Rapid Dendritic Cell Mobilization to the Large Intestinal Epithelium Is Associated with Resistance to *Trichuris muris* Infection


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Rapid Dendritic Cell Mobilization to the Large Intestinal Epithelium Is Associated with Resistance to *Trichuris muris* Infection


The large intestine is a major site of infection and disease, yet little is known about how immunity is initiated within this site and the role of dendritic cells (DCs) in this process. We used the well-established model of *Trichuris muris* infection to investigate the innate response of colonic DCs in mice that are inherently resistant or susceptible to infection. One day postinfection, there was a significant increase in the number of immature colonic DCs in resistant but not susceptible mice. This increase was sustained at day 7 postinfection in resistant mice when the majority of the DCs were mature. There was no increase in DC numbers in susceptible mice until day 13 postinfection. In resistant mice, most colonic DCs were located in or adjacent to the epithelium postinfection. There were also marked differences in the expression of colonic epithelial chemokines in resistant mice and susceptible mice. Resistant mice had significantly increased levels of epithelium-derived CCL2, CCL3, CCL5, and CCL20 compared with susceptible mice. Furthermore, administering neutralizing CCL5 and CCL20 Abs to resistant mice prevented DC recruitment. This study provides clear evidence of differences in the kinetics of DC responses in hosts inherently resistant and susceptible to infection. DC responses in the colon correlate with resistance to infection. Differences in the production of DC chemotactic chemokines by colonic epithelial cells in response to infection in resistant vs susceptible mice may explain the different kinetics of the DC response. *The Journal of Immunology*, 2009, 182: 3055–3062.

Although diseases of the large intestine are among the most prevalent in the world, surprisingly little is known about how immune responses are initiated, activated, and regulated in the large intestine. The epithelium lining the large intestine is composed of a single layer of cells that act as a physical barrier between commensal bacteria in the intestinal lumen and the immune cells in the underlying lamina propria. The interaction between the intestinal flora and the epithelium is complex and important for epithelial homeostasis (1, 2). Colonic epithelial cells (CEC) can actively sense and respond to commensal bacteria, microbiota, and pathogens via expression of pattern recognition receptors (3, 4). Ligation of pattern recognition receptors in epithelial cells drives secretion of effector molecules such as chemokines and cytokines (3, 4), which have the potential to recruit and activate immune cells including dendritic cells (DCs) from the colonic lamina propria to the epithelial layer.

DCs direct immune responses by responding to the pathogen and directing the appropriate T cell responses. DCs activate and direct T cell bias (Th1, Th2) and the type of immune response (inflammation vs tolerance), but little is known about how DCs function in the tissue microenvironment of the large intestine. This contrasts with the considerable body of literature on tissue-resident DCs in other regions of the gastrointestinal tract including the small intestine (5–7). Although it is possible that there are some commonalities between mucosal responses and DC function in the small and large intestine, this seems unlikely given the clear differences in their anatomy, physiology, and function. For example, the large intestine has a distinct and more complex microbiota several magnitudes greater in number than that of the small intestine. In the small intestine, DCs are found primarily in two compartments: Peyer’s patches; and the lamina propria. The colon lacks conventional Peyer’s patches and has few M cells, although structures such as colonic patches and isolated lymphoid follicles may be analogous to Peyer’s patches and represent primary sites of Ag uptake in the colon (8). Within the Peyer’s patches, DCs access luminal Ags indirectly via specialized intestinal epithelial M cells (5–7); whereas in the lamina propria, CX3CR1 expressing DCs may directly sample lumen Ags via the extension of transepithelial dendrites (9, 10). DCs with transepithelial dendrites are not found constitutively in the terminal ileum. However, they can be induced in response to infection (11). In contrast, there are few DCs in the naive large intestine (12). Also, the ability of colonic DCs to extend dendrites into the intestinal lumen has not been demonstrated...
for colonic DCs suggesting that this may be a rare or nonevent (13). The importance of colonic DCs is, however, demonstrated in infection in which clearance of bacteria is dependent on cecal DCs (13). There is therefore significant regional diversity in DC subset and function and most likely in epithelial/DC cross-talk throughout the gastrointestinal tract.

