Influence of the Tissue Microenvironment on Toll-Like Receptor Expression by CD11c+ Antigen-Presenting Cells Isolated from Mucosal Tissues

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It is recognized that functional activities of antigen-presenting cells (APCs) in mucosal tissue sites differ from those of systemic APCs; however, it is unknown whether there are further differences between APC populations residing in different mucosal sites. In this study, we directly compared murine CD11c+ APCs isolated from colon, lung, and spleen and found that APCs isolated from these tissues differ considerably in Toll-like receptor (TLR) expression and responses to in vitro TLR ligand stimulation. We also provide evidence that tissue microenvironments dictate distinct patterns of TLR expression by CD11c+ APCs in different mucosal tissues. Moreover, CD11c+ cells isolated from different tissues have varied capacities to induce the development of T helper 1 (Th1), Th2, or regulatory CD4+ T cells. Thus, unique tissue microenvironments have a significant influence on determining TLR expression by CD11c+ cells that migrate to and reside in each mucosal tissue and are likely to modulate their functional activities.

Antigen-presenting cells (APCs), such as dendritic cells (DCs), have the ability to control immune outcomes, and a number of factors are thought to be involved in determining the types of immune responses induced. The nature of the antigen/pathogen is likely to be a strong determinant of the type of immune response that is induced. For example, T helper 1 (Th1) responses are preferentially induced when the host is infected with intracellular pathogens, such as mycobacteria (4). Conversely, helminth infections tend to induce Th2 responses (39). In this regard, DCs appear to utilize pattern recognition receptors, such as Toll-like receptors (TLRs), to distinguish between various molecules to induce suitable immune responses (33). The type of immune response induced may also be influenced by distinct subpopulations of APCs that are involved in priming the appropriate T cells. DCs are heterogeneous populations of cells in any given tissue (40), and in the mouse, CD8α+ DCs are thought to be specialized for inducing Th1 responses, whereas CD8α− DCs are thought primarily to induce Th2 responses (28). Moreover, it has been shown that mouse CD8α+ and CD8α− DCs preferentially present antigens to CD8+ and CD4+ T cells, respectively (14). In contrast to DCs, macrophages are thought to be associated with immunoregulation and tolerance rather than immune induction (1, 19).

The microenvironment in a tissue is thought to have a major impact on the nature of immune responses induced at each tissue site (25). Different mucosal tissues, such as those of the respiratory and gastrointestinal or genitourinary tracts, have distinct physiological functions, are exposed to unique antigen and pathogen milieus, and have diverse cellular compositions; therefore, APCs that reside in each mucosal tissue must be adapted to handle such differences. Earlier work showed that splenic microenvironments can induce differentiation of mature bone marrow-derived DCs and hematopoietic stem cells to regulatory DCs (45, 53) and also negatively regulate activated plasmacytoid DCs (27). Also, thymic stromal lymphopoietin, which is expressed in lung and skin, is believed to have a Th2-skewing effect on DCs and has been associated with development of allergic airway inflammation (20, 41, 54). Furthermore, DCs isolated from different tissue sites have functional variations. Mouse DCs isolated from Peyer’s patches preferentially induce Th2-type immune responses, whereas spleen DCs tend to induce Th1-type immune responses, even though these two compartments have similar subset compositions (22). Also, DCs isolated from mouse liver are able to produce interleukin-12 (IL-12), regardless of their CD8α expression, whereas in the spleen, CD8α+ DCs are much superior at IL-12 production compared with CD8α− DCs (32). These observations strongly suggest that tissue microenvironments impose a significant influence on the function of DCs and subsequent immune responses.

Even though APCs from some mucosal tissues such as the lung, small intestine, colon, and genital tract have been studied in isolation and compared to spleen counterparts (1, 6, 8, 44), few comparisons have been reported on APCs isolated from different mucosal tissues. Knowledge of similarities and differences between APCs residing in various tissues is important, since immunity is likely regulated differently in each mucosal tissue site (34).

