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In Vivo Depletion of CD11c$^+$ Cells Delays the CD4$^+$ T Cell Response to *Mycobacterium tuberculosis* and Exacerbates the Outcome of Infection[1]

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Although dendritic cells (DC) are potent APC that prime T cells against many pathogens, there is no direct evidence that DC are required for immunity to *Mycobacterium tuberculosis* (Mtb) infection. The requirement for DC to prime the CD4$^+$ T cell response following Mtb infection was investigated using pCD11c-diptheria toxin receptor/GFP transgenic mice, in which DC can be transiently ablated in vivo. We show a critical role for DC in initiation of the CD4$^+$ T cell response to the mycobacterial Ag early secretory Ag of tuberculosis 6. The delay in initiating the Ag-specific T cell response led to impaired control of Mtb replication. Interestingly, DC were not required for the secondary CD4$^+$ T cell response following Mtb infection in peptide-vaccinated mice. Thus, this study shows that DC are essential for the initiation of the adaptive T cell response to the human pathogen Mtb. ***The Journal of Immunology,*** **2005, 175: 3268–3272.**

Mycobacterium tuberculosis (Mtb)[3] is a facultative intracellular bacterium that infects and replicates in macrophages (Mϕ). Following intracellular infection, Mtb resides in the phagosome where it is able to survive because it prevents acidification and phagolysosome maturation, while maintaining access to nutrient molecules such as iron via the early endocytic pathway (1, 2). Initially, the innate immune response directed by activated Mϕ dominates the host response to Mtb (3). However, optimum host defense against Mtb requires establishment of Th1 adaptive immunity to ultimately control the infection (4, 5). Although both Mϕ and dendritic cells (DC) can process and present Ag to T cells, DC are unique because of their role in priming the T cell response against many pathogens (6). DC are well suited for this function because, following uptake of a pathogen or its Ags, DC migrate from peripheral tissues to draining lymph nodes (LN). During this process, DC undergo maturation and upregulate costimulatory molecules that promote effective T cell activation (7).

Mtb can infect and replicate intracellularly in murine lung DC and bone marrow-derived DC in vitro (8, 9). It is not yet resolved whether activation renders DC more effective at limiting intracellular mycobacterial replication as has been observed for Mϕ. Although human monocyte-derived DC are not permissive of Mtb replication, Mtb does grow similarly in murine bone marrow DC and Mϕ (9, 10). Although the mycobacterial phagosome found in human DC and Mϕ is similar, there is reduced intracellular vesicular trafficking between the phagosome and other endocytic pathways in DC. Because Mtb depends on the delivery of important nutrients from the endoplasmic reticulum and recycling endosome, mycobacterial replication may be impaired if the delivery of essential nutrients to the phagosome is restricted by human DC (11).

Although several investigators have shown that Mtb infects DC in vitro, fewer studies have addressed whether Mtb infects DC in vivo. Lagranderie et al. (12) found that intranasal delivery of bacille Calmette-Guerin (BCG) to mice leads to infection of alveolar Mϕ and DC. Similarly, infected DC and Mϕ were detected in the spleens of mice following high-dose i.v. BCG administration (13). These studies show the potential for Mtb to physiologically infect DC in vivo; however, there is no direct evidence that DC are required for immunity and host resistance in vivo. We used pCD11c-diphtheria toxin receptor (DTR)/GFP transgenic (Tg) mice to determine whether DC have a role in priming the CD4$^+$ T cell response following Mtb infection in vivo (14). Because these mice express the simian DTR in CD11c$^+$ cells, treatment with diphtheria toxin (DT) transiently ablates DC in vivo (14). Using this model, we show a critical role for DC in priming the CD4$^+$ T cell response to the early secretory Ag of tuberculosis 6 (ESAT6). Furthermore, our data indicate that a transient delay in initiating adaptive immunity impairs control of Mtb replication. Thus, our study demonstrates that DC play a pivotal role in initiation of T cell immunity to the human pathogen Mtb.

