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The intracellular protozoan Toxoplasma gondii infects ~20–50% of the U.S. population and is one of the most common parasitic infections worldwide. After an acute disseminating phase that originates from infection in the intestinal mucosa, long-term latency is established in tissues of the skeletal muscle and CNS (1). The parasite is normally asymptomatic, but it can reactivate, causing life-threatening disease in immunocompromised populations (including AIDS patients) (2). Congenital infection with Toxoplasma can cause catastrophic birth defects (3). Toxoplasma is currently the second most frequent cause of death from foodborne infections in the United States (4).

Investigation of the host defense response following oral Toxoplasma inoculation has yielded many insights into function and dysfunction in the intestinal mucosal immune system (5, 6). For example, under low-dose infection conditions, the parasite triggers a protective Th1 response mediated by inflammatory monocytes in the intestinal mucosa (7, 8). This response enables host survival, parasite dissemination, and establishment of latent infection. High-dose infection in the intestine triggers proinflammatory intestinal pathology from which mice do not recover. In this case, intestinal damage is associated with a collapsing regulatory T cell (Treg) population and epithelial lesions, concomitant with overgrowth and translocation of gram-negative species (9–11).

The parasite is known to influence several lineages of innate immunity, including dendritic cells (DC) (12). DC critically bridge innate and adaptive immunity through their exquisite capacity to drive Ag-specific T cell activation and effector subset differentiation. Furthermore, DC are central players in determining tolerance versus immunity during inflammation and infection (13). For the case of Toxoplasma, DC are obligatory for IL-12–dependent Th1 induction and resultant host resistance to T. gondii (14–16).

DC are understood to be a heterogeneous population with distinct surface markers, transcription factor requirements, and functions (17, 18). All DC originate from a common bone marrow progenitor, but they subsequently differentiate into distinct subsets, including monocyte-derived DC, conventional DC, and plasmacytoid DC. Several key cytokines and transcription factors have been implicated in controlling DC developmental pathways, and recent gene mapping studies have begun to elucidate the order in which these factors become expressed (19). For example, through the activity of β-catenin, transcription factor IRF8 is involved in the generation of splenic CD8α+ and CD11b+CD103+ tissue DC, whereas IRF4 is important for the differentiation of CD11b+CD103+ DC in the intestinal lamina propria (LP) (16, 20, 21). The transcription factor Zbtb46 was recently identified as essential in generation of all classical DC lineages (22, 23). Elegant studies are beginning to identify functional differences among DC subsets. Splenic CD8α+ DC appear specialized in cross-presentation and production of IL-12 during infections with microbes, including T. gondii (15, 24). CD103+ tissue DC possess the unique ability to induce tolerogenic Tregs (25, 26). Nevertheless, there is still much to be understood about how DC subsets differ in function under steady-state conditions, and, importantly, how this changes in response to infection and inflammation.

In this study, we investigated the behavior of distinct subsets of mucosal DC following oral inoculation with Toxoplasma. We analyzed several distinct parameters including infection status, IL-12p40 production, activation state, and retinoic acid production in intestinal DC subsets before and after infection. Our results indicate dynamic changes brought about by Toxoplasma infection that are DC subset specific and that alter the DC compartment as a whole.

Materials and Methods

Ethics statement

All experiments in this study were performed strictly according to the recommendations of the Guide for the Care and Use of Laboratory
**RFP OV A (Pru-RFP) strain. Cysts were generated by an initial i.p. injection of**

**Infections were initiated in 8–12-wk-old mice by oral inoculation of 30 cysts a breeding colony at Cornell University College of Veterinary Medicine. YFP** mice were obtained from The Jackson Laboratory and maintained as

**Included anti-IRF8 PerCP and anti-IRF4 e450 (eBioscience). All samples meabilization buffer (eBioscience). Abs used for intracellular cytokine staining, bated with primary Abs resuspended in the Foxp3/transcription factor per-staining kit fixative (eBioscience, San Diego, CA) and subsequently incu-

tracellular staining, cells were fixed using the Foxp3/transcription factor expression was measured by upregulation of YFP.

