Secretory IgA-Mediated Neutralization of *Shigella flexneri* Prevents Intestinal Tissue Destruction by Down-Regulating Inflammatory Circuits

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**References**

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Secretry IgA-Mediated Neutralization of *Shigella flexneri* Prevents Intestinal Tissue Destruction by Down-Regulating Inflammatory Circuits

Séverine Boullier,*† Myriam Tanguy,‡§ Khalil A. Kadaoui,¶ Cécile Caubet,*† Philippe Sansonetti,‡§ Blaise Cortès,¶ and Armelle Phalipon²‡§

*Shigella*, a Gram-negative invasive enteropathogenic bacterium responsible for bacillary dysentery, causes the rupture, invasion, and inflammatory destruction of the human colonic mucosa. We explored the mechanisms of protection mediated by *Shigella* LPS-specific secretory IgA (SIgA), the major mucosal Ab induced upon natural infection. Bacteria, SIgA, or SIgA-*S. flexneri* immune complexes were administered into rabbit ligated intestinal loops containing a Peyer’s patch. After 8 h, localizations of bacteria, SIgA, and SIgA-*S. flexneri* immune complexes were examined by immunohistchemistry and confocal microscopy imaging. We found that anti-*Shigella* LPS SIgA, mainly via immune exclusion, prevented *Shigella*-induced inflammation responsible for the destruction of the intestinal barrier. Besides this luminal trapping, a small proportion of SIgA-*S. flexneri* immune complexes were shown to enter the rabbit Peyer’s patch and were internalized by dendritic cells of the subepithelial dome region. Local inflammatory status was analyzed by quantitative RT-PCR using newly designed primers for rabbit pro- and anti-inflammatory mediator genes. In Peyer’s patches exposed to immune complexes, limited up-regulation of the expression of proinflammatory genes, including TNF-α, IL-6, Cox-2, and IFN-γ, was observed, consistent with preserved morphology. In contrast, in Peyer’s patches exposed to *Shigella* alone, high expression of the same mediators was measured, indicating that neutralizing SIgA dampens the proinflammatory properties of *Shigella*. These results show that in the form of immune complexes, SIgA guarantees both immune exclusion and neutralization of translocated bacteria, thus preserving the intestinal barrier integrity by preventing bacterial-induced inflammation. These findings add to the multiple facets of the noninflammatory properties of SIgA. *The Journal of Immunology*, 2009, 183: 5879–5885.

An important activity of mucosal surfaces is the production of Abs referred as to secretory IgA (SIgA) that serves as the first line of defense against pathogenic microorganisms (1, 2). SIgA also limits the penetration of commensal bacteria through the epithelium and contributes to shape the density of different bacterial species in the intestinal lumen (3, 4). Mucosal SIgA Ab, a remarkably stable molecule (5, 6), exists in dimeric and polymeric forms (pIgA) comprising the joining chain (J chain protein) added during Ab secretion from mucosally activated plasma cells (7). Transport of pIgA to mucosal surfaces is ensured upon specific binding to the polymeric Ig receptor (pIgR) selectively expressed on the basolateral surface of mucosal epithelial cells (EC) (8). Release at mucosal surface generates SIgA retaining the extracellular domain of the pIgR-termed secretory component (SC) (9). SC, by ensuring binding of SIgA to the mucus lining, is mandatory to the immune exclusion function of this mucosal Ab (10). This function consists of preventing interactions between microbes and the intestinal epithelium upon their agglutination, thus facilitating clearance by peristaltic and mucociliary movements (11). Besides the key role of SIgA in the luminal compartment, protective functions are also ensured by pIgA within the underlying tissue and the intracellular compartment of pIgR-expressing EC (12–15). Recently, luminal SIgA has been shown to translocate, via M cells, to mouse Peyer’s patch (PP) dendritic cells (DCs) and T cells without promoting an inflammatory response (16–19). SIgA thus displays neutralizing as well as immunopotentiating capacities to promote effector immune responses in a noninflammatory context favorable to preserve local homeostasis (20–21).