In this study, we used purified CD11c+ cells from murine colon, lung, and spleen to undertake a direct comparison of their phenotypes and activities. We found that even though CD11c+ cells from all tissues examined are able to carry out typical APC functions, they have distinct cytokine production profiles, modulation of T-cell responses, and regulatory T-cell induction, along with different TLR expression patterns. More-
over, our data suggest that this differential TLR expression is the direct result of the mucosal tissue microenvironments in which cells reside.

**MATERIALS AND METHODS**

Mice. C57BL/6 mice 6 to 8 weeks old were from Charles River Laboratories. CD45.1 congenic B6 mice 6 to 8 weeks old were from Jackson Laboratories, and OT-II transgenic mice were bred at McMaster University’s Central Animal Facility. All procedures were approved by the Animal Research Ethics Board of McMaster University.

Isolation of CD11c+ cells from colon and lung tissues. CD11c+ cells from colon and lung were isolated using magnetic-activated cell sorting (MACS) as described previously (44). Briefly, large intestine was washed, cut into small pieces, and incubated at 37°C in EDTA-supplemented medium to remove epithelial cells and then digested with collagenase type VIII (Sigma-Aldrich) for 1 h at 37°C. Colonic cells were further purified using 43%/63% Percoll (GE Healthcare) density gradient centrifugation, and cells at the interface were collected. Lungs were perfused to remove red blood cells, cut into small pieces, and digested for 1 h at 37°C with collagenase. Colon or lung CD11c+ cells were then purified using CD11c microbeads (Miltenyi Biotec) and running the samples through MS² columns twice to consistently achieve an enriched population of >90% CD11c+ cells. Where specified, exhaustive bronchoalveolar lavage (BAL) was performed as described previously (37) prior to lung tissue being processed for CD11c+ cell isolation, to reduce alveolar macrophages in the interstitial cell preparation. Splenocyte isolation. Spleens were infused with collagenase-supplemented RPMI, cut into small pieces, and digested for 30 min at 37°C. The resulting cell suspension was treated with M-lyse buffer (R&D Systems) for red blood cell lysis. Spleen CD11c+ cells were isolated by MACS as described above. Splenic T cells were purified using a CD4+ T-cell isolation kit (Miltenyi Biotec) according to the manufacturer’s instructions. For some experiments, CD25+ cells were depleted using CD25-specific magnetic beads prior to T-cell isolation to obtain CD4+ CD25+ T cells. Purified T cells were routinely >90% pure based on CD4 staining.

Ex vivo CD11c+ cell culture. A total of 1 × 10⁶ CD11c+ cells in 200 μl were incubated at 37°C in complete medium supplemented with 40 ng/ml granulocyte-macrophage colony-stimulating factor and 200 U/ml gamma interferon (IFN-γ) and 100 U/ml granulocyte-macrophage colony-stimulating factor (37, 47) in the presence of PGN, LPS, recombinant FliC, and CpG ODN, ligands for TLR-2, -4, -5, and -9, respectively, in the presence of granulocyte-macrophage colony-stimulating factor and IFN-γ and analyzed the production of IL-12p70, TNF-α, and IL-10. While DCs from all tissues produced IL-12p70 when stimulated with each of the ligands under these conditions, only CpG ODN induced a robust response, with production of IL-12 by colon and spleen CD11c+ cells being significantly greater than with lung DCs (Fig. 1). On the other hand, TNF-α was produced by lung CD11c+ cells in copious amounts when stimulated with all of the ligands tested, but colon and spleen CD11c+ cells did not produce this cytokine. Levels of TNF-α production by lung CD11c+ cells were similar for PGN, LPS, and Flt3 stimulation but significantly lower following stimulation with CpG ODN (Fig. 1). IL-10 was spontaneously produced by colon and lung CD11c+ cells under these culture conditions, even in the absence of added stimuli, and the level of production stayed relatively constant even upon stimulation with TLR ligand, while spleen DCs required stimulation for IL-10 production (Fig. 1). Thus, it appears that different tissue CD11c+ cells, including those from different mucosal tissues, respond uniquely to TLR ligand stimulation.