**Ly transport (50 mg/ml); Sigma-Aldrich, St. Louis, MO) and rotated at 150 rpm for 45 min. The resulting suspension was passed through a 40-μm filter, washed, and maintained on ice until use. Mesenteric lymph nodes were minced in PBS, cultured with 0.1 mg/ml Liberase TL (Roche Diagnostics, Indianapolis, IN) for 20 min at 37˚C, passed through a 40-μm filter, and washed to create a single-cell suspension. Splenocyte single-cell suspensions were prepared by crushing spleens through a 40-μm filter and lysing RBCs with ammonium-chloride-potassium buffer.

**Flow cytometry**

Single-cell suspensions were washed in PBS prior to resuspension in Zombie Aqua viability dye (BioLegend, San Diego, CA) for 15 min at room temperature to exclude dead cells. Primary Abs (anti-CD11c eFlour610, anti-CD80 Pacific Blue or allophycocyanin-Cy7, anti-CD11b allophycocyanin-Cy7, anti-CD103 BV421, anti-MHCII Alexa700 or FITC, anti-CD64 allophycocyanin, anti-CD54 PE-Cy7, Ly6C BV421, IAB PE-Cy7) re-suspended in ice-cold FACS buffer (1% BSA/0.01% NaN3 in PBS) were added directly to the cells for 30 min. Pre-RRP parasites were detected in the PE channel. IL-12p40-YFP was detected in the FITC channel. For intracellular staining, cells were fixed using the Foxp3/transcription factor staining kit fixative (eBioscience, San Diego, CA) and subsequently incu-

bated with primary Abs resuspended in the Foxp3/transcription factor permeabilization buffer (eBioscience). Abs used for intracellular cytokine staining included anti-IRF8 PerCP and anti-IRF4 e450 (eBioscience). All samples were run on an LSRII flow cytometer (BD Biosciences, San Jose, CA), and the data were analyzed using FlowJo software (FlowJo, Ashland, OR).

**Ex vivo soluble tachyzoite lysate stimulation**

LP leukocytes were harvested into a single-cell suspension from naive Yet40 reporter mice and stimulated ex vivo with 50 μg/ml soluble tachyzoite lysate Ag (STAg). Cells were incubated at 37˚C for 4.5 h, and IL-12p40 expression was measured by upregulation of YFP.

**Retinoic acid metabolism measurement**

To measure retinoic acid metabolism, an AldeFluor assay kit was used according to the manufacturer’s instructions (StemCell Technologies, Vancouver, BC, Canada). Cells were resuspended in the provided assay buffer, and 5 μl AldeFluor reagent was added with or without 5 μl of the DEAB inhibitor. The cells were then incubated for 45 min at 37˚C, washed with assay buffer, and then incubated in primary Abs resuspended in assay buffer for 20 min at 4˚C. Cells were washed once more in assay buffer and then analyzed with a LSRII flow cytometer (BD Biosciences).

**Annexin labeling**

For Annexin V labeling, cells were stained following the manufacturer’s instructions for the Annexin FITC kit (eBioscience). Cells were washed in 1X binding buffer, resuspended in 100 μl binding buffer, and 5 μl Annexin V FITC was then added. Cells were incubated for 10–15 min at room temperature and washed with binding buffer prior to analysis with an LSRII flow cytometer (BD Biosciences).

