Secretory IgA Mediates Bacterial Translocation to Dendritic Cells in Mouse Peyer's Patches with Restriction to Mucosal Compartment

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Secretory IgA Mediates Bacterial Translocation to Dendritic Cells in Mouse Peyer’s Patches with Restriction to MucosalCompartment

Khalil A. Kadaoui and Blaise Corthésy

In addition to fulfilling its function of immune exclusion at mucosal surfaces, secretory IgA (SIgA) Ab exhibits the striking feature to adhere selectively to M cells in the mouse and human intestinal Peyer’s patches (PPs). Subsequent uptake drives the SIgA Ab to dendritic cells (DCs), which become partially activated. Using freshly isolated mouse DCs, we found that the interaction with SIgA was tissue and DC subtype dependent. Only DCs isolated from PPs and mesenteric lymph nodes interacted with the Ab. CD11c+CD11b+ DCs internalized SIgA, while CD11c+CD19− DCs only bound SIgA on their surface, and no interaction occurred with CD11c−CD8α+ DCs. We next examined whether SIgA could deliver a sizeable cargo to PP DCs in vivo by administering SIgA-Shigella flexneri immune complexes into a mouse ligated intestinal loop containing a PP. We found that such immune complexes entered the PPs and were internalized by subepithelial dome PP DCs, in contrast to S. flexneri alone that did not penetrate the intestinal epithelium in mice. Dissemination of intraepithelial S. flexneri delivered as immune complexes was limited to PPs and mesenteric lymph nodes. We propose that preexisting SIgA Abs associated with microbes contribute to mucosal defense by eliciting responses that prevent overreaction while maintaining productive immunity. The Journal of Immunology, 2007, 179: 7751–7757.

Mucosal surfaces are endowed with powerful defense mechanisms, which selectively handle harmful or innocuous Ags to ensure local homeostasis. Constitutive physiochemical and mechanical features (mucus, glycoalyx, lactoferrin, defensins, peristalsis) are backed up by a highly adaptive immune system, whose controlled action contributes to shape proper adaptive immunity (1, 2). An important activity of mucosal epithelia is the production of the special type of Abs referred to as secretory IgA (SIgA). SIgA is produced predominantly as a dimer containing the J chain and is complexed with a secretory component (SC) from epithelial origin. Bound SC is the cleared extracellular portion of the polymeric Ig receptor, that transports dimers and larger polymers of IgA (collectively called pIgA) into mucosal secretions (4). The classical view is that SIgA reinforces the first line of defense against microorganisms by agglutinating potential invaders and facilitating their clearance by peristaltic and mucociliary movements, a mechanism called immune exclusion (5).

In the intestine, Peyer’s patches (PPs) and associated M cells represent the primary site for uptake and presentation of ingested Ags (6). In rabbit, exogenously administered SIgA has been observed in association with the apical surface and inside the intraepithelial pocket of M cells (7). SIgA injected into a mouse ligated ileal loop bound selectively to M cells, whereas IgG or IgM did not (8). Uptake of SIgA by PPs targeted this class of Ab to dendritic cells (DCs) in the subepithelial dome (SED) region and T cells in the interfollicular regions (IFRs), and this had raised the question of the immunological consequences of such an interaction in modulating ongoing mucosal immune responses and/or control of local homeostasis (9). In the mouse, oral delivery of exogenous SIgA comprising human SC and mouse IgA induced hSC-specific Ab and cellular responses in mucosal and peripheral tissues (10). This occurred in the absence of the prototype mucosal adjuvant cholera toxin. Specific immune responses were accompanied by sustained IL-10 and TGF-β expression in draining mesenteric lymph nodes (MLNs) and spleen. Because DCs in the SED region are anatomically positioned to sample Ags from the intestinal lumen and contribute to the regulation of local immune responses (11), these observations provided a link between DC targeting and possible immune regulation by SIgA. This was further consistent with the phenotypic analysis of DCs within tissues of the gastrointestinal tract that had identified a plethora of distinct subtypes with different spatial distribution and effector functions (12). In the present study, we show specific interaction between SIgA and PP and MLN DCs, as compared with DCs recovered from peripheral lymph nodes and spleen. We demonstrate the capacity of SIgA to transport Ags across the intestinal epithelium comprising a PP using as a cargo Shigella flexneri not capable of spontaneously entering the mouse epithelium. The bacterium is rapidly recovered within PPs and MLNs, with no further spreading, and this takes place with fully preserved intestinal tissue. Our results suggest that selective microbe delivery mediated by SIgA at the level of PPs may serve to trigger mucosal immune responses under neutralizing, noninflammatory conditions.
Materials and Methods