*Shigella*, a Gram-negative invasive enteropathogenic bacterium, causes the rupture, invasion, and inflammatory destruction of the human colonic epithelium. This process accounts for the symptoms of bacillary dysentery (22). Both mucosal SIgA and serum IgG specific for LPS, the major bacterial surface Ag, are elicited upon natural infection and evidence indicates that they are the main actors for protection against reinfection (23). In the absence of a mouse model of shigellosis (22), mechanisms underlying the protective role of anti-LPS Abs have been investigated using a mouse model of pulmonary infection, which mimics the induction...
of the inflammatory process (24, 25). In such a model, both anti-LPS SIgA and IgG were shown to protect the host from tissue destruction resulting from Shigella-induced inflammation (10, 25, 26). In vitro studies using human intestinal crypt cells have also revealed that anti-LPS pIgA and bacterial LPS colocalize in the apical recycling endosome, impairing LPS-induced NF-κB translocation and subsequent proinflammatory responses (15).

The aim of the current work was to extend our understanding of anti-Shigella LPS SIgA–mediated mechanisms of protection in vivo at the intestinal level. Shigella alone or in combination with a specific anti-LPS SIgA were previously used to unravel the capacity of SIgA to mediate Ag transport across PPs in the mouse (19). However, because mice are resistant to intestinal infection with Shigella, the model is not fully appropriate to address the anti-inflammatory properties of SIgA in mucosal tissues. Hence, in the current study, we used the rabbit model of ligated ileal loops infected with Shigella because it mimics both local inflammation and subsequent massive tissue destruction as it occurs during natural infection in humans (24, 27). Although this model has intrinsic limitations due to the restricted number of available immunologic reagents, it turned out to be very helpful to demonstrate that specific SIgA is a key player to reduce the proinflammatory response turned on by Shigella at the level of the intestinal barrier in vivo.

Materials and Methods

Protein production and purification

Hybridoma cells producing IgAC5 specific for S. flexneri serotype 5a LPS (10) were cultured in complete RPMI 1640 medium as described (28). High-density cell cultures were conducted in CellLine-350 bioreactors (Integra Biosciences). Supernatants were harvested twice a week, filtered (10) were cultured in complete RPMI 1640 medium as described (28). Supernatants were harvested twice a week, filtered (10) were cultured in complete RPMI 1640 medium as described (28).

A specific anti-LPS SIgA were previously used to unravel the capacity of SIgA to mediate Ag transport across PPs in the mouse (19). However, because mice are resistant to intestinal infection with Shigella, the model is not fully appropriate to address the anti-inflammatory properties of SIgA in mucosal tissues. Hence, in the current study, we used the rabbit model of ligated ileal loops infected with Shigella because it mimics both local inflammation and subsequent massive tissue destruction as it occurs during natural infection in humans (24, 27). Although this model has intrinsic limitations due to the restricted number of available immunologic reagents, it turned out to be very helpful to demonstrate that specific SIgA is a key player to reduce the proinflammatory response turned on by Shigella at the level of the intestinal barrier in vivo.

Shigella flexneri culture conditions

Both wild-type M90T, an invasive isolate from S. flexneri serotype 5a LPS, and its GFP-expressing derivative (29) were used. Bacteria were grown in LB broth or LB agar plates (1.5% Bacto-agar in LB medium) overnight at 37°C. For each concentration was calculated using the formula: 1 OD600 corresponds to 5 × 10^10 bacteria/ml.

Administration into rabbit ligated intestinal loops

Surgery was performed as described (30). Depending on the experimental conditions, 10^7 wild-type, GFP-S. flexneri, or a combination of S. flexneri and 100 μg SIgAC5 (agglutinating amount) were suspended in 100 μl of PBS and injected into the lumen of a 3 cm-long ligated loop displaying one PP using a 0.5-ml U-50 insulin syringe ( gauge 29G1/2, BD Biosciences). The average number of ileal loops performed in one rabbit was five: usually two were administered with the immune complexes, one or two with SIgAC5 alone, and one or two with Shigella alone; ileal loops receiving PBS were used as control. In total, eight rabbits were included. For localization of radiolabeled SIgA alone or combined to Shigella, two rabbits were used. The experiment protocol received the authorization of the Institute Pasteur Ethical Committee.