**LP DC sorting**

LP leukocytes were subjected to a Pan-DC Enrichment negative selection kit (EasySep) with 3 μg biotinylated anti-EpCAM (eBioscience) added. Resulting enriched cells were resuspended in 50 μl Aqua Zombie viability dye (Bio-Legend) for 15 min at room temperature. Next, 500 μl of a DC Ab mixture in MACS buffer (5 μl each: anti-CD45 PE-Cy7, anti-MHCII FITC, anti-CD11c e610, anti-CD64 allophycocyanin, anti-CD11b allophycocyanin-Cy7, anti-CD103 BV421) was added. Cells were incubated for 30 min at 4˚C, washed in MACS buffer, and passed through a filter-top FACs tube prior to sorting with a FACSaria (BD) for LP-DC populations I–IV. Cells were sorted into DMEM supplemented with 20% BGS, 25 mM HEPES, and 2 mM EDTA and cultured in complete DMEM (DMEM supplemented with 10% BGS, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 0.05 mM β-mercaptoethanol, 300 U/ml penicillin, 300 μg/ml streptomycin, and 30 mM HEPES) with or without STAg (50 μg/ml) stimulation for 24 h. IL-12p40 was measured from the supernatants using an in-house ELISA (27).

**Statistical analyses**

Statistical significance between groups was assessed using Student t test. Differences between three or more groups were determined using one-way ANOVA with Neuman–Keuls posttest.

**FIGURE 1.** Small intestinal LP DC populations are stable during oral Toxoplasma infection. (A) WT mice were orally infected with 30 ME49 Toxo-

plasma cysts, and live CD45+ cells from the SI-LP of naive and Toxoplasma-

infected mice were assessed for MHCII and CD11c expression. Total numbers of MHCII+CD11c+ cells were quantified in the SI-LP of naive and infected mice (n = 6 mice/group). (B) CD64 expression was measured on MHCII+CD11c+ cells harvested from naive and infected SI-LP to distinguish monocytic cells (CD64+), from bona-

fide DC (CD64-). In the bar graphs, percentages of each group were con-

verted to absolute numbers (n = 8 mice/group). (C) After gating on bona fide DC phenotype (LiveCD45+MHCII+CD11c+CD64-) cells were assessed for CD103 and CD11b expression to distinguish subpopulations of SI-LP DC (designated populations I–IV). The bar graph shows average percent-

ages over multiple mice (n = 6 mice/group). (D) Total numbers of inflam-

matory monocytes (Ly6C+CD11b+) were quantified in the SI-LP of naive and day 7–infected mice (naive, n = 3 mice; infected, n = 6 mice). Repre-

sentative data from individual mice and the means and SE for multiple mice are shown. Data are representative of at least three experiments. ***p < 0.001.
FIGURE 2. Compartmentalized IL-12 production during Toxoplasma infection. (A) Yet40 reporter mice were orally infected with 30 ME49 T. gondii cysts, and single-cell suspensions were prepared from spleen (SPL), MLN, and LP 7 d later. IL-12p40 production was then measured in total DC (MHCII⁺CD11c⁺ cells) based on YFP expression. (B) The mean (± SE) percent of IL-12p40⁺ DC calculated from mice (n = 7 mice/group). (C) IL-12p40 production by LP PMN (Ly6G⁺CD11c⁺), inflammatory monocytes (IM; Ly6C⁺CD11b⁺CD11c⁺) and bulk DC (CD11c⁺CD64⁻) was examined in Yet40 reporter mice. (D) Single-cell suspensions were prepared from LP and MLN of naive and day 7 orally infected Yet40 mice. Cells were gated on Live⁺CD45⁺MHCII⁺CD11c⁺CD64⁻, and IL-12p40 expression was determined based on YFP expression in DC populations I (CD103⁺CD11b⁻), II (CD103⁺CD11b⁺), III (CD103⁻CD11b⁺), and IV (CD103⁻CD11b⁻). Representative FACS plots are shown. The percent IL-12-YFP expression by populations I–IV over multiple mice is shown in (E) (LP compartment) and (F) (MLN compartment). (G) DC populations I–IV were FACS purified from LP suspensions prepared from naive mice using a FACSaria (purity ~ 80–95%). DC were then cultured in the presence of media or STAg (50 μg/ml) for 24 h. Supernatants were collected and assayed for IL-12p40 with ELISA. In this experiment, IL-12 production fell below the detection level (1 pg/ml) for media groups and STAg-stimulated populations II, III, and IV. (H) LP suspensions were prepared from naive Yet40 mice and stimulated in vitro with media or STAg (50 μg/ml) for 5 h. Cells were then surface stained for DC markers and evaluated for IL-12p40-YFP expression among each DC subset by flow cytometry. Data are representative of three independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001.
Results