Protein production, purification, and characterization

Polymeric IgA Ab from hybridoma clone IgAC5 specific for S. flexneri serotype 5a LPS (13) was obtained as previously described (14). Purified free hSC was prepared from Chinese hamster ovary cells (15). SIgA molecules were obtained by combining in PBS pIgA molecules with a 2-fold excess of hSC for 2 h at room temperature according to the conditions described in the study of Rindisbacher et al. (16). Hamster anti-mouse CD11c IgG was produced from N41B hybridoma cells (17) and purified by affinity chromatography using protein G-Sepharose beads (Amersham Biosciences). ELISAs for quantification of IgA and SC was performed as described in the study of Rindisbacher et al. (16, 18). The biotinichinonic acid assay protein assay kit (Pierce) was used for protein measurement. Cy3-IgA and Cy3-SIgA molecules were obtained by conjugation to indocarbocyanine (Cy3) using the FluoroLink mAb Cy3 labeling kit (Amer- sham Biosciences) according to the procedure provided by the manufactur er. The same protocol was used for the coupling of the Cy5 fluorochrome (Amersham Biosciences) to purify hamster anti-mouse CD11c IgG Ab.

Mice

Four-week-old female BALB/c mice were obtained from Harlan Breeders and used at the age of 7–10 wk. They were housed in the animal facility of the Centre Hospitalier Universitaire Vaudois under standard conditions. All experiments were approved by the State Veterinary Office.

Purification of DCs from mouse tissues

Mice were killed by euthanasia, the abdomen was incised, and the small intestine was removed and thoroughly rinsed with cold PBS. Macroscopically visible PPs were excised using curved surgery scissors and kept in cold, plain MEM-α for no longer than 30 min. PPs were digested with prewarmed (37°C) collagenase IV (0.5 mg/ml in plain MEM-α; Sigma- Aldrich) for 15 min at room temperature under gentle shaking. After en zymatic treatment, PPs were crushed and forced with a 5-ml syringe pestle through a 70-μm pore nylon mesh cell strainer (BD Biosciences). After centrifugation for 5 min at 400 g at 4°C, cells were then passed through a 40-μm pore nylon mesh cell strainer and resuspended in cold buffer A (PBS [pH 7.3], 0.5% FCS, and 5 mM EDTA) at a concentration of 5 × 106 cells/ml. Total cells were recovered from mouse spleen, MLNs, bron chial lymph nodes (BLNs), and inguinal lymph nodes (ILNs) using the same procedure as for PPs, with the exception that no collagenase was added. Freshly isolated cells (10–25 × 106 cells) were positively selected by two successive magnetic separations (MACS) using anti-CD14 magnetic beads (Miltenyi Biotec).

Flow cytometry analysis of DCs

Whole DC labeling. Freshly isolated DCs (5 × 100–20,000 cells) were resuspended in 200 μl of cold buffer B (PBS [pH 7.3], 0.5% BSA, and 5 mM EDTA) and incubated with FITC-conjugated anti-CD11c mAb (clone HL3, hamster IgG, 1/100 dilution in buffer B; BD Biosciences) for 25 min on ice. FcγRI/IIIRs were blocked for 15 min at 4°C using rat anti-mouse CD16/CD32 mAbs (clone 2.4G2, 1/100 dilution; BD Biosciences). After washing with buffer B, flow cytometry analysis (FACScan flow cytometer, BD Biosciences) was conducted after exclusion of propidium iodide–positive dead cells, and live cells were used to determine the background of fluorescence. Data were processed using the CellQuest software (BD Biosciences).