Materials and Methods

Mice

Hemizygous male B6.FVB-Tg(Itgax-DTR/EGFP)57Lan/J Tg (CD11c/DTR Tg) mice that had been backcrossed to C57BL/6 mice at least five generations were obtained from The Jackson Laboratory (14). Male CD11c/DTR Tg mice were crossed with female C57BL/6 mice (The Jackson Laboratory) to obtain F_1 progeny. The F_1 progeny were screened by PCR (with primers DTR1, 5′-CAAATGTTGCTTGTCTGGTG-3′ and DTR2, 5′-GTCAGTCGAGTGCACAGTTT-3′) to identify mice containing the transgene (Tg$^+$) (14). Statistically, 50% of the mice were Tg$^+$. Mice lacking the transgene were designated as wild-type (WT) littermate controls. For each experiment, mice were matched for age (within 1–2 wk) and gender. Mice were housed under specific pathogen-free conditions and used in a protocol approved by the institution (Dana-Farber Cancer Institute, Boston, MA). To delete CD11c$^+$ cells, CD11c-DTR Tg mice were

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[3] Abbreviations used in this paper: Mtb, *Mycobacterium tuberculosis*; BCG, bacille Calmette-Guerin; DC, dendritic cell; DT, diphtheria toxin; DTR, DT receptor; ESAT6, early secretory Ag of tuberculosis 6; LN, lymph node; Mϕ, macrophage; Tg, transgenic; WT, wild type.

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injected i.p. with 12 ng/g body weight DT (List Biological Laboratories) 4 h before infection (14).

Bacteria and infections

Virulent Mtb (Erdman strain) was grown as described (15, 16). Before infection, an aliquot was thawed, sonicated twice for 10 s using a cup horn sonicator, and then diluted in 0.9% NaCl. Mice were infected by the i.v. route via the lateral tail vein with 10^5 CFU of Mtb. The titer of the Mtb suspension was confirmed by plating serial dilutions onto 7H10 agar plates (REMEL). Infected mice were housed in a biosafety level 3 facility.

CFU determination

Following infection, the left lung, spleen, left liver lobe, axillary LN, and pulmonary LN were aseptically removed from euthanized mice (n = 5/group). The tissue was homogenized in 0.9% NaCl-0.02% Tween 80 with a MiniBead Beater-8 (BioSpec Products) as described (17). Viable bacteria were enumerated by plating 10-fold serial dilutions of organ homogenates onto 7H11 Mitchison agar plates (REMEL). Colonies were counted after 3 wk of incubation at 37°C.

Preparation of cells

$CD11c^+$ cell depletions in the lung and spleen were measured 24 and 48 h after DT treatment. Lung mononuclear cells were prepared as previously described (18, 19). Spleen and LN were minced and pressed through a 70-μm cell strainer followed by RBC lysis to obtain single cell suspensions. $CD4^+$ T cells were purified by positive selection using immunomagnetic beads (Miltenyi Biotec), and purities of >90% were routinely obtained.

In vitro restimulation assays

Splenocytes or pulmonary LN cells (2.5×10^6 cells/ml) were cultured in medium alone or medium containing 1 μg/ml Con A or 1:1000 Mtb H37Ra sonicate as described (19). After 48 h, cytokines in the culture supernatants were measured by ELISA using Ab pairs (BD Pharmingen).

Flow cytometric analysis

Cells were resuspended in staining buffer (5% FBS and 0.02% NaN_3 in PBS) containing 50 μg/ml anti-FcR Ab (clone 2.4G2; American Type Culture Collection). Cells were stained with Abs to CD4 or CD11c (clones GK1.5 and HL3, respectively) or isotype controls (BD Pharmingen) for 20 min on ice. Data were collected using a FACSort (BD Biosciences) and analyzed using the FlowJo software program (Tree Star).