Intestinal monocytes rapidly expand while DC numbers remain stable during oral Toxoplasma gondii infection

To understand the dynamics of intestinal monocytes/DC following oral inoculation with *T. gondii*, levels of CD11c<sup>+</sup>MHCII<sup>+</sup> cells were quantified from the small intestinal LP of naive and mice infected on day 7. Naive mice possessed a relatively small population of CD11c<sup>+</sup>MHCII<sup>+</sup> cells, but that cell population significantly expanded in the intestines of infected mice (Fig. 1A). Myeloid cells in the intestine are composed of CD64<sup>+</sup> monocytes--macrophages and CD64<sup>-</sup> DC. The CD11c<sup>+</sup>MHCII<sup>+</sup> cells from naive mice were primarily CD64<sup>-</sup>, suggesting identity with DC (Fig. 1B). However, the vast majority (~80–90%) were CD64<sup>+</sup> in infected mice, suggesting a monocyte--macrophage origin (Fig. 1B). Indeed, the total number of CD64<sup>-</sup> bona fide intestinal DC was unchanged over the course of infection, whereas there was a clear expansion of CD64<sup>+</sup> monocytes--macrophages in the LP (Fig. 1B, bar graphs).

To assess whether DC were altered by infection at the level of individual subsets, DC (defined as MHCII<sup>+</sup>CD11c<sup>+</sup>CD64<sup>+</sup>) from naive and infected small intestinal (SI)-LP were classified into four groups (designated I–IV hereafter) based on expression of CD103 and CD11b (28) (Fig. 1D). Similar to the unchanged total numbers of DC, numbers of individual DC subsets also remained stable during infection (Fig. 1C). These data suggest that *T. gondii* at 7 d post-infection does not influence levels of DC at the global or individual subset level, whereas monocytes are rapidly expanded by infection.

Inflammatory monocytes, which coexpress Ly6C<sup>+</sup> and CD11b<sup>+</sup>, contribute to host defense in the intestinal mucosa following Toxoplasma infection (7, 8). Indeed, we found a pronounced increase in plasma of three experiments. **p = 2 mice; infected, *n* = 4). Data are representative of three experiments. **p < 0.01, ****p < 0.0001.

FIGURE 3. CD8α<sup>+</sup> DC are the primary IL-12 producers in the spleen during Toxoplasma infection. Yet40 reporter mice were orally infected with 30 ME49 *T. gondii* cysts, and splenocytes were harvested 7 d later. (A) Splenic DC (MHCII<sup>+</sup>CD11c<sup>+</sup>) were assessed for expression of CD8α<sup>+</sup> and CD11b<sup>+</sup> markers. (B) CD8α<sup>+</sup> and CD11b<sup>+</sup> DC from naive and Day 7-infected Yet40 reporter mice were evaluated for IL-12p40-YFP expression using flow cytometry. Shown are representative FACS plots for each subset and the means (±SE) of percent IL-12p40<sup>+</sup> DC in naïve and infected mice (naive, *n* = 2 mice; infected, *n* = 4). Data are representative of three experiments. **p < 0.01, ****p < 0.0001.