In vitro APC–T-cell coculture. CD11c+ cells were incubated with 200 μg/ml ovalbumin (OVA) protein (grade V; Sigma-Aldrich) for 2 h at 37°C and washed well with phosphate-buffered saline. OT-II CD4+ CD25+ T cells were labeled with 5 μM carboxyfluorescein succinimidyl ester (CFSE; Molecular Probes) for 5 min at room temperature and washed well. A total of 5 × 10⁶ T cells per well were cocultured for 4 days with 5 × 10⁶ OVA-pulsed CD11c+ cells. Where indicated, OVA protein or recombinant human transforming growth factor β (TGF-β; 2 ng/ml; R&D Systems) was added to the culture medium for the duration of the coculture. T-cell proliferation was examined by visualizing CFSE dilution by flow cytometry. A mouse regulatory T-cell staining kit (eBioscience) was used to detect CD25+ Foxp3+ regulatory cells. Also, coculture supernatants were analyzed for cytokine levels.

Adaptive transfer of spleen CD11c+ cells. Spleen CD11c+ cells from wild-type B6 mice (CD45.2) were isolated as described above. A total of 15 × 10⁶ cells/mouse were injected intravenously into CD45.1 congenic host mice. After 6 days, lung and spleen cells from host mice were isolated and analyzed by flow cytometry for TLR2 expression in adoptively transferred CD11c+ cells.

**TLR gene expression analysis.** Total RNA was isolated using an RNasea minikit (Qiagen), and cDNA was synthesized using a RETROscript kit (Ambion) according to the manufacturer's instructions. Real-time PCR gene expression analysis was conducted using an ABI Prism 7900HT apparatus (Applied Biosystems) and TaqMan gene expression assays for TLR2, TLR4, TLR5, TLR9, and ribosomal protein L32 (Applied Biosystems). Relative gene expression for TLR2 was calculated by normalizing the data using L32 expression and then applying the ΔΔCt method with the following formula: gene expression = 2−ΔΔCt × 1,000, where ΔΔCt = ΔCt(CD11c+ cells) − ΔCt(total splenocyte) and ΔCt(gene) = Ct(gene) − Ct(L32).

**RESULTS**

**CD11c+ cells isolated from colon, lung, and spleen respond uniquely to stimulation by different TLR ligands.** We stimulated the cells in vitro with PGN, LPS, recombinant Flt3, and CpG ODN, ligands for TLR-2, -4, -5, and -9, respectively, in the presence of granulocyte-macrophage colony-stimulating factor and IFN-γ and analyzed the production of IL-12p70, TNF-α, and IL-10. While DCs from all tissues produced IL-12p70 when stimulated with each of the ligands under these conditions, only CpG ODN induced a robust response, with production of IL-12 by colon and spleen CD11c+ cells being significantly greater than with lung DCs (Fig. 1). On the other hand, TNF-α was produced by lung CD11c+ cells in copious amounts when stimulated with all of the ligands tested, but colon and spleen CD11c+ cells did not produce this cytokine. Levels of TNF-α production by lung CD11c+ cells were similar for PGN, LPS, and Flt3 stimulation but significantly lower following stimulation with CpG ODN (Fig. 1). IL-10 was spontaneously produced by colon and lung CD11c+ cells under these culture conditions, even in the absence of added stimuli, and the level of production stayed relatively constant even upon stimulation with TLR ligand, while spleen DCs required stimulation for IL-10 production (Fig. 1). Thus, it appears that different tissue CD11c+ cells, including those from different mucosal tissues, respond uniquely to TLR ligand stimulation.

