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Virulence of Toxoplasma gondii Is Associated with Distinct Dendritic Cell Responses and Reduced Numbers of Activated CD8⁺ T Cells

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The Toxoplasma gondii population consists of multiple strains, defined by genotype and virulence. Previous studies have established that protective immunity to this organism is mediated by IL-12, which drives T cells to produce IFN-γ. Paradoxically, although type I and type II strains of T. gondii both induce IL-12 and IFN-γ in the mouse, type I parasites are lethal, whereas type II strains establish chronic infection. The cellular basis for these strain-dependent differences remains unclear. To better understand these events, the CD8⁺ T cell and dendritic cell (DC) responses to transgenic, OVA-expressing type I RH (RH OVA) and type II Prugniad (Pru OVA) parasites were examined. Pru OVA-infected mice developed a robust DC response at the site of infection and the draining lymph node and generated a population of endogenous OVA-specific CD8⁺ T cells. In contrast, RH OVA-infected mice had fewer DCs and OVA-specific CD8⁺ T cells. RH OVA-infected mice given preactivated OVA-specific CD8⁺ T cells were protected, suggesting that reduced DC-derived signals contributed to the low OVA-specific CD8⁺ T cell numbers observed during type I infection. Indeed, DC depletion prior to Pru OVA infection resulted in a failure to generate activated OVA-specific CD8⁺ T cells, and IL-12p70 treatment during RH OVA infection modestly increased the number of Ag-specific cells. Together, these data are consistent with a model of immunity to T. gondii in which strain-dependent DC responses shape the generation of Ag-specific CD8⁺ T cells and determine the outcome of infection.

Toxoplasma gondii is an obligate intracellular protozoan parasite that causes disease in immunocompromised individuals and fetuses infected in utero (1–3). Strains of this parasite are generally defined by genotype and virulence in the mouse model (4). Based on current paradigms, the T. gondii population in Europe and North America consists of three clonal strains, types I, II, and III (5). However, in South America, Asia, and Africa, these clonal strains are less dominant, and recombinant and exotic strains are more common. Some of these atypical strains have been associated with the development of toxoplasmosis in immunocompetent individuals (6, 7).

Whereas protective immunity to T. gondii is dependent upon IL-12 (8–12), IFN-γ (13, 14), and IFN-γ-dependent effector mechanisms (15–19), the influence of parasitic virulence on the immune response remains unclear. During murine infection with a type II or type III strain, the host’s immune system effectively controls replication of the tachyzoite stage, resulting in parasite differentiation to the cyst-forming bradyzoite and the establishment of chronic infection (2, 7, 20). In contrast, following challenge with type I strains, mice succumb 9–11 d postinfection (p.i.) as a result of uncontrolled parasite growth and associated inflammation, despite high systemic levels of IL-12 and IFN-γ. Thus, whereas the murine host develops effective IL-12– and IFN-γ–mediated protective immunity to type II and type III strains of T. gondii, the IL-12/IFN-γ response appears to be unable to limit parasite replication during infection with type I strains. In fact, high levels of IL-12 and IFN-γ during type I infection have been proposed to contribute to morbidity and mortality (21, 22), although neutralization of IFN-γ does not reliably provide protection to mice infected with type I parasites (12, 22).

Previous studies have examined parasite- and host-intrinsic factors that contribute to strain-specific virulence, and a number of hypotheses have been proposed to explain virulence. For instance, type I strains replicate slightly faster than type II strains in vitro (7), and type I strains also appear to be better able to migrate across biological barriers, including the lamina propria and submucosa (23). These data establish that replication rate and migratory ability may correlate with increasing virulence. Additionally, type I strains interfere with NF-kB activation and induce less IL-12 from infected macrophages in vitro than type II strains, suggesting that the parasite may be able to modulate the host immune response in a strain-specific manner (24, 25). In concert with this idea, genetic and bioinformatic approaches have identified the T. gondii shptopy proteins ROP16 and ROP18 as serine-threonine kinases that contribute to strain-specific virulence (26–28). These proteins may be key in directly modulating the host immune response and influencing the progression of the infection.
Despite the multiple proposed mechanisms that may underlie the virulence of type I strains, the precise nature of the host–pathogen interactions that determine the outcome of infection is still unclear. Many questions remain regarding whether virulent strains actively prevent the development of protective immunity or simply evade an ongoing response. Of note, the cellular immune responses associated with type I compared with type II infection in vivo have not been extensively compared, raising questions regarding the impact of parasite virulence on the development or maintenance of protective immunity. To address these issues, transgenic type I (RH strain) and type II (Prugniaud [Pru]) T. gondii that express the model Ag OVA (29) (referred to as RH OVA and Pru OVA throughout) were used to compare the CD8+ T cell and dendritic cell (DC) responses to type I and type II strains. Whereas the type II infection induced a robust Ag-specific CD8+ T cell response, significant expansion of this population was not observed during type I infection. Moreover, when RH OVA-infected mice were given preactivated OTI OVA-specific CD8+ T cells, these animals were protected. These observations suggested that insufficient signals from innate cell types contribute to the reduced numbers of Ag-specific CD8+ T cells associated with type I infection. Consistent with this idea, challenge with type I RH OVA was accompanied by an early defect in DC accumulation at the site of infection and in the draining lymph node (DLN). Additionally, a mouse model of transient DC depletion (30) revealed that DCs are essential for the expansion and activation of Ag-specific CD8+ T cells during infection with the type II Pru OVA strain. Finally, administration of murine rIL-12p70 (rIL-12p70) during RH OVA infection resulted in a small increase in the number of OVA-specific CD8+ T cells. Together, these findings support a model in which infection with the virulent RH strain of T. gondii prevents the activation and expansion of DC populations that are required for the development of protective CD8+ T cell responses.