DC subtype-specific labeling. To evaluate the percentage of myeloid and lymphoid DC subtypes or possible CD19+ B cell contaminants after purification, DCs were labeled for 25 min on ice with a combination of either FITC-conjugated anti-CD11c mAb and PE-conjugated anti-CD11b mAb (clone M1/70, rat IgG2b, 1/200 dilution; BD Biosciences), PE-conjugated anti-CD10 mAb (clone 53-6.7, rat IgG2a, 1/200 dilution; BD Biosciences), or PE-conjugated anti-CD19 mAb (clone I3D, rat IgG2a, 1/200 dilution; BD Biosciences), respectively. All Abs were diluted in buffer B. Propidium iodide treatment excluded dead cells from the analysis. Data were processed using the CellQuest software.

Association of SIgA with DCs isolated from PPs

Freshly isolated DCs (15,000 cells) were resuspended in 50 μl of buffer B and incubated with 100 ng or 1 μg of Cy3:SIgA (80–800 ng of Cy3:SIgA/mAb concentration) at room temperature for 5 min before incubation with the Cy3:SIgA. Similarly, dilutions of anti-FcγRIIa receptor (1/50) (19) or anti-transferrin receptor (1/50) (20) mAb capable of labeling cells positive for these two receptors were added 5 min before incubation with the Cy3:SIgA. Cells were then incubated for 25 min at room temperature with a 1/50 dilution of Cy5-conjugated anti-CD11c mAb. DC subtypes associating with Cy3:SIgA were determined as indicated above, with the exception that 1/50 dilutions of each FITC-labeled mAb was used in combination for 25 min at 4°C. Cells were spread onto glass slides, fixed in PBS/2% paraformaldehyde, and mounted with Vectashield (Vector Laboratories) for ob servation by laser scanning confocal (LSC) microscopy.

LSC microscopy

LSC 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, 568, and 647 nm for FITC, Cy3, and Cy5 fluoro rochromes, respectively. Images were taken with a ×20 or ×63 objective and processed using the Leica TCS NT software (Leica Microsystems).

S. flexneri culture conditions

M9OT, an invasive isolate from S. flexneri serotype 5a LPS (21, 22) expressing GFP, was a gift from Dr. A. Phalipon (Pasteur Institute, Paris, France). Bacteria were grown overnight at 37°C on BBL-agar plates (3% BBL-trypticase soy broth, 1.5% Bacto-agar (BD Biosciences), 0.1% Congo Red, and 50 μg/ml ampicillin (Sigma-Aldrich)). Three colonies were picked and cultured in Luria-Bertani (LB) medium (1% Bacto-trypticase, 0.5% Bacto-yeast extracts (Sigma-Aldrich), 1% NaCl, and 50 μg/ml ampicillin) for 45 min at 37°C before being spread onto LB agar plates (1.5% Bacto-agar in LB medium). Bacteria grown overnight as a lawn were recovered in 0.9% NaCl, and their concentration was calculated using formula: 1 OD600 corresponds to 5 × 108 bacteria/ml (15).

Association of immune complexes with freshly isolated PP DCs

Immune complexes were formed by incubating 109 GFP-S. flexneri with 2.5 pmol of either Cy3:SIgAC5 or Cy3:IGG20 in PBS for 25 min on ice, corresponding to ~2000 Ab molecules per bacterium. Freshly isolated DCs were resuspended in buffer B and incubated for 30 min at 4°C with 105 SIgA-bacterium immune complexes (DC:bacterium ratio of 1:20). In control experiments, DCs were incubated with 103 Fluoresbrite Yellow microspheres (diameter 0.2 μm; Polysciences) per cell.