The enteric pathogen Trichuris muris has been used as a model large intestinal infection to address fundamental questions about how immune responses are initiated in the colon and cecum. *T. muris* specifically invades colonic and cecal epithelial cells within the first 24 h of ingesting embryonated eggs (14, 15) eliciting either a Th1 (susceptible) or Th2 (resistant) response depending upon the level of infection or strain of host (14). Here we compare the magnitude and kinetics of DC responses in mice resistant or susceptible to *T. muris* and present evidence for differences in the kinetics and magnitude of the DC response that distinguish between susceptible and resistant animals.

Materials and Methods

**Mice and infections**

BALB/c and AKR mice (Harlan U.K.) were infected orally with 150 embryonated eggs or 20 embryonated eggs (low dose) at 6–8 wk (14, 16). BALB/c given a high dose of *T. muris* are resistant to infection and are termed HD BALB/c or resistant BALB/c throughout this article. BALB/c given a low dose of *T. muris* eggs are susceptible to infection and are termed LD BALB/c or susceptible BALB/c throughout this article. AKR are referred to as susceptible AKR throughout. At least three mice were used per time point. Worm burden counts (days 10–14 postinfection) were taken from each series of infections. For each series of infections, AKR and BALB/c mice were analyzed simultaneously to reduce the variation to *T. muris* and present evidence for differences in the kinetics and magnitude of the DC response that distinguish between susceptible and resistant animals.

**Flow cytometry**

DC-enriched preparations of colonic lamina propria mononuclear cells (12, 17) were stained with Abs to CD11c, CD45, CD80, CD86, MHC II, CD11b, CD8a, CCR7, CCR5, CD103 (BD Biosciences); TLR2, TLR4, CCR2, and CCR6 (provided by Dr. Matthias Mack); CCR6 (Insight Bio-technology); and plasmacytoid DC Ag (Miltenyi Biotec) Abs. Isotype matched Abs of irrelevant specificity were used to determine the level of non-specific staining. Stained cells were analyzed on a FACScalibur flow cytometer using CellQuest software (BD Biosciences). Endocytosis was assessed by measuring the uptake of FITC-dextran (Sigma-Aldrich) by flow cytometry as described previously (12).

**Immunohistochemistry**

Frozen sections (5–20 μm) of cecum and proximal colon were air dried, fixed in ice-cold acetone, rehydrated in PBS, and incubated with anti-CD11c, -MAC-1, -CD4, -B220, -CD8a, -F4/80 (BD Biosciences); -cyto-keratin (Sigma-Aldrich); -claudin 3 (Panomics); -occludin (Zymed Laboratories); -fractalkine receptor (Cambridge Bioscience); and laminin (Abcam) Abs followed by tyramide amplification reagents (PerkinElmer) (12) or secondary Abs conjugated to Texas Red (Abcam) or AF633 (Molecu- lar Probes). Sections were counterstained with the nuclear counterstains 4′,6′-diamidino-2-phenylindole (DAPI) or TO-PRO-3 (Molecular Probes), mounted with Fluormount G (Southern Biotechnology Associates), and viewed using a Zeiss Axiostar 200M microscope (Zeiss) with Axiovision software (Fig. 2, A–F and J) or a Zeiss upright LSM 510 META confocal microscope (Figs. 2, G–I and K; 3, and 4). Specific band pass filter sets for DAPI, FITC, and Texas Red were used to prevent bleed through from one channel to the next. Images were then processed and analyzed using Axiovision software or LSM Zeiss software. Three-dimen-sional data sets (Figs. 3 and 4) were taken from 20-μm-thick sections and then analyzed with Imaris version 4.0.4 software (Bitplane). Negative controls of secondary Ab only and appropriate control IgGs together with a secondary Ab were included in each experiment.

To enumeration of DCs in association with the crypt epithelial cells, only DCs in or immediately adjacent to the epithelial layer (which was identified by either cytokeratin or claudin 3) with evident nuclei (DAPI or TO-PRO-3 stained) were counted. All sections were counted blind and each section was counted two to three times. A minimum of 15 sections per mouse were counted, and a minimum of 3 mice were analyzed per time point.

**Electron microscopy**

Segments of intestine recovered at days 1 and 7 post-peroral infection with *T. muris* were fixed in 1% osmium tetroxide, washed, and stained for 2–4 h in 2% uranyl acetate. After a washing, the samples were dehydrated using acetone, embedded in Araldite resin, and cured at 65°C. Ultrathin sections (100 nm thick) were cut on a Reichert-Jung Ultracut E microtome and collected on a 200 mesh copper grid. After staining with Reynold’s lead citrate, the grid was carbon coated and visualized using a JEOL 1200 EX electron microscope.