Materials and Methods

Mice

Cs7Bl/6J 6– to 8-wk-old female mice were obtained from The Jackson Laboratory (Bar Harbor, ME). OTI TCR transgenic mice, CD45.1 mice, and FVB.B6DTR(Ifgax/EGFP/GFP) transgenic mice that express the simian diapheridina toxin receptor (DTR) under the CD11c promoter (CD11c–DTR mice) were originally obtained from The Jackson Laboratory and/or bred in the University of Pennsylvania animal facility. DPEC599 mice were originally obtained from U.H. von Andrian (Center for Blood Research, Harvard University, Boston, MA) and bred to OTI TCR transgenic mice in the University of Pennsylvania animal facility. For transient DC depletion, CD11c–DTR mice or their wild-type (WT) littermates were given 100 ng Corynebacterium diphtheriae toxin (DT) (Sigma Aldrich, St. Louis, MO) in 100 μl i.p., 24 h prior to infection with T. gondii. For rIL-12p70 treatment, Cs7Bl/6J mice were given 250 ng rIL-12p70 (PeproTech, Rocky Hill, NJ) i.p. on the day of infection with T. gondii and days 1 and 2 p.i. Age- and sex-matched mice were used at 6–10 wk of age. All mice were maintained in specific pathogen-free conditions at the University of Pennsylvania animal facility, in accordance with institutional and federal regulations.

Parasites and infection

Transgenic T. gondii RH and Pru OVA parasites were generated as previously described (29). Briefly, RH OVA or Pru OVA parasites express and secrete a truncated form of OVA, including aa 140–386, into the parasitophorous vacuole. The vector used was based on Bluescript pKS3 (Strategene, La Jolla, CA) and contains a chloramphenicol acetyltransferase-selectable marker and the OVA coding sequence fused downstream of the P30 signal sequence under the T. gondi TUB1 promoter (Supplemental Fig. 1A). Two billion tachyzoites of the RH or Pru Δhx parental strains were transfected by electroporation with 70 μg linearized construct in a 2-mm–gap cuvette (BTX; 1.5-kV pulse, 24 V). Stable transgenic parasite lines were selected and maintained in vitro by serial passage through human foreskin fibroblasts in parasite culture medium (DMEM [Invitrogen, Carlsbad, CA], 20% media M199 [Invitrogen], 10% FBS [Serum Source International, Charlotte, NC], 1% penicillin/streptomycin [Invitrogen]) containing chloramphenicol. Parental parasites were maintained in the absence of selection. Immunofluorescence was used to assess OVA expression in RH OVA and Pru OVA strains. Pru OVA or RH OVA-infected human foreskin fibroblast monolayers were grown on 22-mm–diameter glass coverslips. Coverslips were fixed using 4% formaldehyde in PBS and permeabilized in 0.2% Triton X-100. A rabbit anti-chicken OVA primary Ab (Bethyl Laboratories, Montgomery, TX) diluted 1/1000 in a solution of 10% FBS and 0.1% Triton X-100 in PBS was used to stain for OVA. An Alexa Fluor 488-labeled goat anti-rabbit IgG secondary Ab (Molecular Probes, Eugene, Oregon) was used to visualize OVA labeling. A Zeiss LSM 710 microscope equipped with a 100-W Hg vapor lamp, barrier-filter emission, and an interline transfer chip charge-coupled device camera (Hamamatsu, Bridgewater, NJ) was used to detect fluorescence. Images were analyzed and prepared with Openlab software (Improvision, Lexington, MA). Expression of OVA was roughly similar in RH and Pru OVA strains (Supplemental Fig. 1B). Tachyzoites of RH Pru parental or Pru OVA strains that express OVA were isolated by serial needle passage and filtered through a 0.5-μm pore, 250 μl tachyzoites in 200 μl PBS. Animals were sacrificed at day 6, 7, or 8 p.i.

