Modulation of Pulmonary Dendritic Cell Function during Mycobacterial Infection

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We have previously reported that during mycobacterial infection, naïve CD4+ T-cell activation is enhanced in the lungs. We investigated the role of chemokine receptor CCR7 and its ligands in the ability of CD11c+ lung dendritic cells (DCs) to activate naïve CD4+ T cells during pulmonary infection with Mycobacterium bovis bacillus Calmette-Guérin (BCG). BCG infection resulted in the accumulation and maturation in the lungs of DCs that persisted as the mycobacterial burden declined. Lung DCs from infected mice expressed more major histocompatibility complex class II (MHC-II) than those from uninfected mice. CCR7 expression levels on lung DCs were comparable among uninfected and infected mice. The gene expression of the CCR7 ligand CCL19 progressively increased throughout BCG infection, and its expression was MyD88 dependent. CD11c+ lung cells from BCG-infected mice activated ovalbumin (OVA)-specific naïve CD4+ T cells more than CD11c+ lung cells from uninfected mice. Interestingly, during peak mycobacterial infection, CD11chi MHCIII lung DCs had slightly decreased chemotaxis toward the CCR7 ligand CCL21 and less efficiency in activating naïve CD4+ T cells than DCs from mice during late-stage infection, when few bacilli are found in the lung. These findings suggest that during BCG infection, the inflammation and sustained expression of CCL19 result in the recruitment, activation, and retention in the lung of DCs that can activate naïve CD4+ T cells in situ.

During pulmonary mycobacterial infection, the migration of dendritic cells (DCs) and the dissemination of mycobacteria to draining lymph nodes are thought to be important for a successful cell-mediated immune response (6, 22, 36). Upon the engagement of Toll-like receptors (TLRs) on DCs by mycobacterium-derived, pathogen-associated molecular patterns, DCs undergo a coordinated maturation program that upregulates expression of the chemokine receptor CCR7. Chemokine receptor CCR7 expression is essential for the migration of DCs to the lymph nodes, where they coordinate adaptive immune responses following TLR stimulation (9, 17, 23, 33, 34).

CCR7 and its ligands, CCL19 (Epstein-Barr virus-induced molecule 1 ligand chemokine) and CCL21 (secondary lymphoid chemokine), are important for both the initiation and regulation of adaptive immunity, as they direct the migration of mature DCs, naïve CD4+ T cells, and central memory T cells to secondary lymphoid organs (SLOs) (31, 32). There are two isoforms of CCL21: CCL21-ser and CCL21-leu. CCL19 and CCL21-ser are produced by stromal cells within the T-cell zones of SLOs. CCL21-ser is also expressed in high endothelial venules, while CCL21-leu is expressed only in the lymphatic endothelium. Upon maturation, DCs also express CCL19 (26). Both CCL19 and CCL21 are involved in the organization of lymphoid structures under normal and chronic inflammatory conditions by facilitating encounters among stromal cells, T cells, B cells, and DCs (1, 20, 21).

CCR7 may have a restricted role in immune responses to airborne pathogens. Mice lacking the expression of CCL19 and CCL21-ser (plt/plt) have a delayed immune response that is still protective during influenza virus infection (24). Although CCR7−/− mice can control virulent mycobacterial infection, the roles of CCL19 and CCL21 in pulmonary mycobacterial infection have not been evaluated (14, 35). CCR7 ligands within SLOs facilitate the interactions between naïve T cells and mature DCs (20). Therefore, we examined how pulmonary BCG infection affects lung DCs and the expression of CCR7 ligands in the lungs in order to gain insight into naïve CD4+ T-cell activation in the lung during chronic lung inflammation. Using an attenuated strain of mycobacteria, Mycobacterium bovis BCG, we report that BCG infection induced the pulmonary expression of CCL19 along with the increased activation of DCs in the lungs. The migration of lung DCs toward CCL21 and the activation of naïve CD4+ T cells by lung DCs were slightly less efficient during peak infection than in late infection. All together, our findings suggest that the pulmonary expression of the naïve T-cell chemoattractant CCL19 and the reduced CCR7-mediated migration of activated lung DCs lead to the retention of mature DCs in the lungs that can activate naïve CD4+ T cells in situ during pulmonary mycobacterial infection.