Administration into mouse ligated intestinal loops

Surgery and injection of 108 GFP-S. flexneri or immune complexes in 100 μl of PBS were performed as previously described (9).

Preparation of tissue sections and immunolabeling

Intestinal portions containing one PP were fixed in PBS/4% paraformaldehyde for 2 h at 4°C, with subsequent embedding in PBS/12% sucrose for 90 min at 4°C, followed by overnight incubation in PBS/18% sucrose at 4°C. Intestine portions were flushed into the lumen with OCT (Sakura Finetek), followed by complete immersion. Sections cut at a 7-μm thickness were obtained, and blocking was conducted in PBS containing 5% mouse serum and 2% FCS. DCs were labeled using biotinylated anti- CD11c mAb (1/30 dilution) in PBS/2% FCS and Cy3-streptavidin or Cy5 streptavidin (1/1000 dilution) for 30 min each at room temperature and finally mounted with Vectashield (Vector Laboratories).

Staining with hematoxylin-erythrosin B

Slides were washed for 5 min in H2O and then stained in hematoxylin (Sigma-Aldrich) for 45 s. After a rapid washing in H2O, slides were soaked in 70% ethanol/0.1 M HCl for 5 min. Slides were extensively washed in H2O, then colored in 0.25% erythrosin B (Merck) for 10 s. Dehydration steps were performed by washing slides successively in 70% ethanol, 95% ethanol, 100% ethanol, and xylol during 5 s for each step. Slides were then colored in 0.25% hematoxylin-erythrosin B (Sigma-Aldrich) and observed by light microscopy (Leica Microsystems).

Delivery of immune complexes by the oral route

GFP-S. flexneri (109) alone or immune complexes were resuspended in 150 μl of PBS and administered intragastrically using a metal feeding tube (Harvard Apparatus) to starved mice. Sixteen hours after oral gavage, intestinal PPs, MLNs, spleen, and liver were aseptically removed, crooked, and used at the age of 8–10 wk. They were housed in the animal facility of the Centre Hospitalier Universitaire Vaudois under standard conditions. All experiments were approved by the State Veterinary Office.
following staining with trypan blue. Culturable bacterial counts were determined by plating dilutions of cell suspension on agar plates containing 50 µg/ml ampicillin.

Results

Selective binding of SIgA to freshly isolated PP DCs

Tracking of exogenously delivered SIgA in the intestinal lumen has previously shown selective binding to M cells and subsequent uptake by underlying DCs located in the SED region (8, 9). We first sought to characterize the DC features responsible for SIgA capture using CD11c++ DCs freshly isolated from mouse PPs. Upon incubation with Cy3-labeled pIgA or SIgA, we confirmed that DCs in vitro display the same pattern of association and internalization as DCs in the tissue, as assessed by LSC microscopy (Fig. 1, A and B). Uptake and/or surface binding took place over a large range of pIgA (80–800 ng) or SIgA (100 ng to 1 µg) amounts tested and was completely prevented upon incubation at 4°C (our unpublished observations). Specificity of DC-IgA association was further established in competition experiments by preincubation with 5-fold molar excess of unlabeled pIgA (Fig. 1C). In contrast, neither free SC (Fig. 1C) nor IgG specific for FcγRII and III receptors (our unpublished observations) used in equivalent molar excess competed for SIgA binding, arguing for the presence of a receptor selective for the IgA moiety of SIgA on DCs. Furthermore, no blocking of SIgA binding and internalization was observed by competition with IgM or after addition of mAb specific for either the FcαRI or the transferrin receptor (Fig. 1D). The FcαRI not existing in mice and not involved in human DC binding of IgA (23) can similarly be excluded.