Isolation, in vitro activation, and transfer of OTI T cells

Spleens and lymph nodes (LN) were removed from OTI TCR transgenic female mice (with or without GFP), single-cell suspensions were prepared, and RBC and were lysed using 0.86% ammonium chloride. For naive cell transfer, 3×10^6 t cell-enriched lymph node cell suspensions were isolated according to manufacturer instructions (R&D Systems, Minneapolis, MN), and 1×10^6 cells were transferred i.v. into congenic hosts 24 h prior to infection with T. gondii. For activated cell transfer, isolated cells were washed once and resuspended in 500 μg/ml OVA protein in complete RPMI 1640 (RPMI 1640 [Invitrogen], 10% FBS [Serum Source International], 1% penicillin/streptomycin [Invitrogen], 1 mM sodium pyruvate [MediaTech, Manassas, VA], 2 mM L-glutamine [Mediatech], 1% 2-mercaptoethanol [Invitrogen]) and cultured at 37°C overnight. Cells were washed twice the next day with media, resuspended, and allowed to rest at 37°C for 48 h. Cultures were then supplemented with 100 U/ml IL-2 and split every other day for 5 d. After seven total days of culture, the T cells were isolated, as described above. The isolated OTI T cells were washed and resuspended in PBS, and 5×10^6 cells in 200 μl were transferred i.p. to B6 female mice 24 h prior to infection with T. gondii.

Flow cytometry

Single-cell suspensions were prepared from peritoneal exudate cells (PECs) by peritoneal wash with 5 ml ice-cold PBS. PECs were washed twice with complete RPMI 1640. Single-cell suspensions were prepared from spleens and mediastinal and parathymic LN following injection with 3.5 mg/ml Liberase Blendzyme 3 (Roche, Indianapolis, IN) and incubation at 37°C for 30 min. RBC were lysed using 0.86% ammonium chloride. Cells for staining (3×10^5) were washed twice with PBS and resuspended (PBS, 1 mM CaCl₂, 1 mM MgCl₂, 1% DMSO [Sigma-Aldrich], 2 mM EDTA [Invitrogen]) and blocked in 50 μl Fc-block solution (flow cytometry buffer, 1 μg/ml 24G2 anti-CD16/32, 1 μg/ml normal rat serum [Invitrogen], and 1 μg/ml normal mouse serum [Invitrogen]) at 4°C for 15 min. Cells were then stained in 100 μl total volume at 4°C for 30 min, and were washed twice in flow cytometry buffer for acquisition. For intracellular cytokine staining, 1×10⁶ splenocytes were plated in a 96-well plate and incubated with 1× brefeldin A, with or without 1 μg/ml OVA peptide (CHI Scientific, Maynard, MA), at 37°C for 6 h. Harvested cells were fixed using 2% paraformaldehyde, washed twice with flow cytometry buffer, and permeabilized using 100 μl BD Perm/Wash buffer (BD Biosciences, San Jose, CA) at room temperature for 10 min. Cells were incubated in Fc block diluted in BD Perm/Wash buffer (BD Biosciences) at 4°C for 10 min, and were subsequently stained with cytokine antibodies diluted in BD Perm/Wash buffer (BD Biosciences) in 100 μl total volume at 4°C for 45 min. Cells were washed twice and resuspended in flow cytometry buffer for acquisition. Abs were used FITC anti-CD3e (14-251-2C11 or eBioscience 550A), FITC anti-CD19 (eBio1D3), FITC anti-CD44 (IM7), FITC anti-CD49b (DX5), PerCP-Cy5.5 anti-CD69 (53-6.7), PerCP-Cy5.5 anti-IFN-γ (XM1G2.1), allophycocyanin anti-CD62L (MEL-14), Alexa Fluor 700 anti-MHC class II H-2D^d (MS/114.15.2), allophycocyanin-Alexa Fluor 750 CD11b (1H10/70), allophycocyanin-Alexa Fluor 750 anti-CD45.2 (104), allophycocyanin-Alexa Fluor 750 CD62L (MEL-14), PE anti-human perforin (D9), PE anti-CD44 (IM7), PE anti-CD80 (16-10A1), PE-Cy5 anti-CD86 (GL1), PE-Cy7 anti-CD8α (53-6.7), PE-Cy7 anti-CD11c (N418), and PE-Cy7 CD44 (IM7), supplied by eBioscience (San Diego, CA). FITC anti-CD4 (GK1.5), Pacific Blue anti-CD3e (550A), PE anti-CD8 (53-1.5), and PE anti-CD19 (eBio1D3), were supplied by BD Biosciences. Allophycocyanin anti-human granuzyme B (GB12) was supplied by Invitrogen. MHC I H-2K^d OVA monomer was generously provided by J. Wherry (Wistar Institute, Philadelphia, PA) and labeled to make tetramers.
using PE-streptavidin (Invitrogen), according to standard protocols. Acquisition was performed using a 4-laser 18-color LSR II (BD Biosciences) or a 3-laser 8-color FACSCanto (BD Biosciences). Results were analyzed using FlowJo 8.7.1 (Tree Star, Ashland, OR).