**Mesenteric lymph node (MLN) cultures**

MLN cells were prepared from naive AKR and BALB/c mice or from mice at day 7 postinfection with either a high dose (HD BALB/c, AKR) or low dose (LD BALB/c) of *T. muris*. Cells were plated at 5 × 10^6 cells/ml in the presence or absence of *T. muris* excretory secretory Ag (ES Ag) as described previously (18). After 48 h, supernatants were taken and analyzed on a flow cytometer using the CBA Flex set (BD Biosciences) to measure IL-10, IFN-γ, IL-12 p70, and IL-4.

**PCR and quantitative real-time PCR**

The large intestine was excised from AKR and BALB/c mice on day 0, day 1, and day 7 post-*T. muris* infection separated into cecum, proximal colon, and mid-distal colon for epithelial cell isolation (3, 4). Briefly, sections of intestine (cecum, proximal colon, or mid/distal colon) were cut longitudinally, cleaned to remove fecal debris, cut into 5-mm segments, and incubated in 1 mg/ml dispase 1 in medium for 90 min at 37°C under gentle agitation. The resultant cell suspension was washed, and the purity of the resultant cells was confirmed by flow cytometry. RNA was extracted using Tri-reagent, and 1 μg was used to generate cDNA using Superscript II (Invitrogen). cDNA was amplified with Reddy-mix Taq polymerase (Ab-genec), and quantitative PCR was performed using SensiMixPlus SYBR (Quantace) on an OPTICON DNA engine with OPTICON Monitor soft-ware version 2.03 (Real-Time Systems; MJ Research). β-Actin was used to control for the starting amount of cDNA. Expression levels of genes of interest are shown as fold change over that in naive animals after normalization to β-actin using the ΔΔCt method.

**Primers**

To determine the expression of specific Ags in cecum, we used the following primers (Table 1).

**Statistics**

Data are represented as means ± SEM. Data were analyzed using a Kruskal-Wallis test with a Dunn multiple comparison test.

**Results**

**Rapid recruitment of colonic DCs in resistant but not susceptible mice after *T. muris* infection**

BALB/c mice given a high dose of *T. muris* are resistant to infection and are termed HD BALB/c or resistant BALB/c, whereas BALB/c given a low dose of *T. muris* eggs are susceptible to infection and are termed LD BALB/c or susceptible BALB/c. AKR mice are referred to as susceptible AKR throughout.

Colonic DCs (CD45^+ CD11c^+ MHC II^+ cells; Fig. 1, A and B) were enumerated in AKR and BALB/c mice before and at days 1 and 7 postinfection with 150–200 (high dose) or 20 (low dose) embryonated eggs. The total number of colonic DCs in naive BALB/c mice (~5 × 10^6) was higher than in naive AKR mice (~2.5 × 10^6; Fig. 1C), although this did not reach statistical signif-

### Table 1. Primer information

<table>
<thead>
<tr>
<th>Primer</th>
<th>Forward Sequence</th>
<th>Reverse Sequence</th>
</tr>
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<tbody>
<tr>
<td>T. muris</td>
<td>GCTCCCGGGCGTATTCT</td>
<td>reverse, TTTCCATGTGCTCCAGTTGG</td>
</tr>
</tbody>
</table>
susceptible mice at the same time point. BALB/c mice (Fig. 1 D). Strains of mice, the remaining DCs were CD11b+CD45+ CD8− DCs, which represented a minor subset (1–2%) of colonic DCs (Fig. 1 D). At day 7 postinfection, there was a decrease in the proportion of myeloid DCs (∼55%) and a relative increase of CD8α−CD11b+CD11c− colonic DCs (∼30%; data not shown) in resistant BALB/c mice. The proportions of lymphoid and plasmacytoid DCs were unchanged throughout infection.

**DCs localize to the epithelium in resistant but not susceptible mice**

In the colon of naive AKR and BALB/c mice, DCs were rare and localized primarily to the colonic lamina propria with only occasional DCs adjacent to the epithelium (typically 1 or none/crypt; Fig. 2, A, G, and L). In the cecum, a distribution and proportion of DCs similar to that in colon were seen in naïve BALB/c and AKR mice (data not shown). Similar to the flow cytometry data (Fig. 1), fewer DCs were detected in the colon and cecum of naïve AKR mice (Fig. 2G) compared with naïve BALB/c mice (Fig. 2A).

