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Functional Transitions in Macrophages During In Vivo Infection with *Mycobacterium bovis* Bacillus Calmette-Guérin

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Macrophage activation during the immune response to intracellular bacteria is critical for resolution of the infection. We have investigated the pathway of macrophage activation during murine *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) infection. Three distinct phenotypes of macrophages were identified and compared: resident peritoneal macrophages, day 2 postinfection macrophages, and 12-day postinfection macrophages. Compared with resident peritoneal macrophages, day 2 BCG macrophages expressed intermediate levels of the cell surface receptors Mac1 and F4/80 and low levels of MHC class II molecules. These cells were highly phagocytic and produced large amounts of mRNA encoding the chemokine IP-10. In addition, day 2 BCG macrophages did not generate reactive nitrogen intermediates, though they were primed to do so, and did not have increased levels of TNF-α mRNA. Blockade of monocyte influx into the peritoneal cavity using Abs to platelet endothelial cell adhesion molecule 1 had no effect on the appearance of day 2 BCG macrophages, suggesting this cell can differentiate from resident peritoneal macrophages. In contrast to day 2 BCG macrophages, day 12 BCG macrophages were poorly phagocytic, but produced high levels of reactive nitrogen intermediates, IP-10 and TNF-α mRNA, and class II MHC molecules. We propose that day 2 BCG macrophages are specialized for phagocytic uptake of pathogens from the extracellular space, whereas day 12 BCG macrophages are specialized for killing of the internalized pathogens. This functional transition during activation is reminiscent of that seen during maturation/activation of the related dendritic cell lineage induced by bacterial or inflammatory stimuli. *The Journal of Immunology*, 2001, 167: 2227–2233.

Macrophages are a critical component of the innate and adaptive immune response to bacterial pathogens; they internalize and degrade bacteria and induce inflammatory responses. In addition to their effector function, macrophages also coordinate the host response by producing chemokines and cytokines, and by Ag presentation to activated T cells.

Intraperitoneal infection of mice with the intracellular bacterium *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) has been a classical system for the study of macrophage activation. The first interaction of the immune system with BCG is by the resident tissue macrophages, in this case the resident peritoneal (RP) macrophage. During phagocytosis of the bacteria, pattern recognition receptors such as the Toll-like receptors induce a variety of inflammatory responses in the macrophage (1–4). After the initial interactions of the RP macrophages with BCG, a complex series of events ensue that result in macrophage activation. These events include the extravasation of monocytes, NK cells, and T cells into the inflamed tissue, as well as the activation of these cells with macrophages and/or macrophage products (5–8). At the site of infection, NK cells and T cells produce cytokines such as IFN-γ that are critical for macrophage activation (9, 10). BCG-activated macrophages produce high levels of bacteriocidal molecules (11) and cytokines (12), up-regulate expression of MHC class II molecules, and down-regulate the cell surface receptor F4/80 (13).

Previous studies of the pathway by which resting macrophages or monocytes differentiate into the classical BCG-activated macrophage have focused on single time points after infection or on one functional aspect of these cells. Recent studies on the differentiation of dendritic cells (DC), a related cell type, in response to infectious or inflammatory stimuli have suggested that the paradigm of macrophage activation should be revisited. Immature or resting DC are efficient at Ag uptake, while mature or activated DC down-regulate this ability and up-regulate the capacity to stimulate naïve T cells (14). Immature DC acquire Ag through endocytosis, macropinocytosis, and phagocytosis (15–19), while mature DC stimulate naïve T cells through cell surface expression of class II MHC molecules and costimulatory molecules (20–24). This process allows the DC to regulate its functional capabilities during an immune response. DC maturation is induced by a variety of bacteria and bacterial stimuli including bacterial endotoxin (LPS) and mycobacteria (21, 25–27), as well as host inflammatory mediators such as IL-1 and TNF-α (16).

We hypothesized that macrophages also undergo a functional transition during activation induced by bacterial stimuli in vivo and therefore compared the functional capacities of RP macrophages with those activated 2 days or 12 days after BCG infection. These three populations of macrophages are phenotypically and functionally distinct. Day 2 BCG macrophages are highly phagocytic while day 12 BCG macrophages are poorly phagocytic, but produce far higher levels of reactive nitrogen intermediates (RNI) and MHC class II molecules than their day 2 counterparts. We propose that macrophages 2 days post-BCG infection are specialized for bacterial clearance from the extracellular space and that those at 12 days postinfection are specialized for bacterial killing.
Additionally, these two populations of macrophages produce different levels of the cytokine, TNF-α, and the chemokine, IP-10, suggesting that the influence of macrophages on the ongoing immune response differs at distinct times after infection.