Preparation of tissue sections

Tissue sections containing a PP were fixed overnight in 4% parafomaldehyde in PBS, PBS-washed, and embedded in paraffin as previously described (31). Four-mm-thin sections were stained with either hematoxylin/eosin for tissue visualization or Alcian blue (in PBS at pH 2.4, followed by counterstaining with hematoxylin) for mucus staining and processed for light microscopy. For immunohistochemistry, 4-mm-thin tissue sections were incubated in PBS-3.3% H2O2 for 10 min to inactivate endogenous peroxidases. After three washes in PBS and incubation for 30 min with Ultra V blocking agent (Microm), slides were incubated with a mouse polyclonal antisera specific for S. flexneri serotype 5a LPS, followed by HRP-conjugated anti-mouse secondary Ab (EnVision system HRP rabbit; DakoCytomation). Peroxidase activity was revealed with 3-amino-9-ethylcarbazole (DakoCytomation). Tissue sections were counterstained with hematoxylin (Thermo Shandon), and aqueous mounting (Merek) was performed. For experiments using [35S-Cys,35S-Met]-SIgAC5, the tissue sections were treated as previously described (10). Briefly, radiolabeled tissue sections were coated with radiosensitive liquid nuclear emulsion and dried sections were then processed for light microscope autoradiography by attaching nuclear emulsion-coated coverslips. For experiments using GFP-primers (Shigella and SIgAC5), the tissue was pre-incubated by incubation for 90 min at 4°C in PBS containing 12% sucrose (Merek), followed by an overnight incubation at 4°C in PBS containing 18% sucrose. For final embedding, ileal loop portions were immersed in optimum cutting temperature reagent (Sakura Finetek). The tissue was then snap-frozen in isopentane (Sigma-Aldrich) cooled in liquid nitrogen. Frozen sections were generated using a cryostat (Leica Microsystems) and used immediately or kept at −20°C in the dark.

Immunolabeling of rabbit DCs

All subsequent steps were performed in the dark. Glass slides carrying the sections were incubated for 10 min at room temperature before washing by immersion in PBS for 10 min. Incubation with anti-rabbit DC mAb (Chemicon International; 1/200 dilution in PBS-5% FCS) was conducted for 1 h at 37°C. After three successive PBS washings, glass slides were incubated with biotinylated rat anti-mouse IgG1 (BD Biosciences; 1/500 dilution in PBS-5% FCS) for 30 min at room temperature. Slides were washed as above with PBS and incubated with Cy5-labeled streptavidin (Amersham Biosciences) (1/2000 dilution in PBS-5% FCS) for 30 min at room temperature. After a series of washes with PBS, the slides were mounted in Vectashield (Vector Laboratories).

Laser scanning confocal microscopy

Confocal microscopy pictures were obtained using a Leica TCS NT microscope (Leica Microsystems). Excitation was obtained with an argon-krypton laser, with lines set at 488, 567, and 647 nm for FITC, Cy3, and Cy5 fluorochromes, respectively. Images were taken with a 20× or 63× objective and processed using the Leica TCS NT software (Leica Microsystems).

Analysis of cytokine expression by quantitative real-time RT-PCR

Total cytoplasmatic RNA was extracted from freshly isolated PP cells using TRIzol (Invitrogen) and RNAeasy spin columns (Qiagen), according to the manufacturer’s instructions. All RNA samples were treated with on-column RNase free DNase I (Qiagen). Total RNA (100 ng) was converted into cDNA with Superscript II reverse transcriptase (Invitrogen). The nucleic acids were stored at −70°C until assayed by real time RT-PCR. Oligonucleotide primers for Cox-2, TNF-α, IL-6, IFN-γ, IL-10, and the housekeeping gene hypoxanthine phosphoribosyltransferase (HPRT) were chosen based on the rabbit (Oryctolagus cuniculus) cDNA sequences available in GenBank (http://www.ncbi.nlm.nih.gov/Entrez/index.html), and designed using the primer3 software (http://frodo.wi.mit.edu/primer3). Primer sequences were checked with the BLAST (basic local alignment search tool) software (http://www.ncbi.nlm.nih.gov/BLAST) to prevent homologies to undesired genes and other coding sequences. Oligonucleotide primers were synthesized by Eurogentec. The primer sequences and expected PCR product length are shown in Table I. For each gene, a standard curve was established using serial dilutions of an external standard, consisting of the sequence of target genes inserted into the PCR XL-TOPO plasmid (Invitrogen). From the calculated copy number of double-stranded plasmid DNA, serial dilutions (1/10) of known input copy numbers (10^7 to 10^10) for each target gene were used to generate the standard curve. All
amplifications of standard mixes were performed in duplicates that were included on each PCR plate to guarantee quantitation of the tested gene.