Subtype and tissue specificity of DCs associating with SIgA

Closer examination of LSC microscopy photographs led us to observe three main patterns of association between PP DCs and Cy3-labeled SIgA or pIgA: 1) DCs that internalize SIgA, 2) DCs that do not interact with SIgA, and 3) DCs that display binding of SIgA limited to their surface. In the mouse, PPs contain three predominant populations of DCs expressing the CD11c surface integrin (17): the myeloid DC subtype coexpressing the CD11b surface marker (CD11c+CD11b+), the lymphoid DC subtype coexpressing the α-chain of the CD8 receptor (CD11c+CD8α+), and the double-negative subtype (CD11c+CD11b−CD8α−) (24). We speculated that the SIgA-binding properties of DCs are explained by the nature of the three DC subtypes previously identified in PPs. BALB/c mice used in the study yielded 38 ± 10.6% of CD11c+CD11b+ DCs, 36% ± 16.6% of CD11c+CD8α+ DCs, and 45 ± 26.5% of CD11c−CD11b−CD8α− DCs, consistent with previous reports (25, 26). We found that myeloid DCs bind and internalize SIgA, whereas lymphoid DCs did not interact with the SIgA (Fig. 2, A and B). In control experiments aimed at excluding the presence of copurified B cells, we identified the existence of a DC subtype expressing the CD19 marker that is capable of binding SIgA (23) can similarly be excluded.

In addition to subtype specificity, we found that the tissue origin of DCs represents another essential variable that contributes to the specificity of the association with SIgA/pIgA. As for PPs, DCs isolated from MLNs interacted with both SIgA and pIgA, whereas BLNs, ILNs, and spleen DCs did not bind pIgA or SIgA under identical experimental conditions (Fig. 3). The results strongly suggest that the interaction with SIgA is limited to DCs from lymphoid tissues associated with the gut.

FIGURE 1. Internalization of pIgA/SIgA by freshly isolated PP DCs. PP DCs were incubated for 25 min with increasing amounts of pIgA (A) or SIgA (B) at room temperature. Intracellular localization of equimolar amounts of Cy3-labeled pIgA and SIgA was determined by LSC microscopy. C, Inhibition of Cy3:SIgA internalization by a 5-fold molar excess of pIgA or free hSC was analyzed by LSC microscopy. Incubation in the absence of competitor is shown for comparison. D, Internalization is not affected by competing IgM or blocking mAbs against FcμR or transferrin receptors. A representative image of at least 50 observed fields is depicted. Data are representative of three to four separate experiments.

FIGURE 2. Differential association between SIgA/pIgA with freshly isolated PP DC subtypes. Following preincubation with SIgA (upper panel) or pIgA (lower panel), PP DCs were double labeled with Cy5-conjugated anti-CD11c mAb and, respectively: A, FITC-labeled anti-CD11b mAb showing that the myeloid subtype binds and internalizes SIgA/pIgA; B, FITC-labeled CD8α mAb indicating no interaction with the lymphoid subtype; and C, FITC-labeled anti-CD19 mAb mapping surface binding of the novel CD11c+CD19+ subtype. Cells were prepared and analyzed by LSC microscopy as in Fig. 1. A representative image of at least 50 observed fields is depicted.
confirmed by the appearance of yellow spots, was observed on the cell microscopy (Fig. 4).

Reflecting formation of immune complexes was assessed by LSC microscopy. FITC-conjugated anti-CD11c mAb. A representative image of at least 50 observed fields is depicted.

**FIGURE 4.** SlgA-S. flexneri immune complexes and association with freshly isolated DCs. A, Formation of immune complexes by coating of GFP-S. flexneri with Cy3:SlgAC5 or with Cy3:IgGC20 as observed by LSC microscopy. Surface binding of yellow SlgA-S. flexneri immune complexes, along with internalization of free SlgA (B), as well as microspheres (C) by PP DCs. The identity of DCs was confirmed by reactivity toward FITC-conjugated anti-CD11c mAb. A representative image of 20 observed fields is depicted.