ESAT6-specific IFN-γ ELISPOT

An IFN-γ ELISPOT assay was done using the BD ELISPOT kit (BD Biosciences). Briefly, ELISPOT plates were coated with a capture anti-IFN-γ mAb overnight at 4°C. The capture Ab was discarded, and the plates were washed and blocked. Purified $CD4^+$ T cells, 10 μM $ESAT6_{1-15}$ peptide (MTEQQWNFAGIEAAA), and irradiated (3300 rad) naive C57BL/6 splenocytes were added to the wells and cultured for 40 h at 37°C. After the cells were discarded and plates were washed, a biotinylated anti-IFN-γ mAb was added for 2 h, followed by washing. Streptavidin-alkaline phosphatase was added for 1 h, followed by washing and development of a color reaction using the 3-amino-9-ethylcarbazole substrate reagent kit. The reaction was stopped with water when the spots developed. An immunospot analyzer (Series 3A; Cellular Technology) was used to enumerate the spots.

Peptide vaccination

$ESAT6_{1-15}$ (0.5 mg/ml PBS) was emulsified in an equal volume of CFA and injected s.c. into the CD11c/DTR Tg mice.

Results

Intravenous infection with Mtb initiates an immune response in the spleen

Following respiratory infection with Mtb, the bacteria disseminate to the draining LN within 8 days (19). Dissemination precedes initiation of the T cell response, which is detected in the draining LN on day 10 and in the lung on day 12 (19). In contrast, the immune response following i.v. infection develops more rapidly. After i.v. infection, most bacteria are initially deposited in the liver and spleen, and only 1–5% of the initial inoculum reaches the lung (20). In addition, few bacteria reach the peripheral LN (Fig. 1*A*). This suggests that the adaptive immune response might be initiated in the spleen following infection by the i.v. route, bypassing the need for bacteria to be transported from the lung to the draining LN by APC. To examine this in more detail, splenocytes and pulmonary LN cells were obtained from mice 3, 6, and 9 days after i.v. Mtb infection. To determine when the T cell response is initiated, cells were restimulated in vitro with mycobacterial Ags, and the culture supernatants were tested for IFN-γ 48 h later. Production of IFN-γ by splenocytes in response to Ag restimulation was detected within 6 days after infection (Fig. 1*B*). In contrast, no Mtb-specific activation of pulmonary LN cells was detected through day 9 after infection despite a similar response to Con A (Fig. 1*B*). T cell immunity was also evaluated by enumerating $ESAT6_{1-15}$-specific $CD4^+$ T cells in the spleen and peripheral LN

