The Role of Secretory Immunoglobulin A in the Natural Sensing of Commensal Bacteria by Mouse Peyer’s Patch Dendritic Cells*

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Background: Intestinal commensal bacteria are not ignored by the mucosal immune system, yet the mechanisms ensuring homeostatic communication are poorly defined.

Results: Coating of commensals by SlgA mediates their targeting to Peyer’s patch dendritic cells.

Conclusion: SlgA is important for the natural dynamic host-microbiota dialogue.

Significance: Beyond pathogens, immune surveillance function of SlgA applies to the control of commensal bacteria.

The mammalian gastrointestinal (GI) tract harbors a diverse population of commensal species collectively known as the microbiota, which interact continuously with the host. From very early in life, secretory IgA (SlgA) is found in association with intestinal bacteria. It is considered that this helps to ensure self-limiting growth of the microbiota and hence participates in symbiosis. However, the importance of this association in contributing to the mechanisms ensuring natural host-microorganism communication is in need of further investigation. In the present work, we examined the possible role of SlgA in the transport of commensal bacteria across the GI epithelium. Using an intestinal loop mouse model and fluorescently labeled bacteria, we found that entry of commensal bacteria in Peyer’s patches (PP) via the M cell pathway was mediated by their association with SlgA. Preassociation of bacteria with nonspecific SlgA increased their dynamics of entry and restored the reduced transport observed in germ-free mice known to have a marked reduction in intestinal SlgA production. Selective SlgA-mediated targeting of bacteria is restricted to the tolerogenic CD11c+CD11b+CD8α+ dendritic cell subset located in the subepithelial dome region of PPs, confirming that the host is not ignorant of its resident commensals. In conclusion, our work supports the concept that SlgA-mediated monitoring of commensal bacteria targeting dendritic cells in the subepithelial dome region of PPs represents a mechanism whereby the host mucosal immune system controls the continuous dialogue between the host and commensal bacteria.

A particular feature of the gastrointestinal (GI) tract is the presence of a very diverse and dense microbiota comprising as many as 10^{14} bacterial cells, outnumbering the amount of cells composing the human body by a factor of up to 10 (1). In addition to peacefully co-existing with the host, an equilibrium referred to as commensalism, bacteria residing in the gut exhibit numerous protective and metabolic features essential to the function of the epithelial barrier lining mucosal surfaces (2). In contrast to enteropathogens turning on multiple proinflammatory circuits that result in their elimination, commensal bacteria populating the GI tract are not overly inflammatory, ensuring graded or dampened responses essential to their symbiotic survival (3–5). The absence of a virulence program and the differential perception of microbial-associated molecular patterns by epithelial cells may explain such a dichotomy in the host response (6). On the other hand, in IgA-deficient mice, systemic antibody (Ab) responses against commensal species are increased (7, 8), changes in epithelial cell protective responses occur (9), and survival of specific bacteria is promoted (10), a collection of features all arguing for a relevant function of SlgA in the sensing mechanisms of the mucosal environment.

SlgA in mucosal secretions results from the association during transport across epithelial cells from joining (J) chain-containing polymeric IgA with secretory component (SC) (11). The primary function fulfilled by SlgA is to prevent adhesion of pathogenic antigens to mucosal epithelia, a mechanism known as immune exclusion. In addition, SlgA, alone or associated with antigens, is retrotransported back into Peyer’s patches (PPs) across microfold (M) cells (12) in the follicle-associated epithelium (FAE) via a specific receptor expressed at their surface (13). In the subepithelial dome (SED) region, SlgA-based immune complexes associate with dendritic cells (DCs) (14), resulting in the onset of immunomodulatory types of responses associated with quenching of proinflammatory pathways (15–17).

Seminal papers published by the team of John Cebra have demonstrated that commensal bacteria are coated with natural SlgA in the mouse GI tract, a feature instrumental to the maintenance of bacterial homeostasis (18). The use of more sophisticated animal models led to similar conclusions as to the involvement of SlgA in the numerical control of the gut microbiota (8, 19–21). Noteworthy, natural association of commensals...
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with SlgA has also been described in humans at steady-state adult conditions (22). Strikingly, interaction between commensals and SlgA is not only antigen-driven, as natural polyreactive SlgA isolated from human colostrum and mouse hybridomas was shown to associate with commensals in a Fab/Fc-independent, glycan-mediated manner (23). Moreover, complexes made of such SlgA and commensal bacteria potentiate epithelial cell responsiveness to bacteria in vitro (24). The combination of these mechanistic data led us to speculate that SlgA may govern the mucosal sensing of commensals by facilitating communication with partners of the underlying immune system.