**Flow cytometry.** For TLR protein expression analysis, cells were stained with anti-CD11c and anti-TLR2 monoclonal antibodies (BD Pharmingen). Anti-CD45.2 monoclonal antibody (BD Pharmingen) was also used for the detection of adoptively transferred cells. CD4+ Foxp3+ CD25+ regulatory T cells were detected using a mouse regulatory T-cell staining kit (eBioscience) according to the manufacturer's instructions. Nonviable cells were detected using Via-Probe (7-amino-actinomycin D; BD Pharmingen). Samples were run on an LSR II system (BD Biosciences), and data analysis was done using FlowJo software (Tree Star).

**Cytokine measurement.** Cytokine production by cultured cells was determined using enzyme-linked immunosorbent assay kits for murine IL-4, IL-10, IL-12p70, tumor necrosis factor alpha (TNF-α), and IFN-γ (all from R&D Systems).

**Data analysis.** Data are expressed as means ± standard deviations (SD). Statistical significance of the data was determined by using an unpaired, two-tailed Student's t test. P values of <0.05 were considered statistically significant.

**One question that arose was whether the high level of TNF-α production by lung CD11c+ cells was similar for PGN, LPS, and Flt3 stimulation but significantly lower following stimulation with CpG ODN (Fig. 1). IL-10 was spontaneously produced by colon and lung CD11c+ cells under these culture conditions, even in the absence of added stimuli, and the level of production stayed relatively constant even upon stimulation with TLR ligand, while spleen DCs required stimulation for IL-10 production (Fig. 1). Thus, it appears that different tissue CD11c+ cells, including those from different mucosal tissues, respond uniquely to TLR ligand stimulation. IL-10 levels detected in this study were close to the detection limit for the enzyme-linked immunosorbent assay used, but there was a clear trend toward mucosal CD11c+ cells having a greater capacity to produce IL-10 in the steady state, compared with systemic (spleen) CD11c+ cells.
significantly greater than that elicited by spleen CD11c<sup>+</sup> cells (Fig. 2), suggesting that lung DCs are likely to be a robust source of TNF-α, producing significantly greater levels of TNF-α than colon and spleen DCs. Importantly, IL-12p70 production levels by lung CD11c<sup>+</sup> cells isolated from whole lung and from lung tissue after performing extensive BAL were similar (Fig. 2). In other words, the removal of BAL cells did not affect the IL-12p70 production by lung CD11c<sup>+</sup> cells, and the production of IL-12 by only BAL alveolar macrophages was very low (Fig. 2). This is in accordance with our previous report showing that alveolar macrophages produce little bioactive IL-12p70 (49, 50). These data indicate that only alveolar macrophages and not IL-12p70-producing lung DCs were removed as a consequence of conducting prior lung lavage. It will be of interest to further separate lung DCs and alveolar macrophages by fluorescence-activated cell sorting, as done by others previously (26); however, diminished cell viability and adequate recovery levels have limited us from conducting such a study so far.

Lung CD11c<sup>+</sup> cells express significantly greater levels of TLR2 and TLR4. Since CD11c<sup>+</sup> cells derived from different tissues respond differently to TLR ligand stimulation, we wondered if it was due to a difference in their TLR expression and examined this by gene and protein expression in isolated CD11c<sup>+</sup> cells. Intriguingly, lung CD11c<sup>+</sup> cells expressed TLR2 and TLR4 genes at a much greater magnitude than colon and spleen CD11c<sup>+</sup> cells (Fig. 3A). To the contrary, even though TLR5 and TLR9 were expressed by spleen CD11c<sup>+</sup> cells at significantly greater levels than by colon or lung CD11c<sup>+</sup> cells, the extent of the difference was not as striking as what was observed for TLR2 and TLR4. We further measured TLR2 protein expression using flow cytometry and found the majority of lung CD11c<sup>+</sup> cells indeed expressed TLR2 on their surface, reflecting high TLR2 gene expression. Both spleen and colon CD11c<sup>+</sup> cells had fewer cells expressing TLR2 than lung CD11c<sup>+</sup> cells, and spleen CD11c<sup>+</sup> cells had a greater portion of cells expressing TLR2 than colon CD11c<sup>+</sup> cells (Fig. 3B). These results show that lung CD11c<sup>+</sup> cells have a very different TLR expression pattern relative to colon and spleen CD11c<sup>+</sup> cells. Moreover, this high expression of TLR2 and TLR4 by lung CD11c<sup>+</sup> cells may explain the robust TNF-α production observed when they are stimulated with PGN or LPS (Fig. 1). Additionally, the high TNF-α production by lung CD11c<sup>+</sup> cells upon stimulation by LPS, FliC, and CpG ODN, and much lower but detectable levels produced spontaneously under these conditions, may suggest an intrinsic capacity of lung CD11c<sup>+</sup> cells to produce this cytokine.