We next assessed whether similar patterns of IL-12p40 production were observed by SI-LP DC subsets in vitro. The four populations of SI-LP DC were sorted from naive mice and stimulated ex vivo with STAg, a sonicated extract derived from *T. gondii* tachyzoites. As expected, population I (CD103<sup>+</sup>CD11b<sup>−</sup>) DC secreted IL-12p40 after STAg stimulation as determined with ELISA. Surprisingly, and in contrast to the analysis of in situ IL-12p40 production (Fig. 2D, 2E), no detectable IL-12p40 was found in population IV nor in any of the other DC populations (Fig. 2G). To determine whether this result could be replicated using Yet40 IL-12 reporter DC in vitro, LP cells from naive Yet40 mice were stimulated with STAg, and levels of YFP were measured using flow cytometry. Consistent with the sort experiment, IL-12p40-YFP was restricted to population I in vitro, and population IV failed to express cytokine (Fig. 2H). Therefore, whereas CD103<sup>+</sup>CD11b<sup>−</sup> DC are the primary producers of IL-12p40 in response to *T. gondii*, the CD103<sup>−</sup>CD11b<sup>−</sup> DC subset is also capable of upregulating this cytokine during in vivo infection, although this response is not apparent during in vitro culture.

We also examined splenic IL-12p40 production in infected IL-12p40-YFP mice (Fig. 3). Both CD8α<sup>+</sup> and CD11b<sup>+</sup> splenic DC in-
increased IL-12p40 expression after infection. Nevertheless, the CD8α+ DC subset was the major IL-12p40-positive population, which is in agreement with previous studies by others (15, 16, 21, 32). Splenic CD8α+ DC and mucosal population I DC are developmentally related insofar as they depend on transcription factors IRF8 and BATF3 for differentiation, and mice lacking these molecules are defective in IL-12 production during Toxoplasma infection (15, 33). Therefore, our observation that population I (CD103+CD11b−) DC are major sources of IL-12 in the intestinal mucosa is in agreement with these previous studies.

**DC IL-12p40 production correlates with expression of IRF8 and inversely correlates with IRF4**

Previous studies identified roles for IRF4 and IRF8 in respective development of CD103+CD11b+ (population II) and CD103+CD11b− (population I) DC (20, 34, 35). It is also known that IRF8 in splenic DC controls IL-12 production during *T. gondii* infection (16, 21). Therefore, it was of interest to determine intracellular expression of IRF8 and IRF4 in DC populations I–IV. Overall, infection had little or no effect on IRF8 (Supplemental Fig. 2A, 2B) or IRF4 (Supplemental Fig. 2C, 2D) levels among DC subpopulations. However, IRF8 expression was greatest in population I (CD103+CD11b−) DC (Supplemental Fig. 2B), whereas this DC subset expressed the lowest level of IRF4 (Supplemental Fig. 2D). Population II (CD103+CD11b+) expressed the highest levels of IRF4 with a low amount IRF8. Interestingly, IRF8 and IL-12p40 expression were not strictly correlated, inasmuch as population IV was a major source of IL-12p40 in SI-LP DC during in vivo infection (Fig. 2E), yet these DC expressed only modest levels of IRF8 compared with population I DC (Supplemental Fig. 2A, 2B). We

**FIGURE 4.** Parasite-negative DC in the small intestine are the source of IL-12 during infection. (**A–C**) Mice were orally infected with 40 cysts of *T. gondii* Prugniaud parasites expressing RFP (Pru-RFP). (**A**) LP cells were harvested from naive and day 7–infected mice and assessed for Pru-RFP fluorescence in four populations of cells differentiated by expression of Ly6C and CD11b (n = 4 mice/group). (**B and C**) LP DC subsets I–IV were then evaluated for RFP fluorescence to determine infection levels. Shown are representative FACS plots (**B**) and means (and SE) of multiple mice (n = 4 mice/group) (**C**). (**D and E**) To determine IL-12p40 production by infected versus noninfected DC, Yet40 reporter mice were orally infected with 40 Pru-RFP cysts, and SI-LP single-cell suspensions were prepared. Populations I (**D**) and IV (**E**) were examined for expression of IL-12p40-YFP. After setting gates on YFP− and YFP+ populations, Pru-RFP fluorescence was determined. RFP expression in both YFP+ and YFP− cells to determine whether IL-12p40 originated from infected cells. The data are representative of two independent experiments, with n = 4 for each group.
also observed relatively heterogeneous IRF4 staining in naive population IV DC, possibly suggesting further complexity in this DC subpopulation.

