP-Sepharose fast flow (GE Healthcare) equilibrated in 20 mM sodium phosphate buffer. Stepwise elution with a range of KCl concentration (0.05–0.5 M) was performed, allowing for elution of SlgAcol between 0.05 M and 0.2 M KCl and SCol between 0.05 M and 0.1 M KCl. Pooled SlgAcol- or SCol-containing fractions were desalted by addition of 10 volumes of PBS and concentrated using an Amicon Ultra 30K cartridge prior to storage at 4 °C. To ensure that Q- and SP-chromatography properly separated them, the resulting absence of lactoferrin was assessed by ELISA (Lactoferrin ELISA kit; Calbiochem). The integrity of recovered SlgAcol and SCol was checked by SDS-PAGE under nonreducing conditions. SlgAcol and SCol-containing labeled with Cy3 as described above for hybridoma-derived proteins.

**Protein Deglycosylation**—Five units of N-glycosidase F (EC 3.5.1.52; Roche Applied Science) were added to either 5 μg of reassociated SlgA-Cy3 or 1 μg of mSC-Cy3 and incubated at 37 °C for 4 h under gentle agitation, resulting in deglycosylated SlgAC5 (SlgAC5dg), deglycosylated SlgASal4 (SlgASal4dg), and SlgA-Commensal Interactions Mediated by Glycan Residues (SC), the extracellular degradation product resulting from cleavage by the epithelial cells of the precursor polymeric Ig receptor ensuring transcytosis of the Ab (17). In secretions, SC is bound covalently, as well as noncovalently, to IgA, and is found also as free SC (18). Both polymeric IgA (pIgA) and SC are heavily glycosylated (19, 20); remarkably, N- and O-carbohydrate residues have already been implicated in the interaction with bacteria, participating in the protection against enteric pathogens (20–25) either as adhesion competitors or in anchoring SlgA in the mucus to ensure optimal biological function. In face of the increasing involvement of glycans in the function of SlgA, we sought to determine their potential role in commensal binding (25, 26). As substitute of natural SlgA and SC, we used nonspecific mouse hybridoma-derived SlgA, pIgA, and free SC, as well as their deglycosylated counterpart, to examine the role of carbohydrates in binding to a selection of bacteria (*Lactobacillus, Bifidobacteria, Bacteroides, or Escherichia coli* strain). The results obtained with recombinant proteins were validated with SlgA and SC purified from human colostrum.
and deglycosylated mSC (mSCdg), respectively. Deglycosylation was examined by SDS-PAGE under reducing conditions, and immunodetection was carried out using antisera against either α chain or SC. To obtain fully deglycosylated SlgAcol (SlgAcoldg) or deglycosylated SCcol (SCcoldg), further addition of 5 units of N-glycosidase F was carried out after 3 h of incubation, and the mixture was incubated for 3 more hours.

**Protein Analysis**—2 μg of protein was used for silver staining (35), 200 ng for α chain detection, and 600 ng for other detections. Samples were mixed with gel loading buffer (100 mM Tris base, 4% SDS, 0.2% bromphenol blue, and 20% glycerol), and for reducing conditions, dithiothreitol (DTT; Applichem) was added to a final concentration of 100 mM. Samples were heated to 95 °C for 3 min and applied onto polyacrylamide gels of appropriate polyacrylamide percentages as indicated in each figure legend. Western blot assay was performed as described (38), with proteins detected by incubation for 1 h with the following primary and secondary Abs in PBS containing 0.05% Tween 20 and 0.5% nonfat dry milk: goat anti-mouse α chain (1/3000; Sigma), goat anti-human α chain (1/3000; Cappel), and rabbit anti-goat IgG conjugated to horseradish peroxidase (HRP) (1/3000; Sigma); rabbit anti-human κ chain (1/2000; Dako), rabbit anti-human SC (hSC; 1/3000; Dako), rabbit anti-mSC (1/3000) (36) or rabbit anti-J chain (1/1000) (40) followed by HRP-conjugated goat anti-rabbit IgG (1/5000; Sigma); rabbit anti-human γ chain HRP-conjugated (1/5000; Dako). After final washing in PBS containing 0.05% Tween 20, proteins were detected by chemiluminescence using Uptilight detection kit (Interchim) and exposed on autoradiographic films (Konica).