**Uptake of S. flexneri-SlgA immune complexes by freshly isolated PP DCs**

Having established that DCs isolated from PPs specifically bind SlgA/pIgA, we next investigated whether immune complexes consisting of S. flexneri and SlgA in our experimental setting are similarly taken up. Two different types of immune complexes were obtained by incubating S. flexneri-expressing GFP (GFP-S. flexneri) with Cy3:SlgAC5 or Cy3:IgGC20 (both specific for S. flexneri serotype 5a LPS (13, 27)). SlgA Ab coating of GFP-S. flexneri reflecting formation of immune complexes was assessed by LSC microscopy (Fig. 4A). The presence of immune complexes, as confirmed by the appearance of yellow spots, was observed on the cell surface only (Fig. 4B).

Surface binding was identically detected using 0.2-μm latex beads (Fig. 4C), yet they also localized within the cytoplasm of DCs in the SED region in vivo (10). This supports the notion that although PP DCs maintain their capability to internalize SlgA/pIgA in vitro, their phagocytic properties are lost for larger Ags after isolation from the tissue. These unexpected practical limitations prompted us to examine the outcome of immune complexes in vivo.

**SlgA Ab delivers S. flexneri to the SED region of PPs**

In contrast to rabbit and monkeys, intestinal Shigella infection in mice does not develop after oral administration. The mouse model is therefore perfectly suited to examine whether SlgA can mediate the entry of the bacterium in the form of immune complexes. We thus administered the immune complexes formed by GFP-S. flexneri and Cy3:SlgAC5 in a ligated intestinal loop containing a PP. Incubation in mice was allowed to proceed for 45 min. Multiple intense green spots indicative of the translocation of the bacteria were seen in the SED region of PPs and the IFRs when frozen sections were analyzed by LSC microscopy. (Fig. 5A, white arrowheads). In support of the concept that S. flexneri alone does not cross spontaneously the mouse epithelium, we could not detect any bacterium-associated fluorescence in PPs and neighboring epithelium (Fig. 5B, white arrowhead).

Administration of immune complexes formed by GFP-S. flexneri and Cy3-labeled IgGC20 resulted in the same absence of bacterial translocation across the epithelium (Fig. 5C). Consistent with the observation that IgG does not bind to M cells (8), this indicated moreover selective Ag delivery properties for the SlgA Ab.

This represents the first demonstration that SlgA Ab can deliver a sizeable cargo, i.e., whole bacteria, into PPs in vivo. In accordance with the migratory properties of DCs originating from the PPs (28), GFP-labeled bacteria were recovered in the paracortical, T cell-rich region of draining MLNs 4 h after administration in the ligated intestinal loop (Fig. 5D, white arrowheads). Although not a direct demonstration that immune complexes are brought along by DCs, this was in keeping with the in vitro data showing that SlgA binds to DCs isolated from MLNs.

We then sought to determine whether this holds true when SlgA Ab is bound in immune complexes with S. flexneri. After uptake from the intestinal lumen, we observed colocalization of blue CD11c+ DCs in the SED region with yellow spots indicative of preserved immune complexes (Fig. 5E). In many instances, white spots resulting from the superimposition of red SlgA, green bacteria, and blue DCs were observed throughout the SED region. Sustained, although more diffuse, turquoise fluorescence coloring the cytoplasm of DCs was additionally detected, indicating that GFP-S. flexneri was indeed internalized, possibly along degradative pathways. Whether transepithelial transport of S. flexneri through the concerted action of SlgA and M cells affects the integrity of the mucosa was next analyzed.

**Entry of S. flexneri-SlgA immune complexes into PPs does not result from tissue lesion and prevents systemic dissemination**