Materials and Methods

**BCG and infection**

*M. bovis* BCG (strain Pasteur; American Type Culture Collection, Manassas, VA) was a gift from Dr. S. Smith (University of Washington, Seattle, WA). BCG was grown in Proskauer-Beck medium with aeration to 5 x 10^7 CFU/ml and stored in aliquots at −70°C. To infect mice, an aliquot of BCG was thawed, sonicated three times for 30 s in a water bath sonicator, and diluted in PBS. C57BL/6 or FcγRIII−/− mice (The Jackson Laboratory, Bar Harbor, ME) were injected i.p. with 5 x 10^6 CFU of BCG. In some experiments, mice were injected i.v. with 100 μg of anti-platelet endothelial cell adhesion molecule-1 (PECAM-1) Ab 2H8 (28, 29), a gift from Dr. W. Muller (Cornell University), or control hamster IgG (Jackson Immunoresearch Laboratories, West Grove, PA) 1 h before BCG infection.

**Flow cytometry and cell sorting**

Cells were isolated from the peritoneal cavity of uninfected mice or mice at the indicated day after BCG infection by peritoneal lavage with 6 ml of ice-cold PBS. After blocking with 2.4G2 (American Type Culture Collection) supernatant, 5 x 10^5 cells were stained with Mac1 FITC, B220 PE, Gr1 PE, and biotinylated F4/80 (Caltag Laboratories, South San Francisco, CA) or IAb or with Gr1 FITC, B220 PE, and biotinylated Mac1. This was followed by staining with streptavidin TC (Caltag Laboratories) and fixation in 1% parafomaldehyde. All Abs were purchased from BD PharMingen except otherwise noted. FITC-labeled FITC-loaded SRBC ghosts were prepared by incubating FITC-loaded SRBC ghosts with anti-SRBC IgM (Intercell) at a 1:1000 dilution in ice-cold PBS. Isotonicity was restored with 5x PBS and the ghosts were sealed at 37°C for 1 h. Excess FITC-OVA was removed by washing three times in PBS. IgG-opsonized SRBC were prepared by incubating FITC-loaded SRBC ghosts with anti-SRBC IgG (Intercell) at a 1/2000 dilution for 30 min at room temperature. C8b-opsonized SRBC were prepared by incubating FITC-loaded SRBC ghosts with anti-SRBC C8b (F4/80) (Intercell) supernatant. The number of macrophages was determined by multiplying the cells plated per well by the percentage of cells that were macrophages by flow cytometry.

**Real-time PCR**

cDNA from sorted macrophage populations was made as described above and amplified with primers for murine HPRT, IP-10, and TNF-α as indicated below using TaqMan Universal PCR master mix and an Applied Biosystems Prism 7700 Sequence Detector (Applied Biosystems, Foster City, CA) following the manufacturer’s instructions. Genomic DNA contamination was measured by including template that had been mock reverse transcribed and at all times accounted for <10% of the signal. Fold induction was determined from Ct values normalized for HPRT expression and then normalized to the value derived from RP macrophages. Primers were as follows: TNF-α primers: forward, TCCAGCCGCTGGCTCTATGT; reverse, CACCCCAGATTCTAGTAGACAGA; probe, TCAGCTCCTTTCCATTCTGTTGIGG. IP-10 primers: forward, GAGGGTCCTGGTAAACGT; reverse, GGTCCCTCTAGGCCCTATTT; probe, TCACTGCGCCTCTGATAGC. HPRT primers: forward, TGAAAAGA ATGTCCTGATGGTGA; reverse, AGCTGCAACCTTAACCATTGTT; probe, CAAACCTTCTCTCCCTGTTAAGCAGTAGAGC.

**Results**

Activated macrophages are distinguished by cell surface receptor expression

To understand the pathway of macrophage activation during infection with intracellular bacteria, we examined peritoneal macrophages at days 2 and 12 after i.p. infection with live BCG. These cells were compared with RP macrophages. We first wanted to know whether we could phenotypically distinguish day 2 BCG macrophages from both RP macrophages and macrophages at day 12 after infection. Macrophages isolated from the peritoneal cavity of infected or uninfected mice were stained for the cell surface receptors F4/80 and Mac1 and the class II MHC molecule IA^β. As shown previously (29, 30), RP macrophages expressed high levels of both these receptors and at day 12, F4/80 and Mac1 were even further down-regulated (13). RP macrophages were predominantly negative/low for MHC class II molecules (Fig. 1), while day 12 BCG macrophages uniformly expressed high levels of IA^β (13). At day 2 after infection, the macrophages have begun to up-regulate IA^β, but are still expressing very low levels of this protein when compared with day 12 BCG macrophages. Since day 2 BCG macrophages were phenotypically distinct from RP macrophages and from day 12 BCG macrophages, we investigated the functional capabilities of these populations.