The real-time RT-PCR mixture contained 5 μl of sample cDNA (1/10 dilution of the reverse transcriptase reaction in DNase/RNase-free water) and 20 μl of a mix consisting of 2.5 μl of forward and reverse primers (300 μM each), 2.5 μl of tRNA (3 ng/μl), and 12.5 μl of the iTaq SYBR Green Supermix (Bio-Rad). Each sample was tested in triplicate for every couple of primers. Reactions were run on an ABI Prism sequence detection system 5700 instrument (Applied Biosystems). PCR conditions were 40 cycles at 95°C for 15 s and 60°C for 1 min. A post-PCR dissociation curve was run to assess the specificity of the amplified sequences. Results were analyzed with the ABI sequence detection system software. The PCR threshold cycle number for each sample was calculated at the point where the fluorescence signal exceeded the threshold limit, fixed at 10 to 20 SDs above the average background fluorescence. From the threshold cycle number and the standard curves, the number of gene copies was calculated. Normalization of the results was performed using primers targeting HPRT expression. Statistical analysis of the data was done using the Wilcoxon signed-ranks test. Differences between groups were considered significant when p values <0.05 were obtained.

Results
Shigella-specific SlgAC5 prevents bacteria-induced destruction of the intestinal barrier

Intraluminal infection of rabbit ligated ileal loops with Shigella drives the induction of a major destructive inflammatory response (30), mimicking that observed in rectal biopsies of Shigella-infected patients upon natural infection (32). This represents an accurate model to examine at the intestinal level the cellular and molecular consequences of bacterial neutralization mediated by SlgAC5, a monoclonal dimeric IgA specific for S. flexneri serotype 5a LPS O-Ag (25). After administration of the bacteria into ileal loops, massive loss of the architecture of PPs was observed as a consequence of the proinflammatory phenotype of Shigella (Fig. 1A). Elsewhere in the intestine, tissue damages were reflected by effacement of the villus structure (Fig. 1B). In contrast, passive administration of agglutinating amounts of SlgAC5, together with a pathology-inducing amount of bacteria, resulted in the maintenance of the integrity of the intestinal barrier at the level of both PPs and villi during the 8-h incubation (Figs. 1, C and D). These results suggest that the protective efficacy of Shigella-specific SlgAC5 in the intestinal environment is due to immune exclusion. Moreover, these data suggest that SlgAC5-bacteria immune complexes do not dissociate in the harsh intestinal environment during the 8-h incubation (shown later in Fig. 3C), thus supporting the adequacy of the model to investigate the nature of other molecular mechanisms of SlgA-mediated mucosal homeostasis operating, in addition to immune exclusion.

![FIGURE 1. SlgAC5 prevents Shigella induced destruction of the intestinal barrier. Histologic examination of intestinal tissue sections obtained from rabbit ileal loops 8 h postadministration with a single dose of either 10⁹ Shigella alone (A and B) or combined to agglutinating amounts of SlgAC5 (C and D). Following paraffin embedding, H&E staining was performed. Exposure to Shigella triggers inflammation accompanied with destruction of the architecture of PP (compare A and C) and of the epithelial barrier with shortening of the villi length (compare B and D). Typical examples of results of histological analysis of more than five samples are shown. L, Lumen. Magnification, ×100 for A, B, and D and ×40 for C.](http://www.jimmunol.org/)

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\( ^{a} \) bp. Base pairs; F, forward, R, reverse.

\( ^{b} \) GenBank accession number of rabbit cDNA. Available online at http://www.ncbi.nlm.nih.gov.