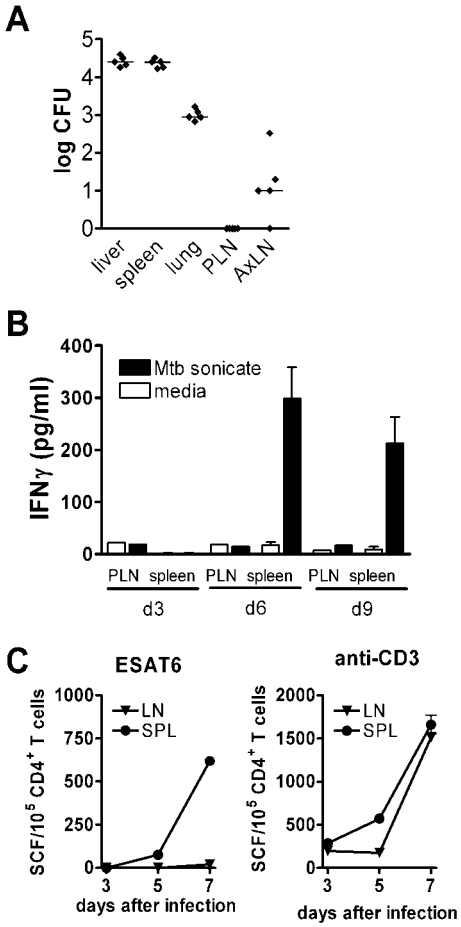

FIGURE 1. Ag-specific response to Mtb following i.v. infection. *A*, Mtb CFU in the liver, spleen, lung, pulmonary LN (PLN), and axillary LN (AxLN) were enumerated 1 day after i.v. infection. Each symbol represents an individual mouse. Line, median. *B*, Splenocytes or PLN cells were obtained from infected mice 3, 6, or 9 days after i.v. Mtb infection and cultured alone (□) or in the presence of Mtb sonicate (1/1000 dilution) (■). IFN-γ in the culture supernatant was measured after 48 h. For all three time points, between 900 and 1800 pg/ml IFN-γ were detected in the supernatant of splenocytes and PLN cells stimulated with Con A. Bars represent means ± SE. *C*, $CD4^+$ T cells were purified from spleen (SPL) or peripheral LN (LN) 3, 5, or 7 days after i.v. Mtb infection. The frequency of the $ESAT6_{1-15}$ $CD4^+$ T cells was enumerated using an IFN-γ ELISPOT assay. As a control, $CD4^+$ T cells were stimulated with anti-CD3 mAb in the presence of syngeneic irradiated APC. Data are expressed as spot-forming cells (SFC) per 10^5 $CD4^+$ T cells. Symbols represent means ± SE. Each graph represents an independent experiment.

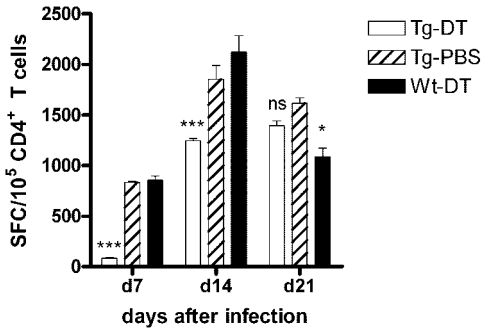

FIGURE 2. In vivo depletion of CD11c$^+$ cells delays the CD4$^+$ T cell response to Mtb. CD11c/DTR Tg mice were treated with DT (Tg-DT) or PBS (Tg-PBS), and non-Tg littermate control mice were treated with DT (Wt-DT), 4 h before i.v. infection with Mtb. At weekly time points after infection, purified splenic CD4$^+$ T cells from the three groups of mice were cultured with syngeneic APC and ESAT6$_{1-15}$ peptide. d, day. The frequency of ESAT6$_{1-15}$-specific CD4$^+$ T cells was measured by IFN-γ ELISPOT assay. Data are expressed as spot-forming cells (SFC) per 10^5 CD4$^+$ T cells. Bars represent means ± SE. Statistical testing using a two-way ANOVA with Bonferroni posttest is as follows: ***, $p < 0.001$ Tg-DT group compared with Tg-PBS and Wt-DT; ns, no difference between Tg-DT and Tg-PBS groups; *, $p < 0.05$ between Tg-DT and Wt-DT and $p < 0.001$ between Tg-PBS and WT-DT groups. Data are representative of two to four independent experiments.

following infection. CD4$^+$ T cells were purified 3, 5, and 7 days after i.v. Mtb infection, and the frequency of ESAT6$_{1-15}$-specific CD4$^+$ T cells was measured using an IFN-γ ELISPOT. Although the frequency of IFN-γ-producing cells was similar following stimulation with anti-CD3 at all time points, increasing numbers of ESAT6-specific CD4$^+$ T cells were detected in the spleen as early as day 5 after infection (Fig. 1*C*). ESAT6-specific CD4$^+$ T cells in the peripheral LN were not detected until day 7 after infection, and at that time their frequency was 30-fold less than in the spleen. Thus, following i.v. Mtb infection, immunity is initiated in the spleen and not in the peripheral LN.