Assessment of parasite burden

To assess parasite burden by cytospin count, single-cell suspensions were prepared, as described above, from PECs, and 1 x 10⁶ cells in 200 μl RPMI 1640 with 10% FBS were spun onto glass slides at 500 rpm for 5 min. Slides were stained with Diff Quick (Merck, Darmstadt, Germany) and mounted in CytoSeal (Edmund Scientifics, Tonawanda, NY). The percentage of infected cells was calculated based on counts of at least 200 total cells. Parasite burden was assessed by quantitative real-time PCR detection of parasite DNA, as previously described (31). Briefly, genomic DNA was isolated from 2 x 10⁶ PECs using the High Pure PCR Template Preparation Kit, according to manufacturer’s instructions (Roche Applied Science, Indianapolis, IN). Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA), 500 ng genomic DNA, and primers for the T. gondii B1 repeat region were used in PCRs. Quantitative real-time PCR was performed using the 7500 Fast Real-Time PCR System and analyzed using system software v1.3.1 (Applied Biosystems).

Statistical analyses

To determine statistical significance, Student t test, one, or two-way ANOVA were performed. Where indicated, Bonferroni’s multiple comparison test was used for posttesting following ANOVA testing. Statistical tests used are described in the text throughout. A p value of <0.05 was considered statistically significant and is indicated by an asterisk.

Results

Phenotype of Ag-specific CD8+ T cells during type I and type II T. gondii infection

As part of initial studies to characterize the global CD8+ T cell response to different strains of T. gondii, C57BL/6 mice were infected with type I RH and type II Pru parasites that express a truncated version of the OVA protein (Supplemental Fig. 1), and the total number and activation status of CD8+ T cells were determined. On day 7 or 8 following infection with Pru OVA, there was an increase in the number of total CD8+ T cells in the spleen (Fig. 1A), and an expansion in the number (Fig. 1A) and percentage (Fig. 1B) of activated (CD44highCD62L–) CD8+ T cells. As RH-infected animals produce high levels of the proinflammatory cytokines IL-12 and IFN-γ (21, 22), we expected that infection with RH OVA would induce a similar or greater total frequency and number of activated CD8+ T cells compared with Pru OVA. Surprisingly, on day 7 or 8 p.i., RH OVA-infected mice had a lower total number of CD8+ T cells and a lower number of activated CD8+ T cells (Fig. 1A). The percentage of activated cells from total CD8+ T cells was equivalent to that observed during Pru OVA infection, and the expression patterns of CD44 and CD62L on CD8+ T cells from RH OVA- and Pru OVA-infected mice were similar (Fig. 1B). Thus, there was a defect in the total number of activated CD8+ T cells during RH OVA infection; however, those CD8+ T cells that did become activated during RH OVA infection were activated appropriately as compared with those present in Pru OVA-infected mice.

Recent studies from this laboratory have shown that Pru OVA parasites induce a population of endogenous OVA-specific CD8+ T cells that can be detected using the H-2Kb OVA tetramer (32), providing a tool to track an Ag-specific CD8+ T cell response during toxoplasmosis. When mice were infected with Pru OVA parasites, a distinct population of CD8+ T cells specific for OVA that expressed an activated phenotype emerged in the spleen (Fig. 2A, 2B), LN, and peritoneal cavity (data not shown) by day 7 or 8 p.i. In contrast, following infection with RH OVA, there was only a modest OVA-specific response, illustrated by a low percentage of OVA-specific CD8+ T cells (Fig. 2A) and a very small number of total and activated OVA-specific CD8+ T cells (Fig. 2B). Despite the defect in numbers of total and activated OVA-specific CD8+ T cells in RH OVA-infected mice, the percentage of activated OVA-specific CD8+ T cells and the expression patterns of CD44 and CD62L on these cells were similar in RH OVA- and Pru OVA-infected mice (Fig. 2C). To determine whether the deficient OVA-specific CD8+ T cell response during RH OVA infection was a result of an early difference in parasite burden, mice were infected with a high dose of Pru

![FIGURE 1. Mice infected with RH OVA have a dampened CD8+ T cell response compared with mice infected with Pru OVA. A, On day 7 or 8 p.i., mice infected with RH OVA had a lower total number of CD8+ T cells (mean ± SEM shown, *p < 0.05 by two-way ANOVA) and a lower number of activated (CD44highCD62L–) CD8+ T cells (mean ± SEM shown, *p < 0.05 by two-way ANOVA) in the spleen than mice infected with Pru OVA. B, On day 7 or 8 p.i., mice infected with RH OVA and Pru OVA had a similar percentage of activated CD8+ T cells in the spleen (mean ± SEM shown, *p > 0.05 by two-way ANOVA) and similar expression patterns of CD44 and CD62L. Plots were gated on CD8+ T cells. Graphs show data from three experiments, representative of eight experiments. Flow cytometry plots display representative samples.](http://www.jimmunol.org/)

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OVA (1 × 10^5 parasites). On day 7 p.i., approximately 2% of all CD8^+ T cells were OVA-specific, similar to the results obtained with 1 × 10^3 parasites (data not shown), suggesting that parasite burden and the size of the activated CD8^+ T cell population are not directly associated.