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2228 FUNCTIONAL TRANSITIONS IN MACROPHAGES DURING BCG INFECCTION
Day 2 BCG macrophages are highly phagocytic for IgG- and C3bi-opsonized particles

A key function of macrophages during the immune response is phagocytosis of pathogens. Macrophages engulf pathogens through a variety of phagocytic receptors including those for opsonized particles, such as Fc receptors (FcR) and complement receptors (CR), and those for nonopsonized particles, such as the phagocytic pattern recognition receptors (31). The phagocytic capacity of BCG-activated macrophages both for opsonized (IgG and C3bi) and nonopsonized (zymosan) particles was examined. Day 2 BCG macrophages were highly phagocytic for IgG-opsonized SRBC, while day 12 BCG macrophages were no more phagocytic than RP macrophages (Fig. 2A). The day 2 BCG macrophages were 7-fold more phagocytic than both RP and day 12 BCG macrophages.

We observed a similar pattern when looking at CR phagocytosis of C3bi-coated SRBC (Fig. 2B). It has been well established that resting macrophages do not phagocytose efficiently through their CRs without prior activation of protein kinase C (PKC) (32, 33). This is demonstrated in Fig. 2B; RP macrophages did not efficiently phagocytose C3bi-opsonized SRBC unless they were pretreated in vitro with the PKC activator PMA. Interestingly, day 2 BCG macrophages were constitutively able to phagocytose through CRs in the absence of in vitro PKC stimulation, although they did not phagocytose more C3bi-opsonized particles in the presence of PMA. This suggests that PKC is already maximally activated in macrophages at day 2 after BCG infection, as has been shown for IFN-γ-activated macrophages (34). In contrast, day 12 BCG macrophages are poorly phagocytic for complement-coated SRBC in the absence or presence of PMA. Interestingly, all three populations of macrophages bound C3bi-opsonized SRBC to similar extents (data not shown). For both complement- and FcR-mediated phagocytosis, macrophages at day 2 after infection with BCG have an enhanced phagocytic capacity. Indeed, this increased FcR and CR phagocytosis persists until 4 days postinfection, which is then followed by decreased phagocytosis between days 8 and 14 after infection (data not shown).

We also looked at phagocytosis of zymosan, a yeast cell wall particle, through pattern recognition receptors including the mannose receptor (31). As shown in Fig. 2C, there are only small variations in the ability of macrophages to phagocytose zymosan during BCG infection; day 2 BCG macrophages phagocytose zymosan marginally more effectively than RP or day 12 BCG macrophages.

Receptor levels do not fully explain the modulation of phagocytic capacity in BCG-activated macrophages

We investigated whether changes in receptor levels could explain the enhancement of phagocytosis in day 2 BCG macrophages and the subsequent reduction seen at day 12. The phagocytic CR for C3bi-opsonized particles in macrophages is CR3 which is composed of the integrin CD11b/CD18 also known as Mac1 (35). Fig. 1 shows changes in Mac1 levels seen during BCG activation of macrophages: Mac1 is highest in RP macrophages, intermediate in day 2 BCG macrophages, and lowest in day 12 BCG macrophages.
Although low Mac1 levels in day 12 BCG macrophages may explain the poor phagocytosis of C3bi-opsonized particles by these macrophages, Mac1 levels do not explain the difference in phagocytosis between RP macrophages and day 2 BCG macrophages.