Table I. Primer sequences for rabbit cytokines, Cox-2, IcsA, and HPRT for standard plasmids
FIGURE 2. SIgAC5 preferentially associates with mucus and restricts Shigella in the intestinal lumen. Tissue sections prepared from rabbit ileal loops 8 h postadministration with either Shigella alone, radiolabeled SIgAC5 alone, or a combination of the bacterium and agglutinating amounts of SIgAC5 were analyzed. Treatment with photogenic emulsion shows the luminal, mucus-associated localization of radiolabeled SIgAC5, when administered alone (A. arrows) or combined with Shigella (B. arrows). C. Confirmation of the presence of abundant mucus trapping the Ab is depicted after staining with Alcian blue of the section adjacent to that of the picture presented in A. D. Bacterial staining (in brown) using an anti-S. flexneri 5a LPS polyclonal serum is limited to the epithelial surface in sections of ileal loops administered Shigella-SIgAC5 immune complexes. In contrast, a largely diffuse bacterial labeling revealing massive invasion of the mucosa is observed when the bacterium is delivered alone (E). Examples of typical results of histological analysis of more than five samples are shown. L, Lumen. Magnification, ×100 for all panels.

**Shigella-specific SIgAC5 preferentially associates with mucus in the intestinal lumen and restricts the bacteria in this compartment**

To demonstrate that immune exclusion was indeed taking place, we analyzed the localization of both SIgAC5 and Shigella, alone or in combination, when administered into the rabbit ligated ileal loop. In the mouse respiratory tract, anchoring of SIgA within the mucus via SC was shown to be essential to the function of immune exclusion of the Ab (10). Similarly, we found that radiolabeled SIgAC5 accumulated as clusters in the rabbit intestinal lumen when administered alone (Fig. 2A). Using 5 μm-adjacent histological sections stained with the mucus dye Alcian blue, distribution of radiolabeled SIgAC5 was shown to be limited to areas rich in mucus (Fig. 2C). Consistent with the local absence of mucus, the Ab molecule was barely detectable in the neighborhood of the surface of PPs (data not shown). Immune complexes were also found as clusters (Fig. 2B) and in association to mucus within the lumen (data not shown). In support of the selective localization of SIgA, detection of Shigella with a specific anti-LPS antisera showed a predominant distribution of the bacteria neutralized by SIgAC5 next to the EC surface (Fig. 2D), in contrast to its wide tissue dissemination when administered alone (Fig. 2E). Similar to the lung tissue (10), these results verify that SIgA binding to mucus is a key feature to ensure optimal immune exclusion in the intestine as well.

SIgAC5-Shigella immune complexes are translocated into PPs and colocalized with DCs in the SED region

In mice, SIgA Abs have been shown to enter PPs, then targeting DCs in the SED region (17). In addition, specific SIgA have been reported to promote bacterial translocation into mouse PPs in the form of immune complexes (19). Therefore, we first analyzed whether in the rabbit model highly sensitive to Shigella, SIgA-S. flexneri complexes were also translocated into PPs and whether this impacted on the tissue wellness. As shown in Fig. 3A, moderate bacterial labeling using a specific anti-LPS antisera was observed in the SED region of the PPs exclusively, in comparison with the pronounced accumulation of bacteria-SIgA complexes on the surface of EC (Fig. 2D). Real-time RT-PCR, using expression of the Shigella-specific gene icsA, which encodes the virulence effector responsible for bacterial spreading within tissues (33), was performed to quantify translocated Shigella. Expression of icsA transcripts was found in the PPs of loops administered with the immune complexes (Fig. 3B, open symbols), providing evidence that under these experimental conditions, Shigella was no longer

**Figure 3.** SIgAC5-Shigella immune complexes are translocated into PPs and colocalized with DCs in the SED region. A. PP tissue sections obtained from rabbit ileal loops administered with Shigella combined with SIgAC5 and stained using an anti-S. flexneri 5a LPS polyclonal serum showing brown-labeled bacteria accumulating in the SED region (magnification, ×40 and ×100 for left and right panels, respectively). B. Quantification of Shigella in the PP tissue sections obtained from rabbit ileal loops administered with Shigella alone or combined to SIgAC5 by quantitative RT-PCR using Shigella-specific icsA gene expression. The results were normalized using HPRT gene expression. Symbols correspond to icsA gene expression measured in individual rabbits. *, p value < 0.05. C. Analysis by laser scanning confocal microscopy of PP tissue sections obtained from rabbit ileal loops administered with Shigella combined to SIgAC5. Colocalization of red-labeled Cy3-SIgAC5 and GFP-S. flexneri indicative of the existence of immune complexes within the PP reflects itself in the detection of numerous yellow spots. D. DCs in the SED region detected with Cy5-labeled anti-CD11c were found in association with yellow immune complexes, resulting in the appearance of green-turquoise spots (arrowheads). Typical examples of results of histological and immunofluorescence analysis of more than five samples are shown. L, Lumen; GC, germinal center; RGB, Red Green Blue.
able to damage the neighboring tissues. The level of transcripts was ~300 times lower than that found in the PPs associated with loops administered *Shigella* alone (Fig. 3B, filled symbols), most likely reflecting Ab-mediated blocking of translocation, rather than inhibition of *icsA* gene expression upon SIgA binding.