The ability to produce cytokines and cytolytic capacity are important functional properties of Ag-specific CD8^+ T cells. Initially, attempts were made to analyze the functionality of the endogenous OVA-specific CD8^+ T cells in the spleen and lymph nodes of RH and Pru OVA-infected animals. However, there were too few of these endogenous cells to reliably analyze, particularly during RH OVA infection. Thus, a transfer system was used, in which 1 × 10^6 naive T cells from an OTI TCR-transgenic mouse were transferred to congenic CD45.1 mice 24 h prior to infection with RH OVA or Pru OVA. To examine the functional capacity of the transferred cells, at day 6 p.i., these cells were isolated and restimulated in vitro with OTI peptide and then assessed for production of IFN-γ and granzyme B by intracellular staining. Expansion of the transferred population in Pru OVA-infected mice was greater than expansion of these cells in RH OVA-infected mice (data not shown). However, OVA-specific OTI CD8^+ T cells were equally functional during RH OVA compared with Pru OVA infection (Fig. 2D).

The results described above suggested that RH OVA-infected animals have a reduced ability to prime, expand, or maintain a population of parasite-specific CD8^+ T cells. To distinguish between these possibilities, naive or preactivated OTI CD8^+ T cells were transferred i.v or i.p. into mice that were then infected with the RH parental or RH OVA T. gondii. Preactivated OTI were CD44^highCD62L^low, had the potential to be cytolytic (granzyme B^+), and produced IFN-γ in response to OTI peptide stimulation (Supplemental Fig. 2). In these studies, mice infected with RH OVA or the parental strain given naive OTI cells succumbed to infection on days 8–10 (data not shown), indicating that although these naive transferred cells did secrete IFN-γ on day 6 p.i. (Fig. 2D), they were not protective, perhaps because their numbers were insufficient. For mice given preactivated OTI cells, on day 5 p.i., infected mice was greater than expansion of these cells in RH OVA-infected mice (data not shown). However, OVA-specific OTI CD8^+ T cells were equally functional during RH OVA compared with Pru OVA infection (Fig. 2D).

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parasite burden in the peritoneal cavity (the site of infection) was assessed, and on day 7 p.i., the number and activation status of the transferred OTI cells were examined. In mice that were infected with the RH parental strain and given preactivated OTI, 31.8 ± 2.59% of PECs were infected (similar to control mice not given the transfer). In contrast, mice infected with the RH OVA strain given preactivated OTI cells had parasite burdens of 5.89 ± 1.16% (Fig. 3A). As shown by cytospin analysis, control mice and mice infected with RH parental given preactivated OTI cells had free parasites in the PECs, whereas mice infected with RH OVA given preactivated OTI cells did not (Fig. 3B). Additionally, in mice challenged with the parental strain, transferred OTI cells were not detectable, and in mice challenged with RH OVA, transferred cells were found in the spleen, and retained an activated status on day 7 p.i. (Fig. 3C). Thus, preactivated OTI T cells can persist and mediate protection in RH-infected mice in a process that requires the presence of cognate Ag. These findings suggest that the reduced CD8+ T cell response observed during RH infection is a consequence of a defect in either priming, recruiting, or expanding CD8+ T cells, rather than an inability to maintain these cells or a preferential deletion of this population.

Phenotype of DCs during type I and type II T. gondii infection

In numerous infectious disease models, DCs are vital for the generation of protective CD8+ T cell responses (30, 33–35). Thus, we hypothesized that strain-specific DC responses might contribute to the inefficient generation of Ag-specific CD8+ T cell populations during RH infection. To determine whether there were differences in the DC response following challenge with RH OVA or Pru OVA, DC activation and numbers in the PECs and DLN following infection were examined. By day 3 p.i. with Pru OVA, there was a marked increase in the total numbers of DCs (DCs were identified as CD19−CD3−CD11c+ Dx5+CD11c+) as well as the myeloid-type DCs (CD11b+CD8+CD11c+) in the peritoneal cavity. DC numbers contracted slightly on day 5 p.i., and remained constant through day 7 p.i. (Fig. 4A). In contrast, following infection with RH-OVA, analysis at the local site showed that by day 3 p.i., numbers of total DCs and myeloid-type DCs were lower than those observed during Pru OVA infection, and subsequently declined on day 7 p.i. (Fig. 4A). The pattern of kinetic change in numbers of total and myeloid-type DCs was significantly different, as determined by two-way ANOVA. At the early day 3 p.i. time point, the percentage of myeloid-type DCs of total DCs was 73.5 ± 3.12% in RH OVA-infected mice compared with 80.7 ± 2.33% in Pru OVA-infected mice, indicating that there was a difference in the overall DC population structure (Fig. 4B).