MATERIALS AND METHODS

Mice. Eight- to 10-week-old female BALB/c and C57BL6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME). MyD88−− mice were obtained from Osamu Takeuchi and Shizua Akira (Osaka University, Osaka, Japan) and backcrossed in a C57BL6 background for seven generations. Strain DO11.10 T-cell receptor (TCR) transgenic mice that express TCRs specific for...
the ovalbumin peptide comprising amino acids 323 to 339 (OVA23-35) presented in the context of I-A\(^d\) (25) were obtained from Alan Levine (Case Western Reserve University, Cleveland, OH) and bred on site. Mice were housed in specific-pathogen-free conditions. All studies were approved by the Institutional Animal Care and Use Committee at Case Western Reserve University.

Aerosolized BCG infection. Mice were exposed to aerosolized M. \textit{bovis} BCG in an inhalation exposure system (Glasc-ol, Terre Haute, IN) as previously described (16). Uninfected mice served as controls.

Tissue isolation. Tissues were harvested and processed as previously described (16). Briefly, mice were anesthetized with a lethal dose of tribromoethanol (240 mg/kg). The abdominal cavity was incised, the spleen harvested, and the mouse exsanguinated. The trachea was cannulated, and bronchoalveolar lavage fluid was collected by aspirating it three times with 1 ml of phosphate-buffered saline (PBS). Lungs were perfused with 10 ml of PBS and harvested. Then draining mediastinal lymph nodes were harvested. Single cells were resuspended in complete medium (Dulbecco modified Eagle medium, 10% fetal bovine serum [FBS], 0.05 mM 2-mercaptoethanol, 2 mM HEPES, 1 mM sodium pyruvate, 100 mM nonessential amino acids, 100 U/ml penicillin, and 0.1 mg/ml streptomycin). Lungs were minced and digested with 125 units/ml of type IV collagenase and 30 units/ml DNase for 90 min at 37°C. Lung tissue aggregates were drawn through an 18-gauge needle three times before being passed through a 40-μm nylon filter. Red blood cells were lysed, and lung tissue was resuspended in RPMI medium. Serial dilutions of lung suspension were plated on 7H10 plates to determine the number of bacterial CFU. Lung cells were then positively sorted for CD11c\(^+\) cells using N418 microbeads (Miltenyi Biotec, Auburn, CA). Mediastinal lymph nodes were processed as above.

Cell staining. Single-cell suspensions of tissues and sorted cells were counted, and viability was assessed by trypan blue exclusion. A total of 5 × 10\(^4\) to 1 × 10\(^5\) viable cells were then resuspended in 1% bovine serum albumin (BSA)-PBS solution of FcBlock (BD Pharmingen, San Jose, CA) for 15 min at 4°C. The cells were then stained with anti-CCR7 (BD12), anti-CD11c, anti-CD11b (eBioscience; San Diego, CA), and anti-I-Ad (BD Pharmingen; catalog number 553546). Cells were incubated for 30 min at 4°C and then washed and incubated with the appropriate fluorochrome-conjugated antibody (eBioscience). Cells were washed once with 1% BSA, and pellets were resuspended in 1% paraformaldehyde in PBS. Stained samples were acquired using a BD LSR II flow cytometer. Flow cytometry results were analyzed with FlowJo software (Tree Star, Inc.).