Murine macrophages express three different Fc receptors, two activating receptors, FcγRI and FcγRIII, and one inhibitory receptor, FcγRII (36). Both FcγRI and FcγRIII have short cytoplasmic tails and associate with the FcεRIγ chain to transduce signals. We looked at mRNA levels for these proteins by semiquantitative RT-PCR in sorted macrophage populations at days 2 and 12 after BCG infection to determine whether changes in FcR expression could influence the phagocytic capacity of the macrophages for IgG-opsonized particles. The mRNA for FcγRI was higher in both day 2 and day 12 macrophages from BCG-infected mice than in RP macrophages, whereas there was no difference in mRNA levels between the macrophage populations for the other activating receptor, FcγRIII, and the signaling chain, FcεRIγ (Fig. 3A). It is possible that this up-regulation of FcγRI accounts for the increase in phagocytosis in day 2 BCG macrophages, although this is unlikely since FcγRI requires the FcεRIγ chain, which does not increase in expression, for signaling and surface expression (36). This high level of FcγRI is maintained in day 12 BCG macrophages that have a poor phagocytic capacity for IgG-coated particles (Fig. 3A). Additionally, although there is no change in the levels of the inhibitory receptor FcγRII, between RP and day 2 BCG macrophages, this receptor is expressed at lower levels in day 12 macrophages (Fig. 3A). This would suggest that day 12 BCG macrophages should have the highest phagocytic capacity; they have increased activating receptors and decreased inhibitory receptors compared with RP macrophages. Despite this, the data in Fig. 2A demonstrate that this is not the case, day 12 macrophages have low levels of FcR phagocytosis.

We were surprised that day 2 macrophages did not have decreased levels of FcγRII. Thiglycollate-elicted macrophages, which also enter the peritoneal cavity with a similar time course, have increased phagocytosis of IgG-opsonized particles (37) and have a reduced ratio of FcγRII:FcγRIII in comparison with RP macrophages (38). To confirm that down-regulation of FcγRII does not play a role in the increased FcR phagocytosis by day 2 macrophages, we looked at phagocytosis after BCG infection of FcγRII<−/− mice (38). RP macrophages from FcγRII<−/− mice have an ~3-fold increased phagocytic index when given IgG-opsonized particles in comparison to those from wild-type mice (Fig. 3B). Consistent with the expression data, there was no difference in FcR-mediated phagocytosis between day 2 BCG macrophages from wild-type and FcγRII<−/− mice. We conclude that changes in FcγRII levels do not cause the increase in phagocytosis at day 2 after BCG infection.

Day 12 BCG macrophages actively produce RNI, whereas day 2 BCG macrophages are primed for enhanced RNI production

After phagocytosis of the bacteria, the macrophage must kill the pathogen in the vacuole to resolve infection. We measured production of RNI, the principal mediator of mycobacterial killing, as an indicator of bacteriociactivity (39). As shown in Fig. 4A, only macrophages isolated at day 12 after BCG infection were spontaneously generating RNI directly ex vivo. However, day 2 BCG macrophages were primed for enhanced RNI production, since these cells produced similar levels of RNI to those produced by day 12 macrophages in response to in vitro stimulation with LPS (Fig. 4B). RP macrophages treated with LPS secreted significantly less RNI into the medium than macrophages from BCG-infected mice. Therefore, day 2 BCG macrophages, although not actively producing RNI, are primed to do so by prior BCG infection, whereas day 12 BCG macrophages appear to be highly bacteriocidal in vivo.
Day 2 and day 12 macrophages differ in TNF-α and IP-10 production

Macrophages help coordinate the immune response to bacterial pathogens by secreting cytokines and chemokines which activate other cells of the immune system and/or induce their homing to the site of infection (9, 40). We therefore wanted to look at the kinetics of production of two such proteins, the cytokine TNF-α, and the chemokine IP-10. We measured constitutive TNF-α and IP-10 mRNA levels in sorted macrophage populations by real-time quantitative PCR. There was no increase in TNF-α mRNA levels in day 2 BCG macrophages when compared with RP macrophages, whereas day 12 BCG macrophages showed a 5-fold increase in this cytokine message (Fig. 5 A). In contrast, IP-10 mRNA was already >250-fold induced in day 2 BCG macrophages and was further increased in day 12 BCG macrophages (Fig. 5 B). It is likely that this differential production of TNF-α and IP-10 by day 2 and day 12 BCG macrophages reflects the capacity of these populations to influence the ongoing immune response.

Day 2 BCG macrophages can be derived directly from RP macrophages

To determine whether RP macrophages have the capacity to differentiate into day 2 BCG macrophages, we used mAbs to PECAM-1 (28) to block monocyte extravasation into the peritoneal cavity (41). Mice were treated with anti-PECAM-1 Abs or control IgG 1 h before infection with BCG and macrophages were isolated after 2 days. There was no difference in the capacity of day 2 BCG macrophages isolated from control IgG- or anti-PECAM-1-treated mice to phagocytose C3bi-opsonized SRBC (Fig. 6 A) or IgG-opsonized SRBC (data not shown), or to produce RNI in response to LPS (Fig. 6 B). Confirmation that the anti-PECAM Ab blocked monocyte influx into the peritoneal cavity was obtained by a number of approaches, including the demonstration that the average number of macrophages recovered from the peritoneal cavity of anti-PECAM-1-treated, BCG-infected mice (0.54 ± 0.09 × 10^6), was significantly lower (p < 0.006) than that recovered from control IgG-treated mice (1.21 ± 0.12 × 10^6). These data show that RP macrophages can serve as precursors for day 2 BCG macrophages.