To tackle this open issue in vivo, we investigated in ligated ileal loops the effect of the association with SlgA on the translocation and fate of commensal bacteria into PPs of conventionally raised or germ-free mice. We could demonstrate that targeting of DCs within PPs by two main representatives of the microbiota, i.e. Firmicutes (Lactobacillus rhamnosus) (25) and Bacteroidetes (Bacteroides thetaiotaomicron) was substantially mediated by their association with SlgA. Our data underscore the importance of the Ab in the regulation of the dynamic interaction that takes place between the resident microbiota and the mucosal immune system.

EXPERIMENTAL PROCEDURES

**Mice**—Female BALB/c mice with a conventional microbiota were purchased from Harlan (AD Horst, The Netherlands) and housed in the animal facility of the Centre Hospitalier Universitaire Vaudois under conventional conditions prior to use at the age of 6–8 weeks. Germ-free BALB/c mice were purchased from the French Institute for Agricultural Research (Jouy-en-Josas, France), and kept in sterile incubators at the Nestlé Research Center Animal Facility until use at the age of 6–8 weeks. All experiments were performed upon approval of the State Veterinary Office.

**Source of SlgA**—Culture supernatants of mouse hybridoma cell clone IgAC5, specific for *Shigella flexneri* serotype 5a LPS, grown at 37 °C in RPMI 1640 medium complemented with 10% fetal calf serum (FCS) were used as a source of IgA (24); to reconstitute SlgA, equimolar purified polymeric IgAC5 mAb and recombinant mouse SC were combined as described (26).

**Bacteria**—The commensal strains *L. rhamnosus* (LPR, CGMCC 1.3724 and *B. thetaiotaomicron* DSM 2079 (Bt) were cultured as published (24). Fluorescent labeling was performed by incubating 1 × 10⁹ bacteria in 1 ml of PBS containing 50 μg/ml FITC (Sigma-Aldrich) for 30 min at room temperature in the dark. Washed labeled bacteria were resuspended at a concentration of 4 × 10⁹/100 μl and used freshly as such or after association with 2 μg of reconstituted SlgA for immediate administration into mouse ligated ileal loops.

**Injection into a Mouse Ligated Ileal Loop**—The procedure of Rey et al. was used (12). To comply with Swiss law governing animal experimentation, the investigation protocol had to be limited to 6 h. 100 μl of the solution containing 4 × 10⁹ bacteria associated or not with SlgA was delivered into the lumen of a ligated ileal loop containing a PP. Mice were sacrificed 1, 2, 4, or 6 h later, and ligated ileal loop tissue samples were collected.

**Preparation of Tissue Sections and Immunolabeling**—Intestinal segments containing a PP were immediately fixed in 1 ml of PBS/4% paraformaldehyde (Fluka) for 2 h at room temperature and further processed as described (14). Frozen sections (average thickness of 7 μm) were generated on a Leica cryostat, washed in PBS, and blocked for 20 min with first 5% FCS and then with purified anti-CD16/32 mAbs (1/100; BD Biosciences) at room temperature. Various cell types in PPs were detected upon incubation in PBS-5% FCS with biotinylated anti-CD11c mAb (1/10; BD Biosciences), purified anti-CD4 rat IgG (1/50; BD Biosciences), or purified anti-CD45R rat IgG (1/50; Invitrogen), followed by Cy3- or Cy5-labeled streptavidin (1/500; both from Amersham Biosciences) or anti-rat IgG conjugated to Alexa Fluor 647 (1/100; Invitrogen). Endogenous SlgA was detected with rabbit anti-mouse SC antisera at 1/200 dilution (26) and goat anti-rabbit IgG coupled to Alexa Fluor 647 (1/200; Invitrogen). M cells were stained with rhodamine-coupled UEA-1 (Vector Laboratories, Burlingame, CA) used at a concentration of 20 μg/ml. The DNA stain 4′,6′-diamidino-2-phenylindole (DAPI) was used at a concentration of 100 ng/ml. Sections were finally washed with PBS and mounted with anti-fading Vectashield reagent (Vector Laboratories).