CD11c\(^+\) lung cell chemotaxis assay. Lung cells were sorted with magnetic CD11c (N418) beads (Miltenyi Biotec) as described above. In 24-well transwell plates with 8-μm-pore-size polycarbonate inserts (Corning Costar Corp., Cambridge, MA), a total of 4 × 10\(^4\) viable CD11c\(^+\) cells were added to inserts, and 600 μl of chemotaxis medium (0.1% BSA in serum-free HL-1 medium) containing 100 ng/ml of CCL21 (R&D) was added to the lower chambers. After 5 h of incubation at 37°C, cells from the lower chambers were collected and pelleted. Migrated cells were stained with anti-CD11c, anti-CD11b, and anti-I-Ad (BD Pharmingen; catalog number 553546). Cells were incubated for 30 min at 4°C and then washed and incubated with the appropriate fluorochrome-conjugated antibody (eBioscience). Cells were washed once with 1% BSA, and pellets were resuspended in 1% paraformaldehyde in PBS. Stained samples were acquired using a BD LSR II flow cytometer. Flow cytometry results were analyzed with FlowJo software (Tree Star, Inc.).

RESULTS

BCG infection results in the accumulation and maturation of lung DCs beyond peak bacterial burden in the lungs. BALB/c mice were infected by aerosol with BCG. The BCG burden in the lungs peaked 4 to 6 weeks after infection and declined to <100 bacilli/lung by 12 to 14 weeks (Fig. 1A). To determine the effect of BCG infection on the number and maturity of DCs in the lungs, the mice were divided into three groups: uninfected mice, mice infected for 4 to 6 weeks, and mice infected for 12 to 14 weeks. The mice were sacrificed, and their lungs harvested. Lung cells were stained for CD11b, CD11c, CD80, and I-Ad to measure the maturation state of myeloid DCs (CD11b\(^hi\)CD11c\(^+\)) in uninfected mice (Fig. 1B). To determine the maturation state of myeloid DCs (CD11b\(^hi\)CD11c\(^+\)) in infected mice compared to those in uninfected mice (Fig. 1C). Inter-estingly, the maturation and accumulation of mature and late DCs in the lung persisted up to 12 to 14 weeks after infection, when the lung bacterial burden had decreased to below the limit of detection.
FIG. 1. Accumulation and maturation of DCs in the lung during BCG infection. (A) Lungs from BALB/c mice at 1 day, 4 to 6 weeks, and 12 to 14 weeks after aerosolized BCG infection were processed, and aliquots were plated to enumerate lung CFU. Mean numbers of CFU ± standard deviations (SD) from a single BCG infection of three mice per time point are shown. Similar bacterial burdens were measured in three separate experiments. Sorted CD11c⁺ lung cells from uninfected mice, mice infected for 4 to 6 weeks, and mice infected for 12 to 14 weeks were stained for MHC-II (I-Ad), CD11b, and CD11c. (B) Maturation of myeloid (CD11b⁺ CD11c⁺) DCs was assessed by gating on these cells and by examining the geometric mean fluorescent intensity (MFI) of surface MHC-II staining. Uninf., uninfected. (C) After gating on live cells was done, the number of mature (CD11c⁺ MHC⁺) lung DCs was calculated by multiplying the percentage of CD11c⁺ MHC⁺ cells by the total number of live cells, as assessed by trypan blue exclusion. Data in panels B and C are means ± SD of four independent experiments with three to four mice per experiment; #, ##, *, **, P < 0.02 compared to uninfected mice.

BCG infection does not increase surface expression of CCR7 on lung DCs during peak and late stages of infection. The maturation of DCs is accompanied by a coordinated switch in their chemokine receptor expression that may explain the increased retention of DCs in the lungs during BCG infection. Upon maturation, tissue DCs upregulate CCR7 expression and migrate to SLOs (34). To determine if the maturation of lung DCs during mycobacterial infection resulted in the increased expression of CCR7, lungs were harvested from uninfected mice and mice that had been infected with aerosolized BCG 4 to 6 weeks (peak infection group) or 12 to 14 weeks earlier (late-infection group). Lung cells from these different groups of mice were sorted with CD11c beads to enrich for lung DCs. Total RNA was extracted from a total of 2 x 10⁶ to 3 x 10⁶ sorted CD11c⁺ cells from the three groups of mice and used to measure CCR7 mRNA transcript levels by qRT-PCR. BCG infection induced 1.5- to 2-fold increases (P > 0.05) in CCR7 gene expression in CD11c⁺ lung cells compared to that in uninfected mice (Fig. 2A). In addition, CCR7 protein expression levels on lung macrophages (CD11c⁺⁴ CD11b⁻) and myeloid lung DCs (CD11c⁺⁴ CD11b⁻) were assessed using flow cytometry (Fig. 2B). Lung macrophages from the three groups of mice did not express detectable levels of CCR7. Although lung DCs expressed low levels of CCR7, a significant difference in CCR7 surface expression levels between lung DCs from infected and uninfected mice was not detected. The surface expression of CCR7 was also determined by staining with CCL19-Fc ligand (eBioscience); however, no evident shift in mean fluorescent intensity was noted among the three groups with this method (data not shown). Thus, the accumulation of DCs in the lung during infection was associated with an increased amount of CCR7 mRNA in the lung, as measured by qRT-PCR, without causing significant changes in CCR7 protein expression on individual lung DCs or lung macrophages as determined by flow cytometry.