In the rabbit ligated intestinal loop shigellosis infection model, massive leukocyte recruitment caused tissue damage within 4 h (29). In contrast, mice did not develop shigellosis and associated intestinal lesions because no entry of S. flexneri occurred. However, to rule out that artificial passage across the epithelium takes place because of physical damages induced by surgery at the level of the intestinal loops, S. flexneri-SlgA immune complexes were administered by intragastric gavage, and both PPs and adjacent intestinal villus samples were prepared time-wise. Staining of frozen sections at 24 h with H&E showed no histopathological lesion...
FIGURE 5. Uptake of SIgA-S. flexneri immune complexes by PP DCs in vivo. A. Immune complexes made of GFP-S. flexneri and Cy3:SIgAC5 are observed in the SED region of PPs after incubation for 45 min in a ligated intestinal loop. No entry of GFP-S. flexneri alone (B) or GFP-S. flexneri-Cy3:IgGC20 immune complexes (C) occurred. Yellow lines indicate the interface between the intestinal lumen and tissue. D. GFP-S. flexneri are found in association with deep paracortical regions of the MLNs. E. Colocalization in PP sections of GFP-S. flexneri-Cy3:SIgAC5 immune complexes and Cy5-conjugated anti-CD11c mAb indicates targeting to DCs in the SED region. V, Villus. F, H&E coloration of a section of a PP and adjacent villi 24 h after delivery of 10⁶ immune complexes by intragastric gavage. G. Assessment of the penetration of GFP-S. flexneri-SIgAC5 immune complexes into PPs, MLNs, spleen, and liver. Counts of live bacteria are indicated per 10⁶ cells of tissue homogenate. Data are expressed as means ± SD (n = 3).

Discussion

The main function of the SIgA Ab is to avoid the attachment of bacterial Ag to mucus surfaces, a mechanism referred to as immune exclusion. We have previously shown that after selective interaction with M cells, SIgA is targeted to DCs located in the SED region, resulting in limited mucosal and systemic immune responses against a non-self-associated protein Ag. In this work, we have first established that SIgA could direct a bacterial cargo to the SED region and then characterized the subtype of DCs involved in the sampling of the SIgA-Ag immune complex. Finally, we have demonstrated in the physiological context that SIgA restricts Ag mucosal dissemination to PPs and MLNs under noninflammatory conditions.

To examine whether SIgA Ab can deliver an associated bacterial Ag to PPs, the “natural” entry of such an Ag has to be avoided. S. flexneri represents an ideal candidate Ag because it does not infect the mouse by the intestinal route (30). SIgAC5-mediated translocation of S. flexneri represents thus an appropriate mouse model that further benefits from suitable controls, including IgGC20 mAb with the same anti-LPS specificity. The observation that DCs in the SED region bind and internalize SIgAC5-based immune complexes suggests that SIgA can serve as a delivery vehicle for large Ag in the physiological context. However, in face of the large excess of SIgA in intestinal secretions, the passage of immune complexes remains limited. Preferential uptake of SIgA-Ag complexes compared with free SIgA might be due to a conformational change(s) that unmasks the binding site to the M cell receptor. For abundant commensal bacteria similarly coated with SIgA (31), formation of large complexes involving Ab of different reactivities (32) and the interaction with the endogenous protein Fv (33) may dampen binding to M cells and permit detectable entry of Shigella-SIgA immune complexes.

It is conceivable that early in life, immune complexes comprising maternal SIgA might contribute to educate the intestinal immune system of the breast-fed baby toward a tolerogenic or protective type of response, should the Ag be innocuous or harmful. In the case of pathogens, passive transfer of SIgA-based immune complexes could provide a “shield” during a period when the neonatal immune system is not mature enough to respond actively (34). In the context of mucosal vaccination, passive administration of SIgA-based immune complexes might deserve further evaluation, since this has the potential to direct small amounts of Ag to sampling and processing sites such as PPs in the absence of aggressive mucosal adjuvant. The stability of the SIgA molecule along with its delivery properties should favor the establishment of oral immunity through the contribution of effector
and/or regulatory pathways characteristic of the intestinal mucosal compartment.