**Laser Scanning Confocal Microscopy (LSCM)**—LSCM images were obtained using a Zeiss LSM 710 Quasar confocal microscope (Zeiss, Göttingen, Germany) in multi-track mode. Raw images were analyzed and processed on the Zen 2009 software. All of the images presented in the paper are representative of 5–10 sections prepared from at least three individual experiments. Bacterial counts in the FAE and the SED region were obtained from three-dimensional acquisitions generated from six individual sections prepared from three mice. Numbering of fluorescent bacteria on sections was blindly carried out by three trained individuals.

**Flow Cytometry**—Independent mice were administered 4 × 10⁸ FITC-LPR (n = 3) or PBS as control (n = 3) into a ligated ileal loop containing a PP. Mice were sacrificed 6 h later, and the three individual PPs per condition were pooled and digested with Liberase (Roche Applied Science) in RPMI 1640 medium complemented with 2 mM CaCl₂ for 30 min at 37 °C. Cells were resuspended in medium containing 2% FCS and 2 mM EDTA prior to labeling with mAbs (all from BD Biosciences) including anti-mouse CD45-PE-Cy7 (1/500 dilution), anti-mouse CD8-Pacific blue (1/200 dilution), anti-mouse CD11c-APC (1/100 dilution), anti-mouse MHC class II-phycocerythrin (1/200 dilution), anti-mouse F4/80-biotinylated (1/100 dilution, Invitrogen), anti-mouse CD11b-biotinylated (1/100 dilution); the last two were followed by strepavidin-PerCP-Cy5.5. Labeled cells were analyzed on a LSR II flow cytometer (BD Biosciences), and data were processed with FlowJo software (BD Biosciences).

**Statistics**—Statistical analysis was performed using Prism software version 5.0 (GraphPad, La Jolla, CA). Data were analyzed with the Mann-Whitney test.

**RESULTS**

Preassociation of LPR with SlgA mAb Impacts on the Dynamics of Bacterial Entry into PPs and Subsequent Uptake by Underlying DCs—LPR-SlgA complexes were generated by co-incubation of FITC-LPR with purified SlgA molecules. After washing of the excess of free SlgA, the formation of FITC-LPR-SlgA complexes was visualized by LSCM via detection of coating
SIgA with anti-mouse SC antiserum and secondary Cy3-labeled Ab (Fig. 1A). The importance of SIgA in driving in vivo bacterial sampling by PPs was evaluated by administering bacteria alone or in the form of complexes with SIgA into a mouse ligated ileal loop. Visualization of bacterial entry was performed by LSCM on tissue sections harvested at time points ranging from 1 to 6 h after administration of FITC-LPR. Despite the existing abundant microbiota in conventional mice, bacterial adsorption on the surface of the epithelium was detected at 1 h for either the bacteria given alone or in complex with SIgA (Fig. 1B). Two h after administration, FITC-LPR-SIgA complexes were observed in the FAE layer, whereas the distribution of bacteria delivered alone remained mostly limited to its surface, as observed for the 1-h time point (Fig. 1B). Four h after administration, bacteria delivered as SIgA-based complexes accumulated in close association with DCs present in the SED region (yellow arrowheads). At 6 h, bacteria given alone (data not shown) or in complex with SIgA were observed at the interface between the FAE and the SED region. An orthogonal projection of the three-dimensional acquisition along the x- and y-axes further confirmed the presence of FITC-LPR administered as FITC-LPR-SIgA complexes in the ligated ileal loop restricted to the lumen in areas devoid of PPs. Intraepithelial DCs are visualized in red. V, villus; L, lumen.
of bacteria beneath the cell plasma membrane and hence their unambiguous intracellular localization (bottom right). The contribution of SlgA in the entry process was further stressed upon enumeration of FITC-LPR in the FAE and SED regions at short time points (Fig. 1D). Compared with bacteria administered alone, significantly more bacteria were counted at early time points when delivered in complex with SigA. Interestingly, after 6 h, the number of FITC-LPR present in the PPs reached a similar value (approximately 40 bacteria/section) in both conditions, despite the fact that 10^6 bacteria had been administered, arguing for tightly regulated entry. Moreover, translocation of bacteria appeared to be limited to the PPs; sections devoid of such a structure displayed no green staining within the neighboring lamina propria and villi (Fig. 1E), although occasional FITC-LPR were observed on the luminal surface. Consistent with our previous observations that only DCs in the SED region are capable of internalizing exogenously delivered SlgA molecules (12), we were unable to detect any co-localization after staining of sections with antibodies directed against B220 (B cells) and CD4 (T cells), yet bacteria had reached the SED dome region (Fig. 2, A and B). The different dynamics of entry identified via the experimental setting used offers a first clue to ascribe to SlgA a function in promoting the transport of commensal bacteria into PPs and further implies DCs in the SED region as the privileged target cell type for selective capture.