IL-12p40 production by SI-LP DC is confined to noninfected cells

Production of IL-12 is a critical step in establishing immunity to Toxoplasma, but it is unclear whether parasite-positive or parasite-negative DC produce IL-12 (36–39). To gain insight into this issue, we inoculated mice with fluorescent Pru-RFP parasites and examined cells in the intestinal LP 7 d later. We first asked which cells harbored parasites overall based on Ly6C versus CD11b expression. Interestingly, although neutrophils (CD11b+Ly6C+; population a) composed a minor proportion of LP leukocytes (2%), there was a clear preferential infection of this cell type in that ~4% harbored parasites (Fig. 4A). CD11b−Ly6C+ cells (population b), likely activated T cells (40), and CD11b+Ly6C+ inflammatory monocytes (population c) both exhibited low levels of infection (0.2–0.3%). The macrophage/DC subset (CD11b+Ly6C−; population d) also contained a subpopulation of infected cells (0.2–0.3%).

Because of the importance of DC-derived IL-12 during Toxoplasma infection, and given the ability of T. gondii to produce molecules triggering this cytokine, we examined infection status as a potential correlate of IL-12p40 production in each DC subset. As shown in Fig. 4B and 4C, we could readily identify subpopulations of infected DC among each of the four DC subsets. Interestingly, there was a preference for infection in population III DC (CD103+CD11b+), with a less striking tropism for infection in population IV DC (CD103−CD11b−). Notably, the most heavily infected DC subset (population III) was not a strong producer of IL-12p40 during infection (Fig. 2E).

Population I and IV DC are the major sources of IL-12p40 among DC subsets (Fig. 2E); therefore, we specifically examined the correlation between infection status and cytokine production in these subsets using Pru-RFP infection of IL-12p40-YFP reporter mice. In Fig. 4D, we identified ∼5% IL-12p40-expressing population I DC. After gating on YFP− and YFP+ cells, we found that all Pru-RFP-positive cells were contained within the IL-12p40-negative population (Fig. 4D). Similarly, we identified 6% IL-12p40-positive population IV DC (Fig. 4E). After gating on YFP+ and YFP− cells, there were no detectable parasites within the IL-12-expressing population.

We next examined the correlation between infection and IL-12 production in splenic DC. As shown in Fig. 5A, ~18% of CD11c+ DC expressed IL-12p40. Interestingly, when we gated on YFP− (no IL-12) and YFP+ (IL-12 expressing) DC, tachyzoite infection was found with >10-fold greater proportion in the YFP+ DC subset (Fig. 5A, 5B). This could also be demonstrated using the reverse approach (Fig. 5C, 5D). Thus, ~0.3% of splenic DC were parasite Pru-RFP-positive; however, when we compared IL-12 expression in RFP− and RFP+ DC, ∼14% of the noninfected DC population expressed IL-12p40, whereas >50% of parasite-positive DC expressed IL-12p40 (Fig. 5C, 5D). Thus, in the spleen most of the infected DC also produce IL-12p40, although there are clearly noninfected DC that produce IL-12p40.