The patterns of DC recruitment observed at the site of infection were mirrored in the draining parathymic and mediastinal LN. During Pru OVA infection, there was a steady accumulation of total DCs and myeloid-type DCs throughout the time course, starting at day 3 p.i. (Fig. 5A). In RH OVA-infected mice, there were fewer total DC and fewer myeloid-type DCs recruited to the DLNs compared with Pru OVA-infected mice in a time course of infection (Fig. 5A) (determined by two-way ANOVA comparing the kinetic changes in the DC population). Specifically, on day 3 p.i., mice infected with RH OVA had significantly fewer total DCs and myeloid-type DCs than mice infected with Pru OVA (Fig. 5B), and there was a significantly lower percentage of myeloid-type DCs of total DCs as well (Fig. 5C).

The data described above suggested that day 3 p.i. represented an important turning point in the DC response in RH OVA versus Pru OVA infection, as this time point was the peak of the DC influx into

![Image](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org)
the site of infection, and marked the initiation of detectable recruitment to the DLN. In addition, at day 3 p.i., parasite burden does not significantly differ in Pru OVA- infected mice as measured by either counting infected cells using light microscopy or by real-time PCR for *T. gondii* DNA in the PECs (Supplemental Fig. 1C, 1D), suggesting that strain-specific DC responses at this time point are not due to large differences in parasite burden. Thus, day 3 p.i. was chosen for further analysis of DC phenotype. Recently published work from this laboratory (36) showed that cross presentation may play a significant role in modulating DC phenotype. From these data do not establish a direct causal relationship between early DC responses and the generation of Ag-specific CD8+ T cells. To correlate whether the early defects in DC numbers during RH infection contribute to the dampened Ag-specific CD8+ T cell responses, studies were performed to assess whether DC depletion in mice infected with Pru would result in a CD8+ T cell response similar to that observed during RH infection. To deplete DCs, we used mice that carry the simian DTR under the CD11c promoter (CD11c-DTR mice), rendering CD11c+ cells (primarily DC subsets) susceptible to transient depletion upon exposure to DT (30). A recent report used this approach to understand the role of DC in resistance to *T. gondii* and showed that DCs are required for resistance to a type II strain. In these studies, the impact of the depletion on endogenous CD8+ T cell responses was not addressed (37).

To assess the role of DC depletion on the generation of the Ag-specific CD8+ T cell response, CD11c−DTR mice and WT littermates were treated with 100 ng DT 24 h prior to infection with 10^7 Pru OVA parasites. At the time of infection, CD11c−DTR mice treated with DT were depleted of $\approx$90% of DCs in peripheral blood, and WT DT-treated mice showed no changes in the DC population at this site (data not shown). Mice depleted of DCs succumbed to Pru OVA infection between days 9 and 11 p.i., whereas undepleted controls survived (data not shown). At day 7 p.i., CD11c−DTR and WT controls were sacrificed, and the parasite burden, numbers, and activation status of endogenous OVA-specific CD8+ T cells were examined. CD11c−DTR mice treated with DT had higher parasite burdens (21.1 ± 5.09%) in the PECs than WT DT-treated controls (5.48 ± 5.01%) (Fig. 6A), and cytospin analysis revealed free parasites in the PECs of depleted mice (Fig. 6B). There were a lower frequency (Fig. 6C) and total number (Fig. 6D) of OVA-specific CD8+ T cells in the spleens of DC-depleted CD11c−DTR mice as compared with WT DT-treated control mice. There was also a lower number of activated OVA-specific CD8+ T cells in depleted mice (Fig. 6D). In contrast to the trend observed in RH OVA infected with Pru OVA infection, there was a lower percentage of activated (CD44*CD62L*) OVA-specific CD8+ T cells in depleted mice compared with WT controls (Fig. 6E). Whereas DC depletion does not exactly mimic the DC status during RH OVA infection, the absence of DCs early on in type II Pru OVA infection results in a CD8+ T cell phenotype similar to that observed during disease caused by RH OVA.
Effect of IL-12p70 treatment during type I T. gondii infection