As observed by flow cytometry, in resistant BALB/c mice at day 1 postinfection, more colonic DCs were detected than in susceptible BALB/c and AKR mice (Fig. 2, B, E, and H). This trend was also seen in the cecum (data not shown). In addition, DC localization was also altered in resistant BALB/c postinfection, with the majority residing in large aggregates within lymphoid follicle-like aggregates or, in close proximity to the epithelium along the full length of the crypt. Indeed, quantification of colonic DCs revealed that significantly more DCs (p < 0.0001) were found in close association with CECs (5 ± 0.4 DC/crypt; Fig. 2L) on day 1 after T. muris infection than in naïve mice or susceptible mice (typically one or none per crypt). Also, consistent with flow cytometry analysis (Fig. 1), there were more DCs in the large intestine of resistant BALB/c mice (Fig. 2C) than in that of susceptible AKR mice (Fig. 2, I and L) or susceptible BALB/c mice (Fig. 2F) at day 7 postinfection. In these animals, colonic DCs were clustered around the epithelium, although they tended to be in aggregates associated with the epithelium rather than single cells, as seen at day 1 postinfection. Overall, the number and size of the lymphoid follicles increased during infection in resistant BALB/c mice but not in susceptible AKR mice, consistent with previous reports of lymphoid aggregates developing later after infection in AKR mice (19).
at days 2, 3, and 4 postinfection with a similar trend showing increased colonic DCs in resistant but not susceptible mice (data not shown). To rule out the possibility of a general defect in DC recruitment in AKR mice, a later time point postinfection was assessed. At day 13 postinfection in AKR mice there was an increase in the number of DCs with many clustering around the epithelium (Fig. 2J) similar to that in BALB/c mice on day 1 postinfection (Fig. 2B).

Collectively, these data demonstrate that there is a delay in the DC response in susceptible mice compared with resistant mice. Furthermore, DC expansion correlates with an increase in the proportion of the DCs adjacent to the epithelium.

**DC-epithelial cell interactions in resistant mice postinfection**

Electron microscopy, confocal imaging, and three-dimensional tissue reconstruction were used to investigate the possibility that DCs make contact with epithelial cells during *T. muris* infection in resistant BALB/c mice. A small proportion (<5%) of DCs appeared to be in contact with epithelial cells (Fig. 3). Analysis of the tight junction proteins claudin 3 and occludin revealed that the cell surface of these DCs colocalized with or coexpressed tight junction proteins (Fig. 3C and data not shown). Among the DCs in the epithelial layer, the majority had long cellular dendritic processes extending between adjacent epithelial cells (Fig. 3). In some instances, the dendritic processes extended into the lumen (Fig. 3, A–D) with some containing electron-dense material resembling apoptotic cellular debris (Fig. 3D).

Transepithelial dendritic processes among epithelia-associated colonic DCs were observed only in resistant BALB/c mice after infection. In naive mice or susceptible BALB/c and AKR mice, very few DCs were seen adjacent to the epithelium with the majority residing deeper within the lamina propria. From >50 sections of 6 or more naive mice (BALB/c and AKR), only one DC was found to have short dendrite projections that extended between epithelial cells. This suggests that the formation of transepithelial dendrites is associated with a postinfection event and too rare to be observed in naive mice.

Postinfection, a large proportion of DCs were found directly underneath the epithelial layer (Fig. 3). To assess whether DCs were in the epithelial layer, the epithelial basement membranes were stained using anti-laminin Abs (Fig. 4). Before infection, no DCs had breached the epithelial basement membrane and crossed the epithelial layer (Fig. 4, A and B). In contrast, postinfection, several DCs were observed to have breached the basement membrane and were in direct contact with the epithelium (Fig. 4, C–E). These data confirm the confocal and electron microscope images in Fig. 3 and demonstrates that postinfection DCs are recruited into the epithelium, many of which had dendrite processes extending between epithelial cells (Fig. 3, A–D). However, although DCs were clearly seen in the epithelium, the laminin staining showed that postinfection the majority of DCs were adjacent to the epithelial basement membrane and had not crossed it.