Vitamin A metabolism by SI-LP DC is downregulated during T. gondii infection

A main feature of gut DC is maintenance of immune tolerance, mediated in part by the ability to metabolize vitamin A into retinoic acid using aldehyde dehydrogenases (ALDH), leading to Treg induction (25, 26, 41). Previous studies have shown that T. gondii infection triggers inflammation and disappearance of Tregs (11, 42). Therefore, it was important to determine the effect of Toxoplasma on vitamin A metabolism in population I–IV DC. We used a flow cytometry–based assay that measures ALDH enzymatic activity through production of a fluorescent product (Aldefluor) (43). In naive mice, all subsets displayed ALDH activity, with population II (CD103+CD11b+) DC demonstrating the strongest levels (23%; Fig. 6A). This finding indicates that each SI-LP DC population produces retinoic acid under homeostatic conditions, albeit potentially to differing degrees. However, we found a profound effect of T. gondii infection on DC ALDH activity. Thus, ALDH activity was strongly downregulated by each DC subset during T. gondii infection (Fig. 6A). Reinforcing these data, Fig. 6B shows results of multiple mice and also demonstrates specificity of the effect, because inclusion of the inhibitor DEAB blocks Aldefluor staining. Thus, our data indicate that Toxoplasma infection globally quenches vitamin A metabolism in DC populations in the small intestine.

T. gondii infection induces apoptosis in intestinal DC

Toxoplasma infection has been reported to both promote and inhibit apoptosis in various immune cells, namely lymphocytes and...
macrophages (44–47). To address the effect of T. gondii infection on early programmed cell death in DC, Annexin labeling was performed on SI-LP DC subsets 7 d postinfection to determine cells in early stages of apoptosis. Annexin V was expressed on relatively few SI-LP DC harvested from naive mice, consistent with low levels of apoptosis in steady-state DC (Fig. 7A, 7B). However, the post-infection proportion of Annexin V+ DC sharply increased among all LP DC subsets, suggesting that T. gondii triggers increased apoptosis among DC in the gut (Fig. 7A, 7B). We also examined Annexin staining in CD8α+ and CD11b+ splenic DC before and after infection. In contrast to mucosal DC populations, Toxoplasma infection resulted in a decreased percentage of cells displaying an early apoptotic phenotype (Fig. 7C).

Discussion
The function of DC subsets in immunity and tolerance and the effects of infection on their steady-state properties are important areas of continuing investigation (48, 49). In this study, we examined how oral inoculation with Toxoplasma affected DC subpopulations present in the intestinal mucosa. In agreement with others, we identified four CD11c+ MHCII+ CD64− DC populations in the LP and mesenteric lymph node compartments based on expression of CD103 and CD11b (28). In terms of numbers, these populations remained remarkably stable during infection. This contrasted with a striking infection-induced influx of macrophages into the LP, at least some of which we presume are CCR2-dependent antimicrobial inflammatory macrophages characterized previously (7, 8, 50).

Functional specialization of mucosal DC subsets is not well understood. There is evidence that CD103+CD11b+ DC promote Th17 polarization, and that both CD103+CD11b+ and CD103+CD11b− can induce Th1 differentiation (20, 51, 52). It also appears that CD103+CD11b− DC specialize in cross-presentation to CD8+ T cells (31). The least well-characterized intestinal DC are those that do not express CD103. Nevertheless, CD103−CD11b+ DC can...
trigger both IFN-γ and IL-17–producing effector T cells. In addition, there is evidence that CD103^+CD11b^− DC originate from gut-associated lymphoid tissue, whereas CD103^+CD11b^+ may be resident within the intestinal LP (53).

DC are critical for IL-12 production and induction of protective immunity to Toxoplasma (14). In the spleen, CD8α^+ DC are a major source of this cytokine during infection (15). Among the DC subsets in the intestinal mucosa, two DC subsets (population I, CD103^+CD11b^−; population IV, CD103^+CD11b^+) were the major sources of infection-induced IL-12p40. Population I DC are believed to be developmentally related to splenic CD8α DC, insofar as they share a requirement for transcription factors Id2, IRF8, and Batf3 for their differentiation (54–56). Furthermore, IRF8 itself is implicated in parasite driven DC IL-12 production (16, 21). Thus, our findings that mucosal CD103^+CD11b^− DC produce IL-12p40 in response to Toxoplasma is in line with these previous studies.