**Protein Association with Bacteria**—2 × 10⁷ bacteria were mixed with 200 ng of SlgA, SlgAdg, or plgA, or with 40 ng of mSC and mSCdg, in a final volume of 400 μl of PBS and incubated for 1 h at room temperature under gentle agitation. Bacteria-protein complexes were washed three times in PBS, laid onto 8-well slides (Marienfeld), fixed in 2% paraformaldehyde in PBS for 25 min, and mounted in Vectashield (Vector Laboratories). Complexes were observed using a Zeiss LSM 510 Meta confocal microscope (Carl Zeiss) with a 63 × objective (Cellular Imaging Facility, Lausanne University, Switzerland) and processed using the Zeiss LSM 510 Meta software.

**Quantification of Bacterial Coating by Antibodies**—Quantification of bacterial coating by antibodies was performed with ImageJ software 1.41 (National Institutes of Health). We measured on each series of pictures the area covered by bacteria (bacterial area) using the differential interference contrast channel. In parallel, in each area associated with bacteria, we quantify the area containing Ab- or SC-linked pixels with a fluorescence intensity superior to 15 units (fluorescent area) defined in our experimental settings as the background signal (signal range extends from 0 to 256 units using transformation into 8-bit gray scale). The following formula was used to quantify the percentage of bacterial surface covered with fluorescent molecules: 100 × (fluorescent area)/bacterial area.

**Statistical Analysis**—The results are given as means ± S.E. Two-tailed nonparametric Mann-Whitney U test analysis was performed using the GraphPad 5 Prism software. Differences were considered as significant when p values < 0.05 were obtained.

**RESULTS**

**SlgA Binds Bacteria in a Fab- and Fc-independent Manner**—Coating of bacteria has been previously observed in feces samples recovered from human and mouse (12, 41), suggesting that this takes place at all times in the gut lumen. We made the assumption that nonspecific SlgA serving as substitute of natural SlgA can bind to commensals in a Fab-independent fashion. We tested this hypothesis by incubating in vitro a battery of
bacterial strains including Gram-positive LPR, ST11 and BL, as well as Gram-negative 
*E. coli* strains D2241 and Nissle and *Bacteroides* Bt with fluorescently labeled reconstituted SIgA, pIgA, SC, and IgGC20. Panels of Fig. 1 show the data obtained with LPR; similar, if not identical results were produced with the strains ST11 and BL. Complexes with bacteria were detected by laser scanning confocal microscopy (LSCM) after 1 h of incubation in the presence of either SIgAC5, SIgASal4, or SIgAHNK20 (Fig. 1). Bacteria were almost entirely coated by nonspecific SIgA molecules, indicating that the formation of complexes relied on Fab-independent binding mechanisms. Interestingly, incubation with SC alone led to the same observation, pointing out that Fab- but also Fc-independent associations sustain the coating of bacteria by SIgA. No bacterial coating could be detected using pIgA alone (data not shown), indicating that different types of interactions are involved as a function of the bacterial surface.

### Glycan Residues Are Involved in the Interaction between Bacteria and SIgA—Glycosylation is known to be involved in the innate defense against pathogens (25). Because SIgA and SC are highly glycosylated proteins (19, 20), we performed deglycosylation cleaving all types of N-branched glycan residues bound to asparagine. Contrary to most of other methods, N-glycosidase F removes carbohydrate residues by preserving the conformational structure of the protein (23). Clipping of all seven branched glycans carried on pIgA-bound or free SC was confirmed by analysis on polyacrylamide denaturing gels (Fig. 2A). The shift of the apparent molecular mass from 80 kDa down to 62–65 kDa is indicative of fully deglycosylated SC (24). In contrast, carbohydrate moieties present on the α chain were insensitive to the action of the enzyme, suggesting a nonaccessible anchoring of the carbohydrates in pIgA (Fig. 2B). Representative pictures of association between deglycosylated proteins and LPR are depicted in Fig. 2C. Deglycosylation of either free SC or bound to pIgA led to a drastic drop in colocalization with bacteria: only a few isolated red dots of weak fluorescence intensity were observed by LSCM. Experiments performed with ST11 or BL yielded to the same dramatic decline in SIgA and mSC reactivity toward bacteria (data not shown). Taken together, these data underline the paramount role of glycan residues and in particular those present on SC in ensuring bacterial coating by SIgA and SC. In sharp contrast to *Lactobacillus*...
and Bifidobacterium, interaction of the three tested Gram-negative bacteria with deglycosylated or native proteins resulted in similar high level binding, indicative of a selective role of carbohydrates in the binding of Gram-positive bacteria only (data not shown).