The lung tissue microenvironment induces TLR2 expression by adoptively transferred spleen CD11c<sup>+</sup> cells. To test whether the microenvironment influences the greater levels of TLR2 in lung CD11c<sup>+</sup> cells, spleen CD11c<sup>+</sup> cells isolated from wild-
type mice (CD45.2) were adoptively transferred to CD45.1 congenic host mice. A small number, but very distinct population, of CD11c\(^{+}\) CD45.2\(^{+}\) cells was recovered at 6 days post-transfer, from both lung and spleen of the hosts, and examined for their TLR2 expression (Fig. 4A). CD11c\(^{+}\) cells from both CD45.2\(^{+}\) and CD45.2\(^{-}\) cell populations found in the host’s lung highly expressed TLR2 (Fig. 4B). Since CD45.2\(^{-}\) cells are resident host lung DCs, it is not surprising that they have high TLR2 expression. CD45.2\(^{+}\) cells, originally spleen DCs with lower levels of TLR2 (Fig. 3B), express high levels of TLR2 when found in lung (Fig. 4B). To rule out any artifact of adoptive transfer, we also examined the host’s spleen and found that both CD45.2\(^{+}\) and CD45.2\(^{-}\) CD11c\(^{+}\) cells expressed lower levels of TLR2 (Fig. 4B). The difference in TLR2 expression by adoptively transferred CD45.2\(^{+}\) cells found in the host’s lung and spleen was statistically significant, whereas CD11c\(^{+}\) cells found in the same tissue (lung or spleen) expressed similar levels of TLR2 (Fig. 4C). We have also conducted an experiment in which spleen CD11c\(^{+}\) cells were labeled with CFSE and adoptively transferred to host mice. It was possible to visualize a greater number of donor cells by flow cytometry; however, strong autofluorescence found in the lung samples made the analysis more complex. Nonetheless, we observed comparable results to what is described above (unpublished observation). These data suggest that lung tissue microenvironments can induce TLR2 expression by CD11c\(^{+}\) cells that migrate to that tissue.

**CD11c\(^{+}\) cells isolated from colon, lung, and spleen induce unique CD4\(^{+}\) T-cell responses.** Priming of CD4\(^{+}\) T cells is one of the key functions of APCs to induce immunity or tolerance, and since CD11c\(^{+}\) cells isolated from colon, lung, and spleen have distinct responses and phenotypes, we compared the abilities of tissue-derived CD11c\(^{+}\) APCs to prime antigen-specific CD4\(^{+}\) T cells by examining the induction of T-cell proliferation. Both colon and spleen CD11c\(^{+}\) cells pulsed with OVA protein induced proliferation of OVA-specific OT-II CD4\(^{+}\) T cells efficiently in vitro (Fig. 5A). Contrary to this, lung CD11c\(^{+}\) cells did not induce CD4\(^{+}\) T-cell proliferation as effectively (Fig. 5A), and this did not change when cells were cocultured in TLR ligand-containing medium (Fig. 5A).