The data described above show that DCs are vital for generating a CD8+ T cell response to type II T. gondii. Previous studies using this DC depletion model concluded that DCs are a necessary source of IL-12 during T. gondii infection (37), and IL-12 is an important factor in generating effective CD8+ T cell responses [reviewed in Mescher et al. (38)]. Thus, during RH OVA infection, a failure to expand DC populations may lead to insufficient IL-12 levels, resulting in low numbers of activated OVA-specific CD8+ T cells. To test this hypothesis, we administered rmIL-12p70 to RH
OVA-infected mice on the day of infection and days 2 and 3 p.i., and assessed the number and activation status of OVA-specific CD8+ T cells at day 8 p.i. Compared with untreated RH OVA-infected mice, IL-12p70–treated RH OVA-infected mice had a slightly higher number of total and activated OVA-specific CD8+ T cells (Fig. 7A). However, these numbers in IL-12p70–treated RH OVA-infected mice were still much lower than those observed during Pru OVA infection, and IL-12 treatment did not significantly reduce parasite burden (Fig. 7B). These data show that insufficient IL-12 signals early in RH OVA infection can influence the size of the OVA-specific CD8+ T cell population to some extent, but indicate that an IL-12 defect does not completely explain the CD8+ T cell phenotype observed during RH infection. Thus, low DC numbers early in RH OVA infection may contribute to low numbers of activated Ag-specific CD8+ T cells through multiple mechanisms, including limiting access to cytokine signals and restricting T cell priming.

Discussion
Clinically, susceptibility to T. gondii has been associated predominantly with acquired defects in T cell function that permit latent infection to reactivate. More recently, there is an increasing awareness that parasite strain can play an important role in determining virulence in humans, and that infection with certain isolates can lead to severe clinical disease in immunocompetent individuals (6, 7). In the last 3 y, forward and reverse genetics have been pivotal in beginning to dissect the molecular basis for virulence (26–28), but there are still major gaps in our knowledge, and a number of interesting paradoxes exist in the body of literature related to T. gondii virulence. For example, the current paradigm that explains protective immunity to T. gondii, which is based on studies with type II strains in murine models, dictates that a robust IL-12 and IFN-γ response is required to control parasite replication (8–11, 13–19). However, there are multiple reports that a type I strain of T. gondii also elicits systemic IL-12 and IFN-γ, but this
FIGURE 7. RH OVA-infected mice given rmIL-12p70 have slightly increased numbers of Ag-specific CD8+ T cells. A. On day 8 p.i., mice infected with RH OVA given rmIL-12p70 had slightly elevated total (mean ± SEM shown, p > 0.05 by two-way ANOVA) and activated numbers of OVA-specific CD8+ T cells (mean ± SEM shown, p > 0.05 by two-way ANOVA) in the spleen compared with untreated RH OVA-infected mice. B. On day 8 p.i., mice infected with RH OVA given rmIL-12p70 had similar parasite burdens in the PECs compared with untreated RH OVA-infected mice (mean ± SEM shown, p > 0.05 by two-way ANOVA). Graphs show data from two experiments.

Previously published work has investigated whether there are strain-dependent differences in DC responses, including the upregulation of activation and maturation markers, migratory ability, and cytokine production (24, 39–41). Our laboratory and others have observed that there do not appear to be strain-specific differences in how bone marrow-derived DCs upregulate activation and maturation markers early in infection (41) (data not shown). In this study, we show that multiple parameters of the DC response in vivo at the site of infection and in the DLN are deficient during type I RH infection. Regarding DC migratory ability, there are conflicting reports describing strain-dependent differences in migration properties of human and murine DC. Murine DCs infected with type I parasites exhibit a migratory phenotype (40); however, in other studies, human DCs exposed to type II parasites demonstrated an enhanced migratory phenotype compared with DCs exposed to type I parasites (39). Finally, experimental evidence from in vitro studies suggests that there is no impairment of IL-12p40 production in type I- as compared with type II-infected splenic DCs as is observed in macrophages (24, 25), but Bzik and colleagues (42) have recently reported that IL-12p70 levels are low during RH infection. As DCs are a critical source of IL-12p70 during toxoplasmosis (37), and numerous studies have established that IL-12 is important in the development of protective CD8+ T cell responses (38), the early deficiencies in the DC population during RH infection might limit CD8+ T cell access to important IL-12 signals. We show that IL-12p70 treatment during RH OVA infection can slightly increase the number of activated Ag-specific CD8+ T cells, although this treatment does not come close to fully restoring this response. Thus, reduced numbers of DCs, partial defects in costimulatory and MHC I expression, and deficient DC-derived IL-12p70 signals may result in suboptimal CD8+ T cell responses during type I infection.

There are multiple mechanisms by which type I T. gondii might modulate the DC response in a strain-specific manner. One possibility is that RH replicates slightly faster than Pru in vivo and selectively targets DCs for lysis (20). However, we have shown that defects in the DC population can be observed early in infection, before there are large disparities in parasite burden (Supplemental Fig. 1C, 1D). Additionally, very early monocyte recruitment to the site of infection appears to be different in RH- compared with Pru-infected mice (E.D. Tait, unpublished observations). Recently, Lee et al. (43) showed that type I and type II parasites induce different panels of chemokines at the site of infection, suggesting that strain-dependent chemokine environments may lead to the recruitment or retention of different populations of cells during type I versus type II infection. Consistent with this idea, the Sibley laboratory observed that type II PTG parasites, but not type I RH parasites, strongly induced a population of CD68+Gr-1+ monocytes that were vital for controlling infection, dependent upon interaction between the chemokine receptor CCR2 and its ligand (44–46). These data indicate that strain-specific induction of chemokines may influence the recruitment and migration of DCs and DC precursors to mediate downstream immune responses.