**Distinctive colonic DCs in infected resistant and susceptible mice**

Colonic DCs from BALB/c mice expressed higher levels of CD80, CD86, and MHC class II (MHC II) than DCs from AKR mice (Fig. 5, A and B, and data not shown). Only expression of MHC II was statistically significantly higher in naive BALB/c mice compared with AKR mice (*p < 0.01*). In susceptible mice, expression of CD80 and CD86 by colonic DCs was unchanged during infection (Fig. 5B and data not shown). By day 1 postinfection, CD86 expression increased significantly on colonic DCs from resistant BALB/c mice (Fig. 5B; *p < 0.05*); whereas MHCII expression remained unchanged (Fig. 5A). Consistent with an immature phenotype, DCs at day 0 and day 1 were endocytic (Fig. 5F) with <5% expressing the chemokine receptor CCR7 (Fig. 5E). At day 7 postinfection, MHC II expression increased almost 2-fold on colonic DCs in resistant BALB/c mice but changed little among DCs from susceptible mice (*p < 0.01*; Fig. 5A). Consistent with colonic DCs from day 7-infected HD BALB/c mice having a mature phenotype the majority expressed CCR7 (Fig. 5E) and had relatively low levels of endocytic activity, with 50% fewer DCs taking up FITC-dextran (*p < 0.05*) compared with naive animals (Fig. 5F). In contrast, DCs from susceptible BALB/c mice showed

![Figure 3](http://www.jimmunol.org/)
FIGURE 4. In response to infection DCs cross the epithelial basement membrane. Immunofluorescent images of sections of colon from resistant BALB/c mice before (A and B) and 1 day postinfection (C–E) with a high Ag dose of *T. muris* (BALB/c HD). Sections of colon were stained for DC (CD11c, green), basement membrane (laminin, red) and counterstained with DAPI (blue) A–D: A and C, the original images overexposed to visualize autofluorescent epithelial cells (red); B and D, higher magnification of the insets in A and C showing DCs beneath the epithelial basement membrane before infection (B), but after infection they are breaching the basement membrane and are in the epithelial layer (D and E). L, Lumen; e, epithelium. E, shows isosurface volume rendering of the confocal Z-sections from C. Grid squares, 5 μm². Isosurface volume rendering was conducted with the Surpass module within Imaris version 5.7 (Bitplane).

no up-regulation of MHC II (Fig. 5A) during infection with <5% of DCs expressing CCR7 (Fig. 5E) consistent with their being immature. DCs from AKR mice also had low levels of MHC II and costimulatory molecules throughout infection, and endocytic activity was unaltered throughout infection (Fig. 5F). However, at day 7 postinfection, DCs from AKR mice did up-regulate CCR7 expression (Fig. 5E), suggesting that these DCs represent semimature DCs. The proportion of MLN DCs was assessed at day 0, day 1, and day 7 postinfection (Fig. 5G). There was no increase in the proportion of DCs in the nodes of BALB/c mice and in AKR mice postinfection (Fig. 5G). The DCs in the MLNs were MHC II⁺ DCs that expressed moderate levels of CD80 and CD86 consistent with a mature phenotype. To investigate the possibility that DCs in MLNs at day 7 postinfection promote T cell-mediated immunity, the ability of MLN cells to respond to *T. muris*-derived ES Ag was investigated. In cultures of MLN cells stimulated with ES Ag, there was no induction of either Th1 (IFN-γ and IL-12) or Th2 cytokines (IL-4 or IL-10) (data not shown). These observations support the finding that at day 7 postinfection, DCs are not being recruited to the MLNs to promote T cell activation in either the AKR or BALB/c mice.

Expression of TLR2 and TLR4 was analyzed on DCs pre- and postinfection in BALB/c and AKR mice. TLR2 was constitutively expressed by ~60% of DCs (Fig. 5C) in both strains of mice. Following infection, TLR2 expression did not change significantly in resistant or susceptible animals. In contrast, TLR4 expression was up-regulated by 2-fold more DCs in naive BALB/c mice than in AKR mice (Fig. 5D), although this was not statistically significant. TLR4 expression was unchanged in all mouse strains at day 1 postinfection. Up to day 7 postinfection, there was a substantial increase in the number of DCs expressing TLR4 in both resistant BALB/c and susceptible AKR mice (Fig. 5D; p < 0.05 compared with naive animals).