DCs are known to have the ability to control immune outcome, and we questioned whether CD11c\(^{+}\) cells isolated from different tissues would have different abilities to induce Th1 or Th2 T-cell responses (3, 25). We examined supernatants from APC–T-cell cocultures for representative type 1 and type 2 cytokines, IFN-\(\gamma\) and IL-4, respectively. We found that colon

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**FIG. 3.** TLR expression by purified tissue-derived CD11c\(^{+}\) cells. (A) Relative TLR gene expression by tissue CD11c\(^{+}\) cells was determined by real-time RT-PCR utilizing \(\Delta\Delta C_t\) method. Results are representative of at least three experiments and are presented as mean ± SD. #, \(P < 0.05\) relative to corresponding colon samples. *, \(P < 0.05\) relative to corresponding spleen samples. (B) TLR protein expression was determined by flow cytometry. Purified CD11c\(^{+}\) cells were labeled for TLR2 along with other surface markers. Open histograms indicate TLR2 specific staining and shaded histograms indicate isotype control staining. Numbers indicate percentages of TLR2\(^{+}\) cells. Representative histograms from at least three experiments are shown.
and spleen CD11c<sup>+</sup> cells were able to induce IL-4 production by T cells to a greater extent than lung CD11c<sup>+</sup> cells, with or without TLR ligand stimulation (Fig. 5B). IFN-γ production by the T cells was rather low when cells were cocultured in medium alone, but a robust induction was observed when the TLR2 ligand PGN was added to the medium, with lung DCs exhibiting the greatest IFN-γ production (Fig. 5B). IFN-γ production was also induced with CpG ODN in the medium, but the responses were not as robust as with PGN (Fig. 5B). Based on these data, CD11c<sup>+</sup> APCs from different tissues induce distinct responses by antigen-specific CD4<sup>+</sup> T cells, and although both colon and lung CD11c<sup>+</sup> cells are mucosal tissue APCs, they induce very different T-cell responses. Furthermore, colon CD11c<sup>+</sup> cells induced T cells to produce the type 2 cytokine IL-4 much more readily than lung or spleen counterparts.

Lung CD11c<sup>+</sup> cells induce a greater level of CD25<sup>+</sup> Foxp3<sup>+</sup> regulatory T-cell development. Recent studies have shown that DCs isolated from small intestine lamina propria or mesenteric lymph nodes (MLNs) have a superior capacity to induce the development of CD25<sup>+</sup> forkhead box protein P3 (Foxp3<sup>+</sup>) regulatory T cells compared with spleen DCs (30, 43). We cocultured CD11c<sup>+</sup> cells isolated from colon, lung, or spleen with OT-II CD4<sup>+</sup> T cells in the presence of OVA protein, with or without TGF-β supplementation. As reported previously, we found that addition of TGF-β was necessary for the in vitro development of CD25<sup>+</sup> Foxp3<sup>+</sup> regulatory T cells induced by
DCs from any of the tissues examined (Fig. 6A). Lung CD11c+ cells were the most efficient inducers of regulatory T cells, and colon CD11c+ cells were the least efficient of the three tissue APCs we compared (Fig. 6A). These differences were also found to be statistically significant (Fig. 6B) and suggest that CD11c+ APCs from different mucosal tissues have different capacities to induce the development of regulatory T cells, and colon DCs, unlike DCs isolated from small intestine or MLNs (30, 43), are not robust inducers of regulatory T cells.

**DISCUSSION**

Increasingly, studies of immune responses in various tissues highlight the importance of local tissue microenvironments in directing local immunity (34). In this study, we directly compared the functions and TLR expression levels for CD11c+ cells from two distinct mucosal sites, the colon and lung, and we have provided evidence for distinct differences from each other and of both from CD11c+ cells in the spleen. We also show critical involvement of the tissue microenvironment in regulating TLR expression by CD11c+ cells. CD11c+ cells found in the colon and spleen are likely to be DCs, since no other cell type in these tissues in steady state is known to express CD11c. In contrast, lung CD11c+ cells include both DCs and macrophages, because alveolar macrophages also express CD11c (10, 26). As such, isolated CD11c+ cells from the lung are a mixture of lung DCs and alveolar macrophages and can be referred to as lung CD11c+ APCs.