**FIGURE 2.** T and B cells do not co-localize with LPR 4 h after administration in a ligated ileal loop containing a PP. LSCM imaging demonstrates no interaction between free or FITC-LPR-SigA (green) and red CD4⁺ T cells (A) or red B220⁺ B cells (B). Yellow arrows indicate the bacteria located outside the labeled cells. V, villus; L, lumen.

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LPR Given Alone Are Coated with Endogenous SlgA before Their Internalization by PPs—The fact that FITC-LPR administered as such are internalized by PPs as well, although with some delay compared with FITC-LPR-SigA complexes, led us to speculate that coating by endogenous SlgA may occur under steadily normal conditions and hence participate in limited entry of bacteria. In an attempt to track endogenous IgA in complexes with bacteria, we faced the technical limitation that massive hiding of epitopes on α and κ chains prevented reliable detection with Abs specific for these two polypeptides (data not shown and Ref. 26). In contrast, we established that recognition of bound SC by a specific antiserum (26) remained efficient; this approach further presented the advantage to discriminate between SigA in luminal secretions from IgA abundantly found in the lamina propria. Following administration of FITC-LPR alone into a ligated ileal loop containing a PP, bound endogenous SlgA was observed by LSCM on sections as soon as 1 h after bacterial delivery (Fig. 3A). Remarkably, despite the high quantity of LPR administered, a significant proportion of bacteria were found coated with SlgA, underlining the presence of sufficient amounts of endogenous SlgA in intestinal secretions to coat the exogenously delivered bacteria.

We then sought to determine whether translocation into the SED region of bacteria injected alone was linked with coating with SlgA. At 6 h after intraluminal administration, most of internalized SlgA detected with anti-SC antiserum co-localized with FITC-LPR in the FAE (Fig. 3B, panel 1), as well as in close contact with the surface of, and within, DCs (Fig. 3B, panels 2 and 3). Noteworthy, various populations of bacteria, i.e. fully, partially, or even not coated with endogenous SigA, may reflect a dynamic removal of the Ab timewise, a phenomenon possibly related to bacterial interaction with DCs, as pinpointed by the appearance of either surface-bound turquoise/white staining and intracellular green rods.

**Reduced Natural Entry of LPR into PPs of Germ-free Mice Is Restored by Pre-formation of Complexes with SlgA**—To confirm further the contribution of SlgA to the transport of FITC-LPR into PPs, the same experiments were performed in germ-free mice, known to have much reduced number of IgA secreting cells in the lamina propria (27). In this model, entry of bacteria administered alone into ligated ileal loops was significantly decreased at 6 h compared with the picture obtained at the same time point in conventional mice (Fig. 4, A and D). Noteworthy, low numbers of translocated bacteria confirmed that the integrity of the epithelial barrier was preserved under these germ-free conditions and that entry remained selective. However, when administered in pre-formed complexes with SlgA, entry of FITC-LPR into PPs was restored to levels resembling those determined in conventional mice at 6 h, further emphasizing the role of SlgA in supporting translocation (Fig. 4, A and D). Moreover, although germ-free mice have underdeveloped PPs, the overall bacterial translocation was not substantially different from that assessed in conventional animals, arguing for functional M cells involved in the process.

**Entry of the Gram-negative Bt Commensal Bacterium Requires Association with SlgA as Well**—The concept of SigA-promoted entry of Gram-positive commensal bacteria prompted us to examine whether the prototype Gram-negative commensal bacte-
The confluence of intestinal bacteria and the host immune system is a highly cooperative relationship that allows bacteria to colonize the gut while maintaining host health. This process is facilitated by the production of secretory immunoglobulin A (SIgA), a major component of the immune system's ability to protect the gut mucosa from invading pathogens. Our study aimed to elucidate the role of SIgA in the host-commensal dialogue, focusing on its importance in governing the passage of bacterial antigens through the intestine.