To further verify whether *Shigella* was associated with SIgAC5 in this compartment, colocalization of red Cy3-SIgA and GFP: *Shigella* was analyzed by confocal microscopy imaging. Stable immune complexes were indeed readily detected in the SED compartment of PPs, as reflected by the appearance of numerous yellow spots (Fig. 3C, arrowheads). After uptake from the intestinal lumen, we observed colocalization of blue Cy5-labeled CD11c⁺ DCs in the SED region corresponding to immune complexes (Fig. 3D). In many instances, turquoise-green spots resulting from the superimposition of yellow SIgAC5-*Shigella* immune complexes and blue Cy5-labeled DCs were observed throughout the SED region. Detectable, although more diffuse, turquoise fluorescence coloring the cytoplasm of a selection of DCs was additionally showing up, suggesting that GFP:SIgA was indeed internalized, leading to release of bacteria-expressed cytoplasmic GFP.

**Binding to SIgAC5 restricts the Shigella-induced inflammatory response in PPs, thus preserving their integrity**

Because no destruction of the PPs was observed (Fig. 1C) despite the presence of *Shigella* in complex with SIgAC5 in the SED region (Fig. 3, A and C), we made the assumption that the proinflammatory pathways usually induced by *Shigella* were quenched when the bacterium was bound to SIgAC5. To test this hypothesis, we measured the expression of a selection of genes encoding pro-inflammatory mediators by cells recovered from PPs exposed to immune complexes. In this compartment, colocalization of red Cy3-SIgA and GFP: *Shigella* was not accompanied by the expected increase of IL-10 known to contribute to intestinal homeostasis (Fig. 4). As a matter of fact, expression of IL-10 remained equivalent in the various experimental settings assayed, and appears thus as a “default” status, which is overcome in inflammatory situations. This result suggests that SIgA Abs exert an anti-inflammatory effect in the PPs, which relies more on a reduction of the proinflammatory responses rather than on the rapid induction of anti-inflammatory pathways.

**Discussion**

In this study, we report that both immune exclusion and SIgA-mediated translocation of *Shigella* into PPs aids the preservation of the intestinal barrier integrity by preventing bacteria-induced inflammation. These data constitute the first demonstration that SIgA specific for *Shigella* LPS are protective in the intestinal environment.

Because SC is crucial for SIgA protective functions (10), we used the optimal and natural molecular form of the Ab, i.e., SIgA, in the rabbit ileal loop model which mimics the intestinal inflammation induced by *Shigella* upon natural infection in humans. However, although 10⁹ bacteria appear to be sufficient in humans to trigger the disease (23), 10⁷ bacteria are required in the rabbit model to induce acute inflammation and the subsequent intestinal damages that peak 8 h postinfection. The amount of *Shigella*-specific SIgA used was therefore dictated by the bacterial inoculum required in this experimental model. Agglutinating amount of SIgA was therefore added to analyze the behavior of *Shigella*-SIgA immune complexes in the intestinal environment. The fact that monomeric IgA is unable to agglutinate bacteria as a consequence of its bivalency as opposed to tetravalency of dimeric IgA (34) and is thus not protective, precludes us from testing this molecular form of the Ab. In addition, previous

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### Table II. Primer sequences for rabbit cytokines, Cox-2, *IcsA*, and *HPRT* genes for real-time PCR

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*bp. Base pairs; F, forward; R, reverse.
studies have shown that protection achieved by SlgAC5 is Ag-dependent (10, 25).