BCG infection upregulates expression of CCL19 in lung in a MyD88-dependent manner. Like the expression of other chemokine receptors, CCR7 surface expression is modulated by its cognate ligands CCL19 and CCL21 via ligand-induced down-regulation (4, 27). To determine if the lack of upregulation of CCR7 surface protein expression during BCG infection could be due to the expression of CCL19 and CCL21 in infected lungs, total RNA from the lungs of uninfected and BCG-infected mice was isolated and CCL19 and CCL21 mRNA levels were measured by qRT-PCR. BCG infection induced CCL19, but not CCL21, gene expression in the lungs (Fig. 3A). Mice infected 12 to 14 weeks earlier expressed higher levels of CCL19 mRNA than mice infected 4 to 6 weeks earlier (P = 0.01). Increased CCL19 expression in the lung during BCG infection may have inhibited the upregulation of CCR7 on DCs.

CCL19 expression is also increased in the lungs of mice infected with virulent Mycobacterium tuberculosis that grows to greater numbers (10⁶ CFU/mouse lung) during late infection (14, 35). Thus, it was surprising that BCG infection upregulated CCL19 mRNA expression during late infection (12 to 14 weeks postinfection), when the lung bacterial burden was reduced to fewer than 10² lung CFU/mouse (Fig. 1A). Recent studies have shown that MyD88 along with other TLR adaptors and cytokines regulate the CCL19 promoter (30). To determine if the recognition of whole mycobacteria or mycobacterial products by TLRs had a role in the increased expression of CCL19 mRNA in BCG-infected mice, wild-type C57Bl/6 and MyD88⁻/⁻ mice were infected with BCG. MyD88⁻/⁻ mice are deficient in the recognition of bacteria and bacterial products through multiple TLRs, including TLR2 and TLR9, that have a role in mycobacterial infection (3). BCG-induced CCL19 mRNA expression was MyD88 dependent (Fig. 3B).

BCG infection modulates CCR7-mediated chemotaxis of lung DCs. The lack of CCR7 upregulation on the surface of lung DCs and the enhanced expression of CCL19 in the lungs of infected mice suggested that the CCR7-mediated migration of lung DCs to draining lymph nodes might be affected by BCG infection. We used transwell assays to examine the ability of
lung DCs from infected and uninfected mice to migrate in response to the CCR7 ligand CCL21, which has affinity for CCR7 similar to that of CCL19 but which causes less down-regulation of CCR7 (4, 27). As shown in Fig. 4A, CD11chi MHChi mature DCs from BCG-infected lungs migrated to toward CCL21 in greater numbers than lung DCs from uninfected mice. Interestingly, lung DCs from mice infected 12 to 14 weeks earlier migrated even more than lung DCs from mice infected for 4 to 6 weeks (P < 0.04), even though both lung DC populations expressed similar levels of CCR7 and MHC-II (Fig. 1B and 2B).

To compare the chemotactic responses of individual mature lung DCs (CD11c\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\hi\h
mature lung DCs were slightly blunted on an individual cell basis compared to those of uninfected mice, even though the accumulation of mature DCs in the lung led to greater numbers of DCs that could migrate in the assay. Thus, the increased number of mature DCs in infected lungs was not due to an intrinsic inability of these cells to migrate during peak infection but rather to a relative change in migratory functions that may have resulted in greater retention of mature DCs in the lung.