In a previous publication, we reported that lung CD11c+ cells have a similar surface marker phenotype to colon but not to spleen CD11c+ cells, where both lung and colon CD11c+ cells were comprised of a large proportion of CD11b+ population but lacked a significant presence of cells expressing CD8α or B220 (44). Based on this information, our initial idea was that APCs residing in mucosal tissues may also share similar functions with spleen APCs. Contrary to this, our findings show that CD11c+ cells isolated from colon and lung respond differently and distinctly to stimulation by various TLR ligands, suggesting that APCs with similar surface phenotypes may function uniquely in different tissues. Alternately, it is also possible that CD11c+ CD11b+ cells found in colon and lung may belong to distinct DC subsets differentially ex-
expressing other surface markers, given the vast number of DC subsets described in recent years (21, 40).

TNF-α is known to be important for type 1 immune regulation and host protection against various intracellular pathogens (15, 31, 52); therefore, the robust TNF-α production by lung CD11c⁺ cells suggests that these cells are optimized to ward off incoming pathogens at the mucosa. In addition, our TLR5 expression and Flt3 stimulation data suggest that there may be intrinsic differences in TNF-α-producing abilities among APCs from different tissues. In contrast, colon CD11c⁺ cells seem to lack the ability to produce a high level of TNF-α upon TLR ligand stimulation, and this may be associated with low-level expression of TLR2 and TLR4. Colonic tissue is highly colonized by commensal bacteria, providing a constant source of TLR ligand to colon APCs in the steady state, and highly colonized by commensal bacteria, providing a constant source of TLR ligand to colon APCs in the steady state, and this environmental circumstance may require colon CD11c⁺ cells to be less inflammatory in nature. Additional evidence to support this possibility is the constitutive expression of IL-10 by colon CD11c⁺ cells and lack of expression by spleen CD11c⁺ cells. Since the spleen is normally a sterile organ, there would be little need for innate protection, but CD11c⁺ cells in the spleen still need to be equipped to regulate immune responses in the event of infection to avoid uncontrolled immune activation. These data support the notion of enhanced immune regulation in the intestinal mucosa, as suggested by others (8, 22). Another interesting finding was the robust IL-12p70 production by CD11c⁺ cells stimulated with CpG ODN. CpG ODN has been used by others to prevent infection at mucosal sites (2, 38); thus, induction of IL-12p70 production by CD11c⁺ cells may be one mode of action by which CpG ODN confers protection against mucosal challenge by pathogens.

We and others have recently shown that colon DCs or lung DCs express TLRs at different levels relative to spleen DCs (6, 44). To our knowledge, the current study is the first report describing heightened TLR2 and TLR4 expression by lung APCs relative to APCs from another mucosal site. Previous studies have shown the importance of TLR2 and TLR4 expression for host protection against respiratory bacterial infection (16, 36); therefore, the high TLR2 and TLR4 expression levels of lung CD11c⁺ APCs may be a critical means of host protection. Together with the apparent enhanced capacity of lung CD11c⁺ APCs to produce TNF-α, this may indicate the unique requirement imposed in lung tissue for APCs to control respiratory infections.