Chemokines are major triggers for DC recruitment therefore we investigated the expression of chemokine receptors on colonic DCs. The majority of colonic DCs expressed CCR5 (65 ± 2.96%) with approximately half expressing CCR6 (51.1 ± 2.2%). CCR2 was expressed on only a subset (22.2 ± 3.07%) of DCs. Given the intraepithelial nature of *T. muris*, the possibility that epithelial-derived signals (chemokines) trigger DC mobilization was explored next.
Increased production of DC chemoattractants by CECs in resistant mice promote DC recruitment. Epithelial cells (n = 4) from the proximal colons of resistant BALB/c, susceptible AKR, and susceptible BALB/c mice were analyzed for expression of CCL2, CCL3, CCL5, CCL20, and TSLP mRNA 0 and 24 h (A) and 7 days (B) postinfection by quantitative PCR. Data are the relative expression of mRNA compared with samples from naive real-time animals. C. To assess whether CCL5 and CCL20 were necessary for DC recruitment in resistant BALB/c mice, BALB/c mice were infected with T. muris and given i.v. neutralizing Abs to CCL5 and CCL20 (left column) or control rat IgG (right column). After 24 h, the DCs were enumerated by flow cytometry. In Ab-treated mice, there was a complete ablation of DC recruitment (***, p = 0.0001, n = 8).

Discussion

We have identified striking differences in the kinetics and magnitude of the DC response in the colon and cecum early in the host response to helminth infection in resistant vs susceptible animals. The T. muris model is well defined with resistance and worm expulsion being dependent on a Th2 CD4 T cell response and susceptibility to infection and inability to shed worms being linked to a Th1 response (20). The mechanisms underlying different Th cell responses are not known. Using two different models of Th1-mediated susceptibility and a resistant mouse model, we found evidence of a delayed DC response in susceptible AKR mice. The lack of an early DC response in AKR mice was not strain specific, given that BALB/c mice rendered susceptible to infection by administration of a low dose of eggs also lacked an early DC response. The early DC response in resistant mice was associated with an up-regulation in the epithelial cell-derived chemokines CCL5 and CCL20, suggesting that the DC response may be driven by epithelial-mediated factors. Little is known about the initial immune response to T. muris and significant CD4+ T infiltrates are only seen at day 14 postinfection. No mouse strain has been found to expel a primary infection before 10 days postinfection. Thus, the early recruitment of DCs in resistant mice occurring several days before the development of adaptive immune responses (20) might facilitate the generation of effector immune responses.

Chieppa et al. (11) have shown that DCs are recruited to the small intestinal epithelium after epithelial TLR stimulation. It is tempting to speculate that recognition of T. muris via epithelial TLRs could contribute to the recruitment of DCs in the large intestine. However, to date no TLRs for T. muris have been identified. We have previously shown that ligation of colonic epithelial TLRs and the interaction of epithelial cells with T. muris ES Ag both result in secretion of chemokines known to be DC chemoattractants (3, 4, 21). Because chemokines are the major mediators of DC migration (22, 23), we explored the possibility that epithelial-derived chemokines in the proximal colon and cecum mediate DC recruitment in resistant mice. In resistant BALB/c mice, there was a strong increase of epithelial-derived CCL2, CCL3, CCL5, and CCL20 24 h postinfection compared with susceptible AKR mice. The secretion of chemokines was highest in the regions of the...
intestine in which *T. muris* larvae reside (i.e., the cecum and proximal colon), whereas the magnitude of induction of chemokine expression was lower elsewhere in the large intestine. The induction of CCL2 seen in BALB/c contrasts with our earlier published findings in which we found no difference in CCL2 secretion (21). This apparent discrepancy may be due to the different isolation strategies used to isolate epithelial cells (EDTA vs dispase) and the fact that we analyzed specific regions of the large intestinal epithelium (cecum, proximal colon, and middistal colon) vs the intact large intestine. We saw differential expression of chemokines between the different intestinal regions, with the most significant changes being in the cecum and proximal colon such that any differences may be masked when analyzing the entire intestinal epithelium in our earlier study (21). EDTA isolation is associated with reduced cell viability compared with dispase isolation and has been shown to specifically enrich for cells from the base of the epithelial crypts (2). The major difference between resistant and susceptible mice was epithelial-derived CCL5 and CCL20. Using C57BL/6 mice, we have shown that a number of chemokines including CCL5 are chemotactic for colonic DCs in vitro (S. M. Cruickshank and S. R. Carding, unpublished findings and Footnote 5). Furthermore, we now show that blocking CCL5 and CCL20 prevented DC recruitment to the colonic mucosa in vivo, emphasizing the importance of these chemokines in DC mobilization. Thus, the differences in epithelial chemokine secretion in resistant vs susceptible mice may be a factor in the enhanced DC mobilization to the epithelium seen in vivo in resistant animals. Accelerated DC responses may contribute to host resistance by facilitating Ag uptake from the epithelium/lumen.