**BCG infection alters the ability of CD11c**

**+ lung cells to present peptide to naïve CD4**

**+ T cells in vitro.** The increased number of mature DCs in BCG-infected lungs along with the expression of naïve T-cell chemotactic CCL19 suggested that naïve CD4**

**+ T cells could occur in infected lungs and result in naïve CD4**

**+ T-cell activation. To measure the antigen-presenting cell (APC) function of lung DCs and their ability to activate naïve CD4**

**+ T cells, CD11c**

**+ lung cells from BCG-infected and uninfected mice were pulsed with the OVA**

**+323-339 peptide and incubated with FAC-sorted naïve (CD4**

**+hi CD62L**

**-hi) CD4**

**+ T cells specific for OVA from DO11.10 TCR transgenic mice. After 48 h, culture supernatants were tested for IL-2 production by enzyme-linked immunosorbent assay. (A) Data are expressed as increased numbers of CD11c**

**+ lung cells added. Wells without naïve OVA-specific T cells had undetectable levels of IL-2 (data not shown). (B) Percentage of CD11c**

**+ MHC**

**+ cells present within the CD11c**

**+ lung cells from the three groups of mice was multiplied by the number of CD11c**

**+ lung cells added per well to obtain the number of CD11c**

**+ MHC**

**+ cells added to each well. Data are representative of two independent experiments.**
cells. At the same time, the in vivo environment during peak infection renders the lung DCs slightly less effective for naïve CD4+ T-cell activation than DCs from the late stage of infection (Fig. 5B).

**DISCUSSION**

We have previously used adoptive-transfer techniques to demonstrate that naïve CD4+ T cells are activated in the lung during ongoing *M. bovis* BCG infection (2). These studies led us to focus on the role of lung DCs in priming naïve CD4+ T cells in the lungs in situ (2). Immune responses ordinarily involve the activation of naïve T cells in the organized microenvironment of SLOs (13, 19). Recently, investigations have demonstrated that mycobacterial and influenza virus infections lead to the development of neolymphoid structures in the lung that could serve as potential sites for the priming of naïve T cells (14, 24, 35). We report here that pulmonary mycobacterial infection results in the accumulation and maturation in the lung of DCs that persist beyond peak bacterial burden for up to 12 to 14 weeks postinfection. The accumulation of mature DCs in infected lungs was associated with a mild reduction in the CCR7-mediated chemotaxis of mature lung DCs during peak infection (4 to 6 weeks postinfection), while surface expression levels of CCR7 remained similar between infected and uninfected mice. The reduction in the CCR7-mediated migration of mature lung DCs suggests that DCs are retained in the lungs during BCG infection. Mycobacterial infection induced the expression of the naïve T-cell chemoattractant CCL19 in the lungs through a MyD88-dependent pathway. OVA-specific naïve CD4+ T cells were readily activated by CD11c+ lung cells from BCG-infected mice. Lung DCs during peak infection (4 to 6 weeks postinfection) were slightly less efficient, on a per cell basis, in activating naïve CD4+ T cells than lung DCs during the late stage of infection (12 to 14 weeks postinfection), despite similar levels of MHC-II on lung DCs at both stages of infection.