Accumulating evidence suggests that the tissue microenvironment influences local immune responses (12). It is likely that APC functions are modulated by many factors that include tissue physiologic functions, the presence/absence of commensal organisms, the antigenic milieu, cytokines and chemokines, tissue matrix and integrin expression, and cellular compositions. The induction of TLR2 expression by CD11c⁺ cells due to the lung tissue microenvironment indicates that the difference in observed functionalities between cells isolated from the colon, lung, and spleen are, at least partially, due to their exposure to different tissue microenvironments. In this regard, a number of differences exist between the colon, lung, and spleen that could potentially modulate APC functions. One of the most notable differences is the sterility of these tissues. The colon is home to a vast number of commensal bacteria, whereas the spleen is normally a sterile compartment. Also, the upper airways and lung tissue are constantly exposed to environmental microorganisms that are present in the air, contributing to a unique lung microenvironment. We speculate that the differences in microorganism compositions between the tissues may direct functional differences seen in CD11c⁺ cells from the colon, lung, and spleen. Additionally, epithelial cells are likely to be involved in modulating DC functions at mucosal tissue sites (5, 35). In support of these possibilities, a recent report has shown in vitro that an intestinal epithelial cell line exposed to commensal organisms directs DCs to become more tolerogenic, expressing lower levels of maturation markers and inflammatory cytokines while having enhanced IL-10 production (51). Also, it has been suggested that the effects of the microenvironment can override the functional differences between DC subsets (13, 24).

Aside from their innate immune functions, APCs are critical for host protection because of their T-cell-priming ability. Additionally, DCs are able to control the adaptive immune response by inducing the development of different types of T cells, including Th1, Th2, and regulatory T cells (3, 25). It is thought that mucosal tissue sites are more Th2 biased than systemic sites, which are more Th1 biased (1, 22). Our colon CD11c⁺ cell data support this notion; however, lung CD11c⁺ cells did not show such expected tendencies in our hands. Interestingly, PGN enabled lung CD11c⁺ cells to induce a robust IFN-γ production by CD4⁺ T cells without a notable increase in T-cell proliferation. This observation correlates with the T-cell stimulatory ability of DCs in the presence of alveolar macrophages where alveolar macrophages have been shown to inhibit the DC’s ability to induce T-cell proliferation while still allowing for the induction of cytokine production by T cells (46). Also, a reduction in alveolar macrophage contribution did not drastically change the cytokine production profile by lung CD11c⁺ cell preparations. Together these data indicate that lung DCs are capable of inducing Th1 immune responses in the presence of an appropriate stimuli. Such characteristics of lung DCs have also been suggested by others (42).

Direct comparison of DCs from different areas of the intestine has not been done thus far, but data from the current study and studies conducted by others collectively indicate that DC functions may differ in colon and small intestine. DCs from colon and small intestine may share some common activities, such as constitutive expression of IL-10 (8, 23). It has recently been shown that blocking IL-10 signaling enhances TLR ligand-induced IL-12-producing ability of small intestine DCs (29). It is thus also possible that IL-10 produced by colon DCs performs a similar role in controlling their functions. Despite such potential similarities, the subset compositions of DCs seem to differ between the colon and small intestine, as CD8⁺ DCs are absent in the colon (44) but present in the small intestine (23). Recent studies have also shown that DCs isolated from small intestine lamina propria and MLNs are superior inducers of Foxp3⁺ regulatory T-cell development in vitro compared to spleen DCs (9, 43). This is in contrast to our current findings using colon DCs, suggesting there are unique DC functions among DCs residing within different regions of the gastrointestinal tract.

It has been reported that TNF-α enhances Foxp3 expression and regulatory T-cell activity (7). As such, the enhanced ability
of lung CD11c+ cells to induce regulatory T-cell development may be due to their capacity to produce high levels of TNF-α. The observation that lung CD11c+ cells are able to induce both IFN-γ production by T cells and regulatory T-cell development may seem contradictory; however, such abilities may allow lung APCs to both induce and regulate T-cell immunity to protect the host against pathogens on one hand and to avoid overwhelming immunopathology on the other.

To our knowledge, this is the first study in which colon and lung CD11c+ cell functions were directly compared in detail, and the data strengthen the concept that different mucosal tissues are immunologically unique. We expect similar differences exist between cells from other mucosal and nonmucosal tissues; thus, more studies are required to clearly understand the nature of APCs residing in different tissues; this knowledge would be instrumental in the development of vaccines and therapeutic methods that are best suited for each tissue site.

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