By performing rabbit ileal loops encompassing a PP, one mimics bacterial crossing of the epithelial barrier via colonic isolated lymphoid follicles, the sampling target site for Shigella upon natural infection in humans (23). As previously reported (10), in the respiratory tract, mucus-mediated anchoring of SlgA to the intestinal epithelium was observed, which emphasized that this mechanism is most likely a general feature of this class of Ab to ensure immune exclusion. Complex and heterologous carbohydrates distributed all over SC (35) might explain the capacity to bind with mucus covering various mucosal tissues. In addition, we confirmed that SlgA is highly resistant to degradation in the harsh intestinal environment (5, 6), as immune complexes are still detectable after 8 h of infection.

Close examination of intestinal tissue sections revealed that even though predominantly present within the intestinal lumen, SlgA/Shigella immune complexes also localized in the SED region of PPs. Apical to basolateral translocation of SlgA across PPs has been demonstrated in the rabbit, human, and mouse intestine (16, 17, 36). However, the capacity of the Ab to transport microorganisms has been reported in mice only (19). A further step to the generalization of the concept, the present work extends the demonstration of this phenomenon to the rabbit. Thus, although the receptor(s) for SlgA on mouse M cells and DCs is (are) in waiting for identification, the biochemical mechanisms underlying adequate targeting of DCs by mucosal SlgA appear to overlap from one species to another. In support of this, we found that SlgA made of human SC and murine plgA are similarly transported and targeted to DCs in mice (19) and rabbit (the present work), either as free Ab or bound to bacteria. Colocalization of SlgA-Shigella immune complexes with DCs marks these cells as the primary APCs in the SED region capable of handling the Ag-Ab immune complexes. In addition, to provide a rationale for the feature of SlgA to selectively target DCs at this site (17), this can participate to limit Ag processing (37), and therefore to activate target T cells, with the ultimate consequence being to bring the mucosal immune response back to homeostatic levels.

Interestingly, we observed that the translocation of SlgAC5-Shigella immune complexes did not induce PP destruction. Preservation of PP integrity can be explained by the limited up-regulation of the expression of proinflammatory cytokines in the cells isolated from PPs of ileal loops administered SlgAC5-Shigella as compared with Shigella alone. We speculate that two plausible mechanisms underlying this protective effect are likely to occur. On one hand, reducing the inflammatory response in PP might be the consequence of SlgA/Shigella immune complexes targeting tolerogenic CD11c+CD11b+ DCs (19), with the subsequent modulation of the host immune response toward anti-inflammatory pathways as previously reported (4, 18, 38). The lack of appropriate anti-rabbit CD11b mAb prevented us from experimentally confirming this hypothesis. The observation that the level of homeostatic IL-10 is slightly affected during inflammation suggests that the default tolerogenic status of the epithelium dominates as long as pathogenic stimuli do not instruct the local immune system to activate proinflammatory circuits. On the other hand, anti-LPS SlgA bound to Shigella might act by blocking pathways of the bacterial virulence program. Indeed, Shigella uses a type III secretion system (TTSS) to inject virulence effectors into human cells, leading to bacterial invasion and a vigorous inflammatory response. Previous results have shown that the TTSS efficiency depends on the length of the LPS chains at the bacterial surface. Strains with truncated LPS are highly proficient at invading cells in vitro, possibly through enhanced access of the TTSS to host cells, whereas the extended LPS isoform impairs TTSS function, which leads to reduced virulence within the gastrointestinal tract (39). Thus, by binding to LPS chains at the bacterial surface and promoting bacterial agglutination, anti-LPS SlgA is likely to impair the accessibility of the TTSS to host cells.

The current study increases our understanding of the mechanisms mediated by anti-LPS SlgA in protection against Shigella infection. We now know that, in addition to immune exclusion (Ref. 10 and the current work) and intracellular neutralization of Shigella LPS by IgAC5 during plgR-mediated transport of the Ab molecule in EC (15), translocation of a small proportion of SlgA-based immune complexes into PP contributes to downregulate proinflammatory responses. This further emphasizes the need to develop adequate strategies to prime mucosal immune responses, including SlgA-mediated Ab response, upon vaccination against enteric pathogens, as it appears from our data that the Ab is involved at different stages in the control of the mucosal homeostasis.

Disclosures
The authors have no financial conflict of interest.

References