Although the studies presented in this work, and others, demonstrate a vital role for DCs in supporting the immune response to T. gondii, CD8+ T cells have also been recognized as being important for long-term control of T. gondii (47–51). Numerous groups have suggested that T. gondii may impair the host CD8+ T cell response via various mechanisms (52, 53), including lysis of cytolytic effectors (54) and induction of apoptosis (55). While our studies were in progress, the Robey laboratory published a report identifying a process by which infected APC facilitate infection of Ag-specific CD8+ T cells, enhancing dissemination of the parasite through the host (56). Our transfer of preactivated, Ag-specific cells into RH OVA-infected hosts demonstrated that at least some of these cells survived and/or expanded to mediate protection; however, these mice did eventually succumb to infection. Thus, deletion or functional impairment of CD8+ T cells during RH infection may be a mechanism by which virulent parasites alter or evade the host immune response.
response. Further studies to investigate these processes will be aided by the use of transgenic parasites that express model Ags (29, 57, 58), and new reagents that identify CD8+ T cells specific for endogenous T. gondii epistopes in the murine host (59, 60).

The study of the immune response to various strains of T. gondii has highlighted the intimate relationship between host and parasite, and focused attention on dissecting ways in which parasite and host affect one another. There has been increasing interest in understanding the molecular mechanisms for strain-dependent virulence and identifying parasite-derived products that may directly modulate host cellular immune responses. The detailed genetic analysis of the progeny of sexual crosses between different strains led to the identification of the serine–threonine kinases ROP16 and ROP18 as important virulence factors (26–28); however, studies to date have not identified parasite products that mediate DC or CD8+ T cell phenotypes during type I or type II infection. Regardless, the work presented in this study illustrates the importance of parasite strain in the development of protective versus nonprotective immune responses in the murine model. We describe a previously unrecognized process by which type I and type II parasites may differentially modulate the host immune response. These studies represent new insights into the development of immunity to T. gondii that have the potential to inform the interpretation of future studies examining virulence.

Acknowledgments
We thank John Boothroyd and David Sibley for discussions during this work, John Wherry for assistance in generating SINFEKL tetramer, and the Woods Hole Biology of Parasitism classes of 2004–2006 for providing early directions for this project.

Disclosures
The authors have no financial conflicts of interest.

References


SUPPLEMENTARY FIGURE 1. OVA transgenic parasite description. A. Depiction of the construct used to create RH OVA and Pru OVA parasites. B. RH OVA and Pru OVA parasites express OVA in the parasitophorous vacuole, as assessed by immunofluorescent staining. C. In a time course of infection, RH OVA- and Pru OVA-infected mice had similar parasite burdens in the PEC by counting infected cells by cytospin at day 1 and day 3 p.i.. On day 5 and day 7 p.i., RH OVA-infected mice had higher parasite burdens (mean ± SEM shown, * p>0.001 by Bonferroni’s Multiple Comparison post-testing comparing RH and Pru OVA on day 5 and 7 p.i. for 2-way ANOVA). D. In a time course of infection, RH OVA- and Pru OVA-infected mice had similar parasite burdens in the PEC, as measure by real-time PCR for parasite DNA at day 1 and day 3 p.i.. On day 5 and day 7 p.i., RH OVA-infected mice had higher parasite burdens (mean ± SEM shown, * p>0.01 by Bonferroni’s Multiple Comparison post-testing comparing RH and Pru OVA on day 5 and 7 p.i. for 2-way ANOVA). Graph in C shows data from 3 experiments, and D shows data from 1 experiment.
SUPPLEMENTARY FIGURE 2. Phenotype of *in vitro* activated OTI prior to transfer. Following restimulation with OTI peptide and incubation with BFA, OTI T cells produced IFN-γ and stained positive for granzyme B by intracellular staining. Plots gated on GFP<sup>+</sup>CD8<sup>+</sup> T cells. Flow cytometry plots display representative samples.
**Supplementary Figure 3**

A

**LN CD11c⁺ % MHC II⁺ D3**

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B

**LN CD11c⁺ MHC II MFI D3**

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**LN CD11c⁺ CD86 MFI D3**

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**SUPPLEMENTARY FIGURE 3.** Mice infected with RH OVA or Pru OVA have similar expression and staining for MHC II and CD86 and similar expression for CD80 in the DLN. A. On day 3 p.i., the percentage of MHC II⁺ and CD86⁺ DC in mice infected with RH OVA and Pru OVA was similar. B. On day 3 p.i., the MFI of MHC II, CD86, and CD80 expression were similar on DC from RH OVA- and Pru OVA-infected mice. Graphs show data from 1 experiment, representative of 3 experiments.