In vivo depletion of CD11c$^+$ cells delays the ESAT6-specific CD4$^+$ T cell response elicited by Mtb infection

DC are extremely potent APC that are uniquely suited to prime naive T cells, although their role following Mtb infection is unclear. To determine the in vivo role of DC in the initiation of Ag-specific CD4$^+$ T cell response following Mtb infection, we used CD11c/DTR Tg mice to transiently delete DC. As previously described, 24 h after i.p. DT injection, >85% of CD11c^{high} cells in the spleen of CD11c/DTR Tg mice are deleted, whereas the Mϕ (CD11b$^+$F4/80$^+$) are unaffected (data not shown and Ref. 14). In contrast, few lung CD11c$^+$ cells are deleted following i.p. DT injection (data not shown). Repopulation of splenic CD11c^{high} cells begins within 48 h after DT injection (14). Because the T cell response is initiated in the spleen after i.v. infection and splenic DC are preferentially deleted in these Tg mice, we used this model to determine whether DC are critical for priming the CD4$^+$ T cell response to mycobacterial Ags following Mtb infection. Three experimental groups of mice were infected with Mtb: 1) CD11c/DTR Tg littermates that received DT (Tg-DT), 2) CD11c/DTR Tg littermates that received PBS (Tg-PBS), and 3) WT littermates that received DT (WT-DT). CD4$^+$ T cells were purified from the spleens of the infected mice, and the ESAT6-specific CD4$^+$ T cells were enumerated by an IFN-γ ELISPOT assay. When measured 7 days after infection, the splenic ESAT6-specific CD4$^+$ T cell response generated by Tg-DT mice was reduced by 90% compared with Tg-PBS and WT-DT mice (Fig. 2). The frequency of ESAT6-specific CD4$^+$ T cells was 88/10^5 CD4$^+$ T cells in the Tg-DT group compared with 833/10^5 CD4$^+$ T cells in the Tg-PBS group. These data show that CD11c^{high} cells are required for initiation of the Ag-specific T cell response to Mtb. Because DT-mediated deletion of CD11c$^+$ cells is only transient, we next asked whether the T cell response recovers or whether there is long-term ablation of the response. Analysis of later time points revealed that the frequency of ESAT6-specific CD4$^+$ T cells began to increase by day 14 after infection, and, by day 21, the frequency of ESAT6-specific CD4$^+$ T cells was similar between the Tg-DT and Tg-PBS mice. These data show that transient deletion of DC significantly delays priming of Ag-specific CD4$^+$ T cells following infection but that, upon repopulation of DC, immune T cells are generated.

Although activated CD4$^+$ T cells have not been reported to express CD11c (J. W. Huleatt, unpublished observations), we verified that ESAT6-specific CD4$^+$ T cells from CD11c/DTR Tg mice are resistant to DT-mediated deletion. CD11c/DTR Tg mice were immunized with ESAT6$_{1-15}$ peptide, and, 10 days later, purified LN CD4$^+$ T cells were stimulated in vitro with ESAT6$_{1-15}$-pulsed irradiated WT APC in the presence or absence of DT. The frequency of IFN-γ-producing ESAT6-specific CD4$^+$ T cells detected by ELISPOT was ~600/10^5 CD4$^+$ T cells and was unaffected by the presence of DT, even at concentrations as high as 5 μg/ml (Fig. 3). Thus, DT treatment did not delete activated CD4$^+$ T cells.

Transient deletion of CD11c$^+$ cells impairs control of Mtb replication

We next determined whether temporary ablation of CD11c$^+$ cells affected control of Mtb replication. CD11c/DTR Tg mice and non-Tg WT littermate controls were treated with PBS or DT as previously described and infected with Mtb. The CFU in the lung and spleen were measured at different time points following infection. To confirm that depletion of CD11c^{high} cells does not affect the initial deposit of viable bacteria in the spleen, we determined splenic CFU 2 days after infection, when DT-mediated DC deletion is maximal. DT treatment did not affect the bacterial burden in the spleens of infected mice (Fig. 4*B*). At later time points, we found that the Tg-DT mice were unable to control the bacterial replication as well as the Tg-PBS or WT-DT control mice. For example, the Mtb CFU in the lungs of Tg-DT mice was 20-fold greater on day 14 and 10-fold greater on day 21 than results for the control mice (Fig. 4*A*). Similarly, bacterial replication was not as