Quantitative Analysis of Bacterial Coating by SIgA—LSCM observations drove us to analyze at the quantitative level the bacterial surface covered by fluorescently labeled proteins. Data in Fig. 3 represent the quantification of the signal associated with Ab- or SC-bacteria complexes using the same set of bacteria incubated with either SIgA, SIgAdg, plgA, mSC, mSCdg, or IgGC20. All Gram-positive bacteria mixed with SIgA or free SC resulted in almost full coating by fluorescently labeled proteins of LPR (95%) and high values of surface labeling for ST11 (88.5%) and BL (73.8%) (Fig. 3, A–C). Interestingly, Lactobacillus strains LPR and ST11 displayed the most contrasted interaction profiles, with deglycosylated molecules leading to a drop in coating of at least 4-fold, confirming at the quantitative level the results obtained on Figs. 1 and 2. Although resulting in a less marked 1.7-fold decrease, data obtained for the Bifidobacterium BL (Fig. 3C) allowed us to reach the same conclusion: glycan residues carried by SC are the keystone of the SIgA Fab- and Fc-independent coating of bacteria. In addition, plgA and SIgAdg exhibited a low level of coating in the case of LPR (3.7%), yet remaining close to 20% for ST11 and varying from 3% to up to 41% for BL. This later observation may reveal a modulable role for extremely heterogeneous glycans present on monoclonal pIgA as a function of the interacting bacterium (Fig. 3, A–C). In sharp contrast, E. coli strains D2241 and Nissle, as well as Bt, were coated to at least 63% following incubations with all forms of proteins tested (Fig. 3, D–F), indicating different molecular patterns involved in binding to Gram-negative bacteria. The very similar results obtained with deglycosylated and native proteins allowed us to conclude on the absence of direct involvement of carbohydrates present on SC in the coating of Gram-negative bacteria by SIgA or SC.
Purification of SlgA and SC from Human Colostrum and Biochemical Characterization—Colostrum and human milk are providential sources of SlgA containing, respectively, up to 12 g and 1 g of the Ab/liter (4, 42). We thus purified SlgA and SC from human colostrum to examine the interaction between natural SlgA and commensal bacteria. The complete purification scheme is depicted in Fig. 4A. Silver staining and Western blot analysis revealed the coelution of SlgA with lactoferrin and IgG (data not shown), two glycoproteins that can potentially interfere with nonspecific binding properties of SlgA (43). IgG was removed by affinity chromatography on protein G-Sepharose resin, whereas elimination of lactoferrin was achieved by ion-exchange chromatographies. Silver staining and Western blot analysis confirmed the integrity and purity of isolated SlgAcol and SCcol (Fig. 4, B and C).