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SIgA, a dimeric antibody, has a crucial role in enabling the passage of bacterial antigens in the gut. In this study, we observed the uptake of pre-formed SIgA-bacteria complexes, where bacteria were detected within and on the surface of DCs in the SED region, suggesting a possible delay in the process of association with the immune system. Although it gets similarly co-localized with endogenous SIgA in the gut, the passage of bacteria from the gut to the lymphoid tissues is controlled by SIgA, as shown by the low amount of LPR observed within PPs by LSCM.

**DISCUSSION**

In this work, we have established the so far undefined mechanism by which SIgA is involved in the interplay between the gut-associated lymphoid tissue and bacteria from the intestinal microbiota. By using the technique of ileal loop, the fate of commensal bacteria in adult conventional mice displaying a heterogeneous community of resident microbiota and a mature immune system, or in germ-free mice, was tracked, leading to a series of novel observations including: (i) the selective passage...
of commensal bacteria across FAE to DCs in the SED region of PPs is related to their association with SIgA in a non-antigen-specific way, as exemplified by the fact that preassociation of bacteria with nonspecific SIgA increased their dynamics of entry and restored the reduced transport observed in germ-free mice; (ii) SIgA-based complexes between endogenous SIgA and bacteria spontaneously form in the intestinal lumen; (iii) immune complexes are detectable in the pocket of M cells at early time points, identifying these latter as the portal of entry and indicating the absence of destructive processing by these cells; (iv) targeting of SIgA-based complexes to DCs in the SED region suggests that this is in this particular form that commensal bacteria are primarily exposed to the antigen-presenting cell.

Interestingly, low quantities of bacteria were found translocated in PPs compared with the hundreds of millions administered. The importance for bacteria to be associated with SIgA to enter PPs may be an intrinsic property of the mouse intestine responsible for their low sampling rate by M cells (14, 22, 30), thus minimizing their interaction with the underlying immune system. First, the differential rates of entry of bacteria alone or in pre-formed complex with nonspecific SIgA into PPs indirectly shed light on the role of the Ab in M cell-mediated sampling; second, experiments performed in germ-free mice further revealed the role of SIgA in the translocation of commensals in the SED region. Moreover, the fact that M cells express a specific receptor for SIgA (13) definitely reinforces the hypothesis of an actively controlled process of intestinal sampling by M cells mediated through SIgA. The contribution of other sampling sites such as isolated lymphoid follicles and villous M cells, or snorkeling DCs present in the lamina propria (31, 32), cannot be excluded at this stage, although addressing them in SIgA-based sampling will be a challenging task in terms of localization and appropriate time of incubation.

Following passage across the FAE, specific targeting of CD11c+CD11b+ DCs in the SED region is fully consistent with the moderate local cellular and humoral responses reported for SIgA working as a carrier for antigens (16). Indeed, this subset produces high levels of IL-10 and induces the differentiation of IL-4/IL-10/TGF-β-producing T cells (33) prone to favor oral tolerance and class switch to IgA secretion (34). The same subset has been involved in cross-tolerance against intestinal antigens (35). The presence of specialized DCs in the SED region that encounter microbiota in a SIgA-mediated manner may be

FIGURE 4. Entry of LPR and Bt is diminished in germ-free mice and is restored after association with SIgA. A, tracking by LSCM of FITC-LPR or FITC-LPR-SlgA after 6 h of incubation in a ligated ileal loop of germ-free mice. Inset, magnification of FITC-LPR-SlgA complex. B, tracking of FITC-Bt or FITC-Bt-SlgA after 6 h of incubation in a ligated ileal loop of conventional mice. Inset, magnification of FITC-Bt-SlgA complex. C, visualization of FITC-Bt administered alone or in pre-formed complex with SIgA 6 h after administration into a ligated ileal loop of germ-free mice. On all panels, bacteria are visualized in green, SIgA in red, and DCs in blue. D, quantification of FITC-bacteria in the FAE and SED region of six individual sections obtained from three independent experiments. Numbers are medians ± S.E. (error bars). V, villus; L, lumen.
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A

FIGURE 5. Interaction of LPR alone and in complex with SlgA with M cells.
A, LSCM image acquired 2 h after administration of FITC-LPR alone in a ligated ileal loop containing a PP. Magnification (×10) of an area rich in M cells demonstrates the association of LPR (green)-SlgA (red) complexes (yellow) with blue-labeled M cell surface. Cell nuclei are white. B, same analysis as in A following administration of pre-formed FITC-LPR-SlgA complexes in a ligated ileal loop. Magnifications of M cell-rich areas demonstrate the presence of LPR-SlgA complexes at the surface and within M cells in three-dimensional reconstructed images (panels 1, 2, and 3). V, villus; L, lumen.