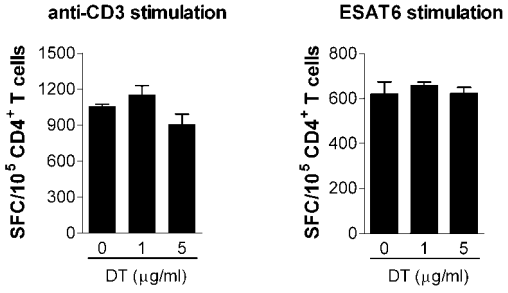

FIGURE 3. DT insensitivity of CD4$^+$ T cells from draining LN of immunized mice. CD11c/DTR Tg mice were immunized s.c. with ESAT6$_{1-15}$. The peripheral LN were harvested 10 days later. Purified CD4$^+$ T cells were cultured with anti-CD3 mAb in the presence of WT APC (*left*) or with ESAT6$_{1-15}$-pulsed WT APC (*right*). DT was added in vitro at the indicated concentrations. IFN-γ-producing cells were enumerated by ELISPOT. Data are representative of two independent experiments. Bars represent means ± SE.

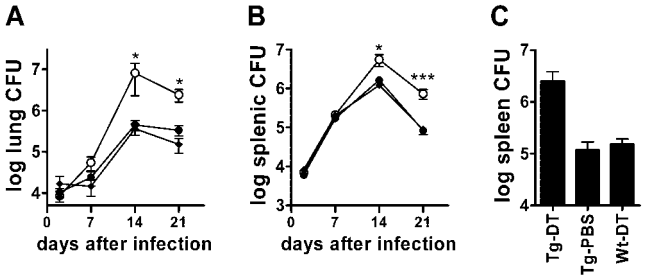

FIGURE 4. Effect of DC deletion on mycobacterial replication following infection. Groups ($n = 5$) of Tg mice treated with DT (Tg-DT; ○) or PBS (Tg-PBS; ●) and WT mice treated with DT (Wt-DT; ♦) were infected with 10^5 CFU of Mtb. CFU in the lung (*A*) and spleen (*B*) were determined weekly as described in *Materials and Methods*. *C*, Splenic CFU were determined 44 days after infection. Each data point represents the mean ± SE. Statistical testing using a one-way ANOVA with Bonferroni posttest is as follows: ***, $p < 0.001$; *, $p < 0.05$ Tg-DT group compared with Tg-PBS and Wt-DT. Data in *A* and *B* are representative of four independent experiments; data in *C* are representative of two independent experiments analyzed 28 and 44 days postinfection.

efficiently controlled in the spleens of the Tg-DT mice (Fig. 4*B*). Ultimately, the Tg-DT mice appear to control the infection, as indicated by the plateauing of the CFU in the lung and spleen, which correlates with their acquisition of T cell immunity (see Fig. 2). However, even 28 and 44 days after infection, the bacterial burden was higher than that for the control mice, indicating that even a transient delay in initiation of adaptive immunity impairs control of bacterial replication (Fig. 4*C* and data not shown).

In vivo depletion of DC does not affect the recall response in peptide-vaccinated mice