Discussion

We have identified macrophages early in the pathway of macrophage activation during infection with *M. bovis* BCG that can be phenotypically and functionally distinguished from the resident tissue macrophage as well as from the typical immune-activated macrophage found at later times after infection. Compared with RP macrophages, these cells, found at day 2 postinfection with BCG, expressed decreased levels of the cell surface receptors Mac1 and F4/80 and slightly elevated levels of MHC class II molecules. Day 2 BCG macrophages were highly phagocytic and produced large amounts of mRNA for the chemokine IP-10. These cells did not produce RNI, although they were primed to do so, and did not have increased levels of TNF-α mRNA compared with RP macrophages. In contrast, day 12 BCG macrophages were poorly phagocytic, but produced RNI constitutively, IP-10 and TNF-α mRNA, and expressed high levels of cell surface MHC class II molecules. We propose that the macrophages at day 2 after BCG infection are specialized for phagocytic uptake of pathogens from the extracellular space, whereas day 12 BCG macrophages are specialized for bacterial killing of internalized pathogens.

This transition in functional capacities seen in BCG-activated macrophages is reminiscent of the maturation pathway of a related cell type, the DC. Immature DC in the tissues are specialized for Ag uptake via endocytosis, macropinocytosis, and phagocytosis (15–19), whereas mature DC in the lymph nodes are specialized for Ag presentation to naive T cells (14). We were struck by the similarities in phagocytic capacity between day 2 BCG macrophages and immature DC and that both cell types subsequently down-regulate this phagocytic capacity. Despite these similarities, we do not have evidence that day 2 BCG macrophages directly differentiate into day 12 macrophages in vivo, whereas it is clear that immature DCs differentiate into mature DCs as this can be observed in isolated cells in vitro.
Mature DC have high levels of MHC class II and costimulatory molecules on their surface to stimulate naive T cells (20–24). Although both day 12 BCG macrophages and mature DC have increased levels of MHC class II and costimulatory molecules on their surface, the function and identity of these proteins appear to differ. Mature DC use high levels of class II and CD86 to stimulate naive T cells in the lymph nodes (14), whereas day 12 BCG macrophages use MHC class II and CD80 to stimulate previously activated T cells at the site of infection (J. A. Hamerman and A. Aderem, unpublished observations). This stimulation of activated CD4 T cells in the peritoneal cavity induces the local production of IFN-γ, a cytokine required for macrophage RNI production and therefore for resolution of BCG infection (39).

It has recently been shown that monocytes can differentiate into DC in vitro and in vivo after phagocytosis of latex beads and zymosan (42, 43). This finding, along with the similarities in phagocytosis between BCG macrophages and DC, induced us to examine whether some BCG-activated macrophages differentiate into mature DC. We did not detect expression of the DC markers CD11c, DEC-205, and CD86 on day 2 or day 12 BCG-activated macrophages (J. A. Hamerman and A. Aderem, unpublished observations). We have not exhaustively examined all DC markers, nor can we rule out the expression of those we have examined on a very small population of cells. It is also possible that some BCG-activated macrophages leave the peritoneal cavity and then differentiate into DC. Randolph et al. (43) demonstrated that after phagocytosing particles, human monocytes reverse transmigrated out of the tissues and then differentiated into DC. Similarly, inflammatory macrophages can leave the peritoneal cavity and migrate to the draining lymph nodes (44).