The stimulation of TLRs by mycobacterial products leads to the maturation of DCs (9, 28). BCG infection caused the accumulation, maturation, and retention of lung DCs even as the lung mycobacterial burden declined. Although BCG infection induced a higher level of surface expression of MHC-II on myeloid lung DCs, CCR7 surface expression was minimally influenced. Moreover, the CCR7-mediated chemotaxis of mature lung DCs was reduced when the bacterial burden peaked in the lungs. A reduction in CCR7-mediated chemotaxis may result in the decreased migration of mature lung DCs to draining lymph nodes and increased retention in the lungs. Naïve CD4+ T cells are present in both lymphoid and nonlymphoid organs such as the lungs (8). The formation of bronchus-associated lymphoid tissue (BALT) during pulmonary mycobacterial infection may facilitate encounters between such naïve T cells and lung DCs (14, 35). The expression of the CCR7 ligands CCL19 and CCL21 is associated with the maturation and organization of neolymphoid follicles (10). Pulmonary BCG infection induced the expression of CCL19 in the lung. However, CCR7 signaling is not essential, since mice deficient in CCR7 or its ligands, CCL19 and CCL21-set (plt/plt), develop BALT during mycobacterial and influenza infections (12, 24). Recently, it has been demonstrated that the formation of BALT in mice and the T-cell responses elicited in such animals are directly affected by the lack of CCR7-mediated migration of regulatory T cells (15). Although the mechanism of BALT formation is still not clearly understood, increased activation and retention of DCs in the lungs along with increased expression of CCL19 may render productive interactions between naïve CD4+ T cells and lung DCs more likely during BCG infection.

We had previously shown that lung inflammation during peak BCG infection caused enhanced pulmonary antigen-specific CD4+ T-cell responses in vivo (2). The enhanced CD4+ T-cell responses in infected lungs could have occurred due to naïve T-cell priming in situ, secondary to increased naïve T-cell recruitment to the lung and the retention of mature DCs in the lung. In the present study, OVA-specific naïve CD4+ T-cell activation by lung DCs was slightly less during peak infection (4 to 6 weeks) than in late infection (12 to 14 weeks) and was not associated with a change in CD80 costimulatory molecule expression. This may represent the modulation of DC function during peak mycobacterial infection that subsides as the infection is controlled. Schreiber et al. have also reported that T cells that are unable to migrate to lymph nodes are recruited to the lungs during pulmonary mycobacterial infection, where they become activated and acquire effector functions after a delay (35). Perhaps the delayed acquisition of effector function by T cells in that study reflects the mycobacterium-induced modulation of lung DC function.

The modulation of the CCR7-mediated migration of DCs may occur at multiple levels. Ligand-induced receptor downregulation is a common mechanism used by chemokine receptors, although CCR7 is more resistant to this form of regulation than other chemokine receptors (33). Once bound by its ligand, CCR7 is endocytosed and recycled back, while bound ligand dissociates and is degraded (27). The expression of CCL19 in the lungs of BCG-infected mice indicated that this mode of receptor downregulation might play a role. However, the reduction in chemotaxis during peak infection was not associated with reduced surface expression of CCR7. In addition, mice had higher expression of CCL19 in the lungs 12 to 14 weeks after BCG infection, yet mature lung DCs from these same mice were more responsive to chemokine-mediated chemotaxis than lung DCs from mice infected for 4 to 6 weeks. Another possible mechanism for the decreased chemotaxis of lung DCs from mice infected for 4 to 6 weeks may involve the CCX-CKR decoy receptor that efficiently scavenges CCL19, CCL21, and CCL25 (7). CCX-CKR does not couple to downstream signaling components; instead, it acts as a chemokine sink. BCG infection may modulate the expression of CCX-CKR and in an in vitro transwell assay could limit the amount of CCL21 available to bind and signal through CCR7.

In summary, BCG infection caused the accumulation and maturation of DCs in the lungs. Surprisingly, the chemotaxis of mature lung DCs was reduced during peak bacterial burden. Our results do not exclude the possibility that acute mycobacterial infection increases the migration of lung DCs to the lymph node, as others have seen with bone-marrow-derived DCs (5). Mycobacteria have been found inside lymph node DCs, but the origin of these DCs has not been determined (12). The ability of infection to impede the migration of DCs is not exclusive to mycobacterial infection. Legge and Braciale...
have shown that lung DCs during pulmonary virus infection become refractory to migration even as inflammation persists and viral replication continues to occur (18). Therefore, the modulation seen in mature lung DC chemotaxis during mycobacterial infection may involve a common set of pathways that recognize pathogen-associated molecular patterns from different pathogens. Additional studies will determine how DCs in the lung become refractory to CCR7-mediated migration and thus become available to activate naïve CD4+ T cells in the lungs during mycobacterial infection.

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