Our data show that DC are required for priming of the ESAT6-specific $CD4^+$ T cell response following infection. Because activated/memory T cells have a less stringent requirement for costimulation, we reasoned that presentation of the $ESAT6_{1-15}$ epitope by infected Mϕ might be sufficient to stimulate activation of Ag-specific $CD4^+$ T cells. Thus, DC may be dispensable for the recall response in vaccinated mice. Groups of CD11c/DTR Tg mice were vaccinated with the $ESAT6_{1-15}$ peptide in CFA or received CFA alone as a control. Two weeks later, the mice were treated with DT or PBS, and 4 h later they were infected with Mtb by the i.v. route. The $ESAT6_{1-15}$-specific $CD4^+$ T cell response in CD11c/DTR Tg mice immunized with CFA alone was similar to that for unvaccinated mice (Fig. 5 and see Fig. 2). The frequency of $ESAT6_{1-15}$-specific $CD4^+$ T cells in Tg-PBS mice was ~1/120, and DT treatment resulted in a frequency of 1/2000 (95% reduction) (Fig. 5). In contrast, DT treatment did not affect the $ESAT6_{1-15}$-specific $CD4^+$ T cell response in mice previously immunized with ESAT6 in CFA. The frequency of $ESAT6_{1-15}$-specific $CD4^+$ T cells in previously $ESAT6_{1-15}$-vaccinated mice increased to ~1/55 for both the DT- and PBS-treated groups (Fig. 5). This result indicates that DC are not required for the secondary $CD4^+$ T cell response following Mtb infection.

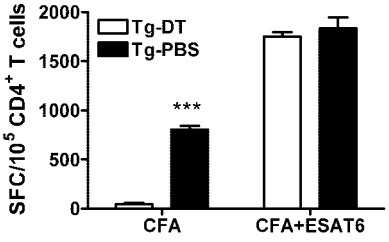

FIGURE 5. Role of $CD11c^+$ cells in the secondary $CD4^+$ T cell response to $ESAT6_{1-15}$. Groups of mice ($n = 3$/group) were immunized with $ESAT6_{1-15}$ in CFA or with adjuvant alone. After 2 wk, the mice were treated with DT or PBS and then infected with Mtb. An IFN-γ ELISPOT was used to enumerate the number of ESAT6-specific $CD4^+$ T cells in the spleen 7 days later. Bars represent means ± SE. ***, $p < 0.001$. Data are representative of two independent experiments.

Discussion

The need to study DC function in vivo led to the development of the pCD11c-DTR/GFP Tg mouse model, which provides a way to ablate DC in adult mice (14). This model has provided considerable insight into the role of DC in the activation of NKT cells (21), the development of airway hypersensitivity (22), and the priming of $CD8^+$ T cells in microbial immunity (14, 23). We have used pCD11c-DTR/GFP Tg mice to demonstrate that DC are essential for priming the $CD4^+$ T cell response following infection with Mtb. Specifically, DC are required for the development of class II MHC-restricted ESAT6-specific $CD4^+$ T cells generated following i.v. Mtb infection. Presumably, the T cell response to other mycobacterial Ags is also dependent on DC, as transient DC ablation significantly impairs control of bacterial replication both in the spleen and in the lung.

Our data do not address the interesting question of whether infected DC are required for initiation of the immune response. Although Mtb infects both human and mouse DC in vitro, it is more difficult to determine whether DC are infected in vivo. Following i.v. infection of mice with BCG or H37Ra-GFP, infected $CD11c^{high}$ splenocytes can be detected by flow cytometry and by immunofluorescence (13) (T. Tian and S. M. Behar, unpublished observations). Even if Mtb-infected DC were definitively demonstrated in vivo, their significance would still be speculative because uninfected DC can acquire mycobacterial Ags by mechanisms other than direct infection. Immature tissue DC can phagocytose dead bacteria, bacteria products, or even apoptotic vesicles that carry mycobacterial Ags (24). For example, splenic DC can take up mycobacterial Ags found in culture supernatants from BCG-infected Mϕ and present these Ags to T cells (25). Regardless of how the Ags are acquired, our data show a critical role for DC in priming the $CD4^+$ T cell response in vivo following Mtb infection and are consistent with either Ag presentation by directly infected DC or following secondary Ag acquisition by uninfected DC.