It is generally viewed that activated macrophages are highly phagocytic (37, 45), although there are some conflicting reports (46). Our results suggest that the difference in these reports may reflect temporal differences in the experiments; in our hands, phagocytic capacity was increased markedly from days 2 to 4 postinfection and was decreased substantially after day 8 (J. A. Hamerman and A. Aderem, unpublished observations). We investigated the mechanism for the modulation of phagocytic capacity by BCG-activated macrophages. For CR-mediated phagocytosis, decreased levels of receptor (Mac1) correlated with decreased phagocytic capacity in day 12 BCG macrophages, although these macrophages can bind similar numbers of C3bi-opsonized particles to both RP and day 2 BCG macrophages. However, the major increase in CR-mediated phagocytosis in day 2 BCG macrophages cannot be explained by receptor levels, day 2 macrophages actually express less Mac1 than RP macrophages. It is likely that increased PKC activation in day 2 BCG macrophages explains this increase in phagocytosis. First, PKC is constitutively activated in day 2 BCG macrophages as judged by the phosphorylation of the PKC substrate MARCKS (A. Aderem, unpublished observations). Second, phorbol ester-induced PKC activation of RP macrophages results in a similar phagocytic index to that seen for untreated day 2 macrophages. In addition to stimulating CR phagocytosis (32, 33), PKC activation has also been shown to enhance FeR phagocytosis in vitro (47), and may also account in part for our data showing increased FeR phagocytosis by day 2 BCG macrophages. As discussed above, it is not likely that differences in FeR expression account for the large increase in FeR-mediated phagocytic capacity in day 2 BCG macrophages. We confirmed that the absence of the inhibitory FeγRII resulted in enhanced phagocytosis of IgG-opsonized particles by RP macrophages (48), but we show here that this receptor was not responsible for the increased FeR phagocytosis seen in day 2 BCG macrophages. It has recently been shown that the Rho family GTPase, cdc42, is important in regulating the differential endocytic capacity of immature and mature DC (49). Therefore, it was attractive to postulate that this mechanism may underlie the differential phagocytic responses observed between BCG-activated macrophages and resident peritoneal macrophages. We attempted to determine whether cdc42 activation differed in BCG-activated macrophages and resident peritoneal macrophages; unfortunately, we could not detect sufficient cdc42 protein in these cells to draw conclusions from this study (our unpublished observations).

Macrophages coordinate the immune response during bacterial infections through cytokine and chemokine production. Although day 12 BCG macrophages have increased mRNA levels for both TNF-α and IP-10, we observed differential regulation of these secreted mediators in the day 2 BCG macrophages. TNF-α mRNA was not increased in day 2 BCG macrophages, although it is well established that macrophage interaction with bacteria induces TNF-α secretion within hours of contact. The result is consistent with that of Stein and Gordon (12) who demonstrated that macrophages from the peritoneal cavity of BCG-infected mice do not release any TNF-α until day 8 after infection. TNF-α has pleiotropic effects on the immune response including neutrophil activation, induction of endothelial cell E-selectin expression, co-stimulation of NK cells for IFN-γ production, and induction of chemokine expression (50). The relevance of the delay in TNF-α production until later times after infection is unclear, but it remains possible that there is a wave of TNF-α production by macrophages immediately after infection that has subsided by day 2.

In contrast to TNF-α production, mRNA for the chemokine IP-10 is already massively induced in day 2 BCG macrophages, and this level is maintained in day 12 macrophages. IP-10 is chemotactic for monocytes, NK cells, and activated Th1 T cells and may be important for the homing of distinct cell types at different times after infection (40, 51, 52). At day 2, IP-10 may induce monocyte and NK cell homing to the peritoneal cavity, while at day 12 it may act predominantly on activated Th1 T cells. IP-10 has been shown to be critical for control of infection with another intracellular pathogen, Toxoplasma gondii, where it is also expressed at both early and late times after infection (53). Differences in TNF-α and IP-10 expression at days 2 and 12 after BCG infection not only further define these macrophage populations, but also illustrates that the way macrophages influence the immune response can change over the course of infection.

To address the identity of the precursor cell of day 2 BCG macrophages, we infected mice with BCG while blocking monocyte extravasation with Abs to PECAM-1 (28, 41). Anti-PECAM-1 Abs did not affect the capacity of day 2 BCG macrophages to increase phagocytosis or to be primed for enhanced RNI production. These data demonstrate that RP macrophages can differentiate into BCG-activated macrophages, though we do not rule out that monocytes can also differentiate into these cells. We also attempted to determine a lineage relationship between day 2 and day 12 BCG macrophages using sustained blocking of PECAM-1 with Abs, but this treatment did not appear to block monocyte extravasation during this extended time period (our unpublished observations).

In this report, we have revisited a classical system used to study in vivo immune activation of macrophages; we have examined the temporal response to BCG infection, identified an early intermediate in the process, and defined a functional transition in these macrophage populations. The results demonstrate an exquisite level of regulation of macrophage function and suggest future directions for further dissection of the pathway.
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