essential to the development of commensal-specific peripheral Treg cells in the gut (36–38) rather than pathogenic effectors. Interestingly, PP macrophages not recognized by SlgA (12) can however engulf LPR; this suggests that SlgA is not a partner of macrophage phagocytic activity and that those cells may do much more than killing translocated commensals in the mesenteric lymph nodes (21). This will represent a topic for further investigation in the light of a recent paper showing that such cells are inflammation adverse (39).

Provision of local SlgA appears to monitor the microbiota in some sort of a self-regulatory loop that would have for ultimate consequence to finely balance the host reaction to its resident microbes and to ensure appropriate mucosal gut homeostasis (40). This appears to be particularly true in the light of the observation that bacteria delivered as such are coated within 1 h with endogenous SlgA. The involvement of both commensal-specific and natural IgA antibodies in maintaining intestinal homeostasis has been reported (41–43). Indeed, as the mouse microbiota comprises Lactobacilli, it is therefore conceivable that part of the endogenous SlgA coating observed in the present study is mediated by cross-reactive Abs recognizing related epitopes on human microbiota-derived LPR. Nonetheless, coating with nonspecific polyreactive SlgA is highly likely to take place in vivo. This is supported by the fact that commensal exclusion remains completely effective in quasimonoclonal neonatal mice, in which the antibody specificity is restrained to the hapten nitrophenol (44). Furthermore, at the biochemical level, carbohydrates carried by SC in SlgA molecules have been shown to account for the Fab-independent association of these latter with a series of commensal species (24). However, whether the manner SlgA associates with bacteria would have an effect on their fate in PPs will have to wait for commensal-specific SlgA reagents.

Unexpectedly, the dynamics of entry of LPR was higher than Bt in conventional mice during the short term protocol, as reflected by the higher number of LPR bacteria in the SED region 6 h after injection in the intestinal loop. Whether this relies on a differential capacity to bind endogenous SlgA in this particular experimental setting, or constitutes effective distinct properties between these two micro-organisms deserves further investigation. Nevertheless, it is worth mentioning that preassociation with exogenous SlgA yielded equivalent results.

As final considerations, it is worthwhile to highlight that experiments performed in germ-free mice may somehow reflect what happens in the underdeveloped epithelium of the newborn; intestinal coating by exogenous SlgA Abs originating from maternal milk during this period may combine the properties of neutralizing newly colonizing bacteria and educate the developing mucosal immune system not to overreact against what will become a symbiont. In support of this, polyreactive SlgA purified from human colostrum have been recently shown to interact strongly with microorganisms isolated from the human GI tract (24). Consequently, the recognition of the perpetual interaction among SlgA, commensals, and the mucosal immune system may find an echo in the correct interpretation of results emanating from germ-free animal models. Indeed, such models only partially reflect the “actual” situation in the GI tract because of the very low endogenous production of SlgA found in those animals, thus impacting on bacterial sampling as deciphered in the present study.

In conclusion, the sum of our data demonstrates that SlgA, in addition to leading to immune exclusion, is involved in the mechanism by which the gut-associated lymphoid tissue permanently checks the commensal content of the intestine. Specific targeting of DCs in the SED region of PPs via the M cell pathway in conventional mice as shown here suggests that SlgA bound to bacteria may indeed serve as the “driving force” to ensure the permanent communication between the content of the gut lumen and components of the neighboring mucosal immune system. The overall goal would be to keep the microbiota confined within the gut lumen, while rare controlled translocation of coated commensal bacteria by natural and/or specific SlgA would thus help the host to trigger an optimal activation of the mucosal immune response (14, 15).
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FIGURE 6. Analysis by flow cytometry identifies CD11b^+ DC subset and macrophages as cells capturing LPR in PPs. A, PP cells from three independent ligated ileal loops incubated with FITC-LPR for 6 h were recovered. Flow cytometry analysis was performed on the three CD45^+ CD11c^+ DC subsets in PPs for both control (CTRL) and experimental settings. B, gating for F4/80^+ CD11c^+ macrophages indicates a capacity to take up FITC-LPR.
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