Bacterial Coating with Natural Purified Colostrum-derived SlgA—To confirm the validity of results obtained with mouse reassociated SlgA, we combined native and deglycosylated SlgAcol and SCcol with the same set of commensal bacteria as illustrated in Figs. 1 and 2. Treatment of SlgAcol with N-glycosidase F resulted in the same deglycosylation pattern of SC as for mouse hybridoma-derived SlgA, although with some delay (Fig. 5A), whereas sugars associated with the α chain remained undigested (Fig. 5B). The lag of sugar clipping observed is probably due to slight structural differences between reassociated SlgA and “naturally” associated SlgAcol in secretions. When SlgAcol was incubated with Gram-positive bacteria, we observed by LSCM bright dots colocalizing with LPR, whereas deglycosylated molecules bound only marginally to bacteria (Fig. 5C and data not shown). Quantification of the bacterial percentage covered by fluorescently labeled proteins confirmed that the use of SlgAcol, SCcol, and control SlgAC5 resulted in similar bacterial coating, whereas deglycosylation of colostrum-isolated molecules triggered the loss of efficient binding to Lactobacillus LPR (Fig. 5D). The demonstration that either human SlgAcol or SCcol and reconstituted mouse SlgA molecules yield similar binding data suggests that the heterogeneity of carbohydrate side chains affords combinational possibilities that are preserved between species. Selective deglycosylation of SC in both murine and human molecules further allows us to conclude as to the critical role of sugar moieties on this polypeptide in the context of commensal binding by the whole SlgA protein.

**DISCUSSION**

The intestinal microbiota have been demonstrated to be involved in the proper development of the intestinal immune system and in particular in the production of a diverse repertoire of SlgA (4) composed of at least 90% of natural SlgA with largely unknown specificity (8, 15, 16). The biological function of such SlgA remains speculative; however, various studies have underscored the natural coating of commensal bacteria by SlgA, a process that may be involved in the homeostatic gut sensing of the microbiota (12, 41, 44).

Nevertheless, the precise biochemical mechanisms underlying the relation between intestinal microbiota and the immune system, in particular, SlgA, remain enigmatic. To tackle this issue, various strains isolated from the human intestinal tract were incubated with a battery of nonspecific SlgA, and the nature of the interaction was investigated qualitatively and quantitatively. The use of purified murine SlgA reconstituted in the test tube or SlgA isolated from human colostrum identified a Fab- and Fc-independent pattern of bacterial coating. Deglycosylation of both SC and SlgA allowed us to conclude as to the pivotal role of carbohydrates, and in particular N-branched glycans, in the interaction with a selection of Gram-positive bacteria. Remarkably, SC alone was able to coat such commensals to the same extent as the whole SlgA. Moreover, deglycosylated SC in the context of SlgA prevented bacterial coating by the Ab, featuring surface-exposed carbohydrate moieties as crucial partners in the process of binding.

It is conceivable that the association of Gram-positive bacteria and SlgA is due to recognition between Ab-bound N-branched glycans and abundant cell wall components such as peptidoglycans, lipoteichoic acid, or teichoic acid available to the environment. Lactobacillus lactis harbors a particular, thick outer layer composed of polysaccharides acting as a protective capsule (45). This polysaccharide envelope conferring to the bacterium a protective barrier against phagocytosis by murine macrophages appears to be restricted to Gram-positive bacteria and might as well serve as a particular constituent involved in
recognition by SIgA or SC. In support of the importance of bacterial surface in selective binding, the presence in the outer membrane of lipopolysaccharides in Gram-negative bacteria masking the underlying peptidoglycan layer could explain why both “native” and deglycosylated Ab and SC exhibit the same pattern of association. This indirectly suggests that other molecular patterns besides glycans on SC contribute to genera-dependent association, opening a new avenue of research at the interface between immunology and microbiology.

The different binding pattern between Gram-positive bacteria LPR, ST11, and BL with respect to Gram-negative D2241, Nissle, and Bt revealed a fine tuning of the bacterial coating by the Ab and SC. Perrier et al. (24) have demonstrated that recombinant SC prevents target HEp2 cells from being damaged by enteropathogenic *E. coli*, a property partly lost after deglycosylation, indicating an important contribution of carbohydrates in this process. These data are in sharp contrast with our results obtained with the nonpathogenic *E. coli* commensal strains D2241 and Nissle for which maintenance of binding could be observed following SC deglycosylation. Because only pathogenic *E. coli* strains including enteropathogenic *E. coli* express outer membrane intimin (46) involved in the interaction with glycans present on SC (24), this may explain differences in SIgA binding. The sum of these data implies that the Fab- and Fc-independent molecular features responsible for