How Ags are taken up by DCs in the large intestine is not known. In the small intestine, two major routes of Ag uptake are indirectly via specialized epithelial M cells within the Peyer’s patches (5, 6) and directly via the extension of transepithelial dendrites on CX3CR1 expressing lamina propria DCs residing immediately beneath the epithelium (6, 7, 9). Colonic DCs are rarely found in the epithelial layer of naive animals as seen here and previously (12). Also, in contrast to the large resident population of DCs found within the lamina propria of the small intestine, few DCs reside in the colon of naive mice. The appearance of DCs with transepithelial dendrites, some of which extended into the colon lumen of *T. muris*-infected resistant BALB/c mice, demonstrates that colonic DCs can form transepithelial dendrites. These transepithelial dendrites may be analogous to those described among DCs in the small intestine, enabling direct sampling of luminal Ags by colonic DCs. Alternatively, dendrites may facilitate Ag transfer from pinocytic CECs. Thus, the rapid recruitment of DCs to the epithelium in BALB/c mice may enhance epithelial-DC interactions, facilitating more rapid or efficient Ag uptake thereby contributing to an effective immune response. Transepithelial dendrites have not been reported in the large intestine before. The fact that transepithelial dendrites were observed only in mice that develop an effective immune response suggests they may be of importance in resistance to infection. The factors underlying the formation of transepithelial dendrites in the large intestine and their function will form the basis for future investigations.

Before DCs can prime T cells, they must undergo a well-characterized process of maturation. There are a number of DC maturation factors including host cytokines and pathogen-derived factors. Maturation of DCs outside the large intestine is associated with induction of CCR7 which facilitates migration to the lymph nodes, increased expression of MHC II, and reduced phagocytic function. In resistant mice, there was clear evidence of earlier maturation of colonic DCs; whereas DCs from susceptible BALB/c mice remained immature. Although DCs from susceptible AKR mice had increased expression of CCR7 postinfection, it is unlikely that they are mature as MHC II expression and endocytic function was unaltered. Because CCR7 expression has been described on a population of semimature DCs (24), it is possible that DCs in infected AKR mice are not fully mature. Although TLR4 expression was increased on DCs from resistant mice, it is not known whether TLRs are involved in recognition of *T. muris*. TLR4-deficient mice (25) are resistant to *T. muris* infection, suggesting that TLR4 expression on DCs is not of primary importance in resistance to infection.

The increased association of DCs with epithelial cells may facilitate epithelial-DC interactions. There is increasing evidence to support the importance of epithelial cells in shaping the ability of DCs to polarize T cell responses. Most notably, epithelial-associated TLSLP has been shown to be important for the generation of Th2 immunity and worm expulsion (26). In keeping with this observation, we found a marked and rapid up-regulation of epithelial-derived TLSLP in resistant BALB/c compared with susceptible AKR mice. Interestingly, this rapid up-regulation in TLSLP expression was also seen in BALB/c mice made susceptible to infection suggesting that there may be other factors involved in polarizing Th2 immunity and that increased production of TLSLP is an innate feature of BALB/c mice in response to injury or infection. Consistent with this observation, we found a marked and rapid up-regulation of epithelial-derived TLSLP in resistant BALB/c mice compared with susceptible AKR mice in which there was down-regulation of TLSLP. Because TLSLP is important for the generation of Th2 immunity (26, 27), it is possible that its down-regulation promotes skewing to a Th1 immune response. However, in BALB/c mice made susceptible to infection and that do not develop Th2 immunity, there was also a rapid up-regulation of TLSLP expression, suggesting there could be other factors involved in polarizing Th2 immunity and that increased production of TLSLP is an innate feature of BALB/c mice in response to injury or infection.

In summary, we have described for the first time key differences in the kinetics of the early innate immune response of epithelial cells and DCs of the large intestine to *T. muris* that might underlie qualitative differences in the subsequent adaptive immune response.

**Disclosures**
The authors have no financial conflict of interest.

**References**

5 S. M. Cruickshank and S. R. Carding. Submitted for publication.