The immune response following respiratory Mtb infection, which represents the dominant route of transmission between people, differs in some important respects from immunity following i.v. Mtb infection. Although we have only shown that DC are required for T cell priming following i.v. Mtb infection, it is also likely that DC are critical following aerosol Mtb infection. DC have a unique role in responding to "danger" signals and transporting microbial Ags from the pulmonary tissue beds to the draining LN, a function unlikely to be mediated by pulmonary Mϕ. Upon trafficking to the draining LN, DC are well suited to efficiently prime naive T cells. Thus, priming mycobacterial Ag-specific T cells following respiratory infection is likely to be critically dependent on DC, as we have observed following i.v. infection.

In contrast to the important role that DC play in priming the T cell response following infection, we found that the secondary recall response of $CD4^+$ T cells following Mtb infection of vaccinated mice was independent of DC. This suggests that presentation of mycobacterial Ags by CD11c-negative APC, such as B cells or Mϕ, may be sufficient to activate memory T cells. Interestingly, using a similar approach, van Rijt et al. (22) found that depletion

of lung CD11c$^+$ DC abrogated the development of allergic hypersensitivity in the lung following priming with OVA. This differs from our results because the development of allergic hypersensitivity in the lungs of OVA-primed mice is thought to represent a secondary response. However, Ag presentation of a small soluble protein is likely to be very different from presentation of proteins secreted by an intracellular bacterial pathogen. This is especially true because Mtb preferentially infects Mϕ, which effectively targets its Ags to a competent APC. In contrast, aerosolized OVA is not selectively targeted to any particular cell type but may accumulate in DC because of their great capacity for pinocytosis. Finally, all alveolar Mϕ and some lung tissue Mϕ also express CD11c, and some are deleted following intratracheal DT administration to CD11c/DTR Tg mice (data not shown and (Ref. 22). These differences may explain why the DC dependence of the recall response to OVA and Mtb differ.

Although tuberculosis is a chronic infectious disease, the early immunological events that occur after Mtb infection may have profound consequences for the outcome of infection and disease. For example, Caruso et al. (26) showed that early production of IFN-γ by CD4$^+$ T cells is a critical determinant of host resistance to Mtb infection. We have previously observed that susceptible C3H mice have delayed dissemination from the lung compared with resistant C57BL/6 mice. Early bacterial dissemination in C57BL/6 mice correlates with early initiation of Ag-specific T cell immunity and recruitment of the immune response to the lung with subsequent granuloma formation (19). Consistent with these data, Mischenko et al. found that T cell recruitment to the lung occurs earlier in resistant mice than in susceptible mice (27). A question raised by these studies is whether a delay in priming or recruitment of immune T cells to the lung affects the outcome of infection. Our experiments using DC-ablated mice show how impeding T cell immunity hinders control of bacterial replication. Following reconstitution of DC, Ag-specific CD4$^+$ T cells are detected in the spleen, leading to improved control of bacterial replication by day 14 postinfection. Although onset of adaptive immunity is heralded by improved control of the infection, the immune response in DC-ablated mice does not appear to completely "catch up," and the chronic steady-state bacterial burden in the spleen remains at a higher level than that observed in normal mice during the first 6 wk of the infection. This finding supports the hypothesis that early initiation of immunity and recruitment of T cells to the lung is crucial for optimum host resistance to Mtb (19). These data show how initiation of the adaptive immune response is linked to control of Mtb infection.

The experiments presented herein evaluate the in vivo requirement for DC during Mtb infection by using the CD11c/DTR Tg mouse model that allows transient ablation of DC. We find that DC are required to prime ESAT6-specific CD4$^+$ T cells because depletion of CD11c$^+$ cells significantly diminishes and delays the emergence of the ESAT6-specific T cell response. Second, deletion of CD11c$^+$ cells and the subsequent delay in adaptive immunity impair control of Mtb replication. Finally, the ESAT6-specific CD4$^+$ T cell recall response elicited by Mtb infection in peptide-vaccinated mice does not require CD11c$^+$ cells. These experiments demonstrate the critical role for DC in the initiation of adaptive immunity to Mtb.

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Disclosures

The authors have no financial conflict of interest.

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