There is currently limited understanding of the function of CD103^+CD11b^− (population IV) DC. Accordingly, it is of interest that these cells were a source of IL-12p40 during intestinal Toxoplasma infection. It is possible that this DC subset is somehow related to the population I DC. However, the distinct pattern of IRF8/IRF4 expression in population I versus population IV DC suggests otherwise. Furthermore, when DC subsets from noninfected mice were subjected to in vitro STAg stimulation, population IV DC failed to respond with IL-12p40 production in marked contrast to population I DC. We propose that population IV DC are triggered to produce IL-12 during in vivo infection because of the intestinal inflammatory milieu triggered by the parasite, rather than a direct response to parasite molecules.

Among intestinal DC responding to T. gondii, we determined that IL-12 production was a response of noninfected rather than infected cells. This determination supports a bystander model of T. gondii–induced IL-12 in which cells respond to soluble parasite factors, parasite debris, or host factors, as suggested by other recent studies (57). In contrast, both infected and noninfected DC produced IL-12p40 in the spleen. In fact, most infected splenic DC were found to be positive for IL-12p40 (Fig. 5). The underlying basis for these differing responses is not clear, but it is interesting to note that GRA15 and GRA24, two Toxoplasma proteins recently implicated in IL-12 induction, are directly injected into the cytoplasm of infected host cells (38, 58). In contrast, T. gondii profilin, an IL-12–inducing TLR11/12 ligand, is believed to function within endosomal compartments resulting from phagocytosis of parasite material (16). Accordingly, we hypothesize that IL-12 production in infected DC could indicate a GRA15/GRA24 dominated pathway, whereas IL-12 production in parasite negative DC might reflect a response driven by profilin, or, especially for the case of population IV DC, a response triggered by host-derived factors. Why these responses might differ could be due to several factors including differences in phagocytic activity or unique patterns of TLR expression among DC subsets. These possibilities are currently under examination.

Retinoic acid has a key role in Foxp3^+ Treg generation in the intestinal mucosa, and CD103^+ DC are believed to be an important source of this vitamin A metabolite (25, 26). We found that in steady state, the four DC subsets all produced retinoic acid, although levels differed for each. Nevertheless, we found that the highest levels of retinoic acid were expressed by the CD11b^+CD103^+ population. Strikingly, there was a profound decrease in retinoic acid expression following T. gondii infection in each DC subset. This finding is consistent with the observed collapse in intestinal Treg and emergence of Th1 activity in the residual population that is associated with intestinal infection with Toxoplasma (11). Loss of retinoic acid producing activity in mucosal DC may underlie the emergence of fulminant proinflammatory pathology that is triggered by high infectious doses of T. gondii (6, 59).

We found an overall increase in DC subsets in the LP compartment undergoing apoptosis, as defined by Annexin V staining. This may be a consequence of the overwhelming proinflammatory cytokine response elicited by Toxoplasma in the small intestine and beyond (60). For example, it has been shown that parasite-triggered apoptosis in the intestinal mucosa is blocked by treatment with anti–IFN-γ mAb (46). It is also possible that apoptosis reflects cytotoxic activity of CD8^+ T lymphocytes and NK cells. In this regard, perforin-triggered egress of parasites from infected cells has been proposed as a method used by T. gondii to promote rapid dissemination during early infection (61, 62). Conversely, the decrease in DC apoptosis in the spleen after infection could be related to the antia apoptotic effects of the parasite in infected cells (47). These issues await further investigation.

Infection with Toxoplasma in the intestinal mucosa can activate innate immune cells and induce highly efficient protective Th1 immunity with little overt inflammatory damage (5). At the other extreme, once an infectious dose threshold is crossed, the parasite triggers dysbiosis and translocation of luminal bacteria that culminates in fulfilment proinflammatory cytokine pathology (6). Understanding how these opposite extreme endpoints are controlled by DC subsets in the intestinal mucosa is an area of ongoing investigation with potential for great translational impact in the clinic.

Disclosures

The authors have no financial conflicts of interest.

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