In contrast to the unique DC population derived in vitro from mouse bone marrow cells, DCs in PPs consist of at least three major subtypes (35). By choosing to work with freshly isolated PP DCs that most likely resemble DCs in situ, we found selective association of SIgA as a function of the subtypes under analysis. Internalization was observed for myeloid CD11b⁺ DCs, while surface binding was detected for the novel CD19⁺ DCs. DCs isolated from MLNs showed SIgA internalization, whereas DCs from other tissues including BLNs, ILNs, and spleen did not interact with SIgA, indicating that the tissue origin represents an essential feature to be considered for analysis of DC function. Myeloid DCs isolated from murine PPs produced high levels of IL-10 and induced naive T cells to differentiate into cells that produced much higher levels of IL-10 than T cells primed with any other DC subset (36). PP DC-mediated induction of IL-10-producing CD4⁺ T cells (37, 38), and expression of TGF-β in the lung and gastrointestinal mucosa, play a particularly important role in maintaining local tolerance and homeostasis (37, 39, 40). Similarly, oral delivery of exogenous recombinant SIgA and SIgA-based immune complexes contribute to orchestrate noninflammatory responses (this study) by triggering secretion of IL-10 and TGF-β by mucosal CD4⁺ T cells (10). Because these cytokines are also involved in IgA class switch, the presence of natural (32) or preexisting SIgA after a recall challenge might permit to keep inducing mucosal Ab production against a variety of microbial Ags, while minimizing any proinflammatory, deleterious effects on the integrity of the mucosal barrier.

The current study demonstrates that immune complexes formed by SIgA and S. flexneri were found in association with DCs in the SED region of PPs. The fact that either Ag-bound or free SIgA are captured by DCs suggests that a specific receptor for this Ab iso-type exists on these cells. We checked that established receptors for IgA appear not to be involved in SIgA binding and uptake. The possible capture of SIgA by DC-specific DC receptors such as Langerin and DC-SIGN and C-type lectin R1 (41) needs further investigation using DCs isolated from PPs. Although the nature of the receptor remains to be elucidated, binding of SIgA does not lead to activation of DCs as reflected by the weak modulation of surface costimulatory molecules CD80/CD86 (10). Limited responsiveness of DCs to the SIgA-Ag immune complexes can possibly be due to the high degree of glycosylation of SIgA that would result in poor processing by DCs (42). Along the same line, SIgA can “seeker” the associated Ag, which leads to insufficient levels of immunostimulatory Ag to efficiently prime naive T cells (43). Differential uptake, routing, and processing of the Ag (44–46) by myeloid CD11c⁺CD11b⁺ DCs that are potent inducers of IL-10-secreting T cells (47) and IgA production from naive B cells (48) might as well contribute to modulate local responses. Because DCs constitutively traffic from the intestinal epithelium and PPs to the MLNs (28, 49), this represents a mechanism whereby soluble immune complexes can be taken to the MLNs to promote T cell responses (50).

Our experiments show that the function of SIgA is not only immune exclusion. Although active in facilitating sampling of luminal bacteria, SIgA restricts penetration at the level of PPs and MLNs, as this has been observed for nonpathogenic commensals (51, 52). In association with SIgA, steady-state Ag acquisition from the luminal content by underlying DCs may favor tolerance induction under homeostatic conditions or induce defense mechanisms under reduced inflammatory conditions in response to mucosal pathogens. From a clinical point of view, the absence of IgA is associated with the development of allergy, autoimmune diseases, inflammatory bowel diseases, and recurrent infections (53–55). In individuals with IgA deficiency, SIgM could compensate for SIgA in terms of immune exclusion, particularly in the gut (2). However, SIgM cannot deliver Ags to PPs because it cannot bind to M cells (8). Should the mother’s SIgA have the capacity to deliver novel Ags such as those associated with foods and the commensal microbiota without damageable consequences, it would be relevant to evaluate the incidence of allergies or autoimmune disorders in infants born from IgA-deficient parents.

The Fc portion of IgA Abs does not confer to the molecule proinflammatory properties or effector functions on target cells (56–58), as this is known for other isotype Abs including IgG, IgM, and IgE. Unique transport features associated with SIgA might permit one to extend this notion to the mucosal compartment and underscores the multitask role of SIgA in protecting against foreign substances and microbes, in regulating the commensal microbiota, while at the same time not subjecting the mucosa to undue inflammation.

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**Disclosures**

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