**FIGURE 5.** Deglycosylation of colostrum-derived SIgA or SCcol and defective association with LPR observed by LSCM. A, immunodetection of the hSC was performed after 0, 2, 4, or 6 h of incubation with 5 units of *N*-glycosidase F from 0 to 3 h and a further 5 units of enzyme for the last 3 h. Samples were separated onto a 10% polyacrylamide gel under reducing conditions. The molecular mass (kDa) of detected polypeptides is marked alongside the lanes. B, samples were separated as in A with immunodetection of the α chain. The unique signal detected at 62 kDa represents nondeglycosylated α chains. C, LSCM imaging of complexes formed between LPR and native or deglycosylated SIgAcol or SCcol was performed as in Fig. 1. SIgAC5 were used as a positive control for the interaction of bacteria with proteins. One representative field obtained from 10 different observations after analysis of 5 different slides is depicted. Scale bars, 10 μm. D, quantification of LPR coating by fluorescently labeled proteins was carried out on pictures of LPR alone (control) or pictures of complexes with SIgAC5, SIgAcol, SIgAcoldg, SCcol, and SCcoldg. Bars represent the mean values ± S.E. Statistically significant differences are indicated above the brackets for intragroup tests: ***, *p* < 0.0001. Data were obtained from 5 to 10 different fields of one experiment repeated 5 times.
bacterial coating are highly plastic and flexible and result in diverse biological consequences.

As SlgA carbohydrates harbor highly heterogeneous structures, these latter can contribute to combinatorial-like, multi-affinity interactions with gut microorganisms (25). This was illustrated in neonatal mice in which antibody immunity fixed to the hapten nitrophenol protected against bacterial challenge (47). Recently, the assessment of the crystal structure of a mucus-binding protein present at the surface of the commensal strain Lactobacillus reuteri identified motifs with potential immunoglobulin binding activity (48). The triad mucus-binding protein–bacteria–SlgA may serve as an organized lattice precluding massive commensal penetration from the intestinal lumen to the underlying tissue, thus ensuring proper host-microbial mutualism. Noteworthy, drug-immunosuppressed and immunodeficient individuals have increased susceptibility to sepsis or chronic intestinal inflammation mediated by the microbiota (49, 50), and this holds particularly true for individuals lacking SlgA.

We found that carbohydrates present on the α chain could not be removed following exposure to N-glycosidase F. This observation is consistent with the masking of N-glycan residues by SC in the context of the whole SlgA molecule (20, 51). It is plausible that these noncleavable carbohydrates would be responsible for the various residual interaction observed upon incubation of Gram-positive bacteria with SlgAdg. However, as any removal of these glycan residues occurs unless harsh conditions (use of reducing agents, exposure to heat) are used (52), this makes removal of these glycan residues not be removed following exposure to harsh conditions (use of reducing agents, exposure to heat) are used (52), this makes removal of these glycan residues.

Our study provides strong evidence that N-branched glycans present on SC are the main component responsible for Fab- and Fc-independent specific binding to Gram-positive commensal bacteria. Due to the presence of LPS on Gram-negative bacteria, motifs other than carbohydrates on SlgA account for binding. It is worth mentioning that introduction of commensals is associated with the induction in the intestine of both strain-specific SlgA (9) and natural SlgA with unknown specificity (8).

Based on these observations, one can argue that SlgA present in human colostrum is a mixture of specific SlgA from maternal origin and natural SlgA. Despite the potential presence of specific SlgA for commensals, identical conclusions were reached with SlgA purified from human colostrum, compared with coating data obtained with monoclonal Ab. Altogether, this contributes to explain at the biochemical level the role of colostrum or milk SlgA with a broad maternal repertoire in shaping the newborn microbiota before maturation of the mucosal immune system (4). Commensal coating by colostrum-derived SlgA would translate into controlled primary gut colonization and the early education of the newborn immune system toward novel antigens prone to become symbiotic partners. We propose that coating of commensals by SlgA adds to the function of mucosal SlgA in maintaining gut homeostasis, and this process integrates potentiation of binding to epithelial cells (28), presentation under noninflammatory conditions (26), and luminal sequestration or limited penetration of host tissues (53, 54).
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