Lung Macrophages Serve as Obligatory Intermediate between Blood Monocytes and Alveolar Macrophages

Limor Landsman and Steffen Jung

*J Immunol* 2007; 179:3488-3494; doi: 10.4049/jimmunol.179.6.3488

http://www.jimmunol.org/content/179/6/3488
Lung Macrophages Serve as Obligatory Intermediate between Blood Monocytes and Alveolar Macrophages

Limor Landsman and Steffen Jung

Alveolar macrophages are a unique type of mononuclear phagocytes that populate the external surface of the lung cavity. Early studies have suggested that alveolar macrophages originate from tissue-resident, local precursors, whereas others reported their derivation from blood-borne cells. However, the role of circulating monocytes as precursors of alveolar macrophages was never directly tested. In this study, we show through the combined use of conditional cell ablation and adoptive cell transfer that alveolar macrophages originate in vivo from blood monocytes. Interestingly, this process requires an obligate intermediate stage, the differentiation of blood monocytes into parenchymal lung macrophages, which subsequently migrate into the alveolar space. We also provide direct evidence for the ability of both lung and alveolar macrophages to proliferate. The Journal of Immunology, 2007, 179: 3488–3494.

The constant exposure to environmental microbial challenges renders the respiratory tract one of the major sites of primary viral and bacterial infections (1). Highlighting this state of permanent alert, the lung, which comprises parenchyma and alveolar space, is seeded with numerous mononuclear phagocytes, including dendritic cells (DC) and macrophages (MΦ) (1, 2). In steady state, MΦ are the major cell type in the alveolar space, representing ~90% of its hematopoietic cellular content (1) and playing a key role in its clearance from pulmonary pathogens and dying cells (3). Pulmonary MΦ, however, are poor T cell stimulators (4, 5), and have even been shown to suppress lung inflammations, probably by inhibiting DC function (4, 6–10).

Alveolar MΦ are bone marrow (BM)-derived cells (11, 12). Interestingly, however, after whole body irradiation and engraftment, their replacement by donor BM cells takes considerably longer than that of most other cells of the hematopoietic system (13, 14). Depending on the irradiation protocol used, the time required for the complete exchange of alveolar MΦ has been reported to vary from several weeks up to 1 year (11, 13–16). This delay in alveolar MΦ replacement by BM-derived cells is discussed as one of the critical causes for the sensitivity of BM transplantation patients to pulmonary infections (17, 18).

Monocytes are BM-derived circulating phagocytes that are considered to be the in vivo MΦ precursors (19, 20). However, specific MΦ populations, such as the brain microglia, have been shown to originate from local precursors, without persisting input from the BM (21). As for the origin of alveolar MΦ, although they clearly belong to the hematopoietic lineage, a direct connection to monocytic precursors remains to be shown (22). Moreover, several studies published during the 1970s and 1980s reported either circulating or local precursor for alveolar MΦ, depending on the method used (for review, see Ref. 22). Based on kinetic studies, Bowden and Adamson (23) suggested, for instance, a dual origin of alveolar MΦ: from blood-borne precursor and from proliferating cells in the lung parenchyma. With the discovery of alveolar DC (24), it became, however, unclear whether this study referred to two distinct cell types, i.e., alveolar DC and MΦ. In addition, the alveolar MΦ population was reported to remain unaffected by depletion of blood monocytes (25), leading to the conclusion that alveolar MΦ are not monocyte derived (26). Because alveolar MΦ are eventually replaced by BM-derived cells (11), the existence of circulating precursor was, however, not ruled out. Taken together, the identity of the alveolar MΦ precursor, as well as its own origin, remains to be revealed (22).

Through an adoptive monocyte transfer approach, we recently showed that parenchymal lung MΦ originate in vivo from blood monocytes (27). Furthermore, we reported that only one of the two major murine monocyte subsets, the Gr1 CX3CR1 CCR2 cells (28), harbors the immediate potential to give rise to parenchymal lung MΦ (27). In this study, we extended these studies and investigated the in vivo origin of alveolar MΦ using a combination of conditional cell ablation and reconstitution. We show that grafted blood monocytes can give rise to alveolar MΦ. However, we provide evidence that alveolar MΦ do not originate directly from blood monocytes, but require a parenchymal lung MΦ intermediate. Lastly, we directly show that both lung and alveolar MΦ can undergo proliferation.

Materials and Methods

Mice

This study involved the use of the following C57BL/6 mouse strains: CD11c.diphtheria toxin (DTx) receptor (DTR) transgenic mice (B6.FVB-Tg(Ifgax-DTR/GFP)57Lan/J) that carry a DTR transgene under the murine cd11c promoter (29); CX3CR1 Tg mice harboring a targeted replacement of the cx3cr1 gene by a GFP reporter (30); and μMT mice (C3H/HeN-Tg(H2bmIcl)J) lacking the membrane exon of the Ig µ chain gene, and therefore are B cell deficient (31). Mice were backcrossed with CD45.1 mice (B6.SJL-Ptprca Pep3b/H).
under specific pathogen-free conditions and handled under protocols approved by the Weizmann Institute Animal Care Committee, according to international guidelines.

**Cell isolations**

Mice were sacrificed, and blood was collected from main artery and subjected to a Ficoll density gradient (Amersham) to remove erythrocytes and neutrophils. For bronchoalveolar lavage (BAL), the trachea was exposed to allow insertion of a catheter, through which the lung was filled and washed four times with 1 ml of PBS without Ca²⁺/Mg²⁺. Lung parenchyma was then collected and digested with 4 mg/ml collagenase D (Roche) for 1 h at 37°C, followed by incubation with ACK buffer to lyse erythrocytes. All isolated cells were suspended in PBS supplemented with 2 mM EDTA, 0.05% sodium azide, and 1% FCS.

**Flow cytometric analysis**

The following fluorochrome-labeled mAbs were purchased from eBioscience and used according to manufacturer’s protocols: PE-conjugated anti-CD11c and anti-CD115 Abs, allopurinocyanin-conjugated streptavidin and anti-CD11b Ab, and biotin-conjugated anti-CD45.1, CD11c, CD115, and MHC II Abs. PerCP-conjugated anti-CD11b Ab was purchased from BD Pharmingen. PE-labeled anti-proliferating cell nuclear Ag (PCNA) Ab was purchased from DakoCytomation and used according to the manufacturer’s protocol for S phase-specific staining, with modifications. Before staining with the anti-PCNA Ab, cells were incubated with indicated biotin-conjugated Abs, followed by methanol fixation. Fixed cells were then incubated with streptavidin-fluorochrome conjugates and stained for PCNA. Cells were analyzed on a FACS Calibur cytometer (BD Biosciences) using CellQuest software (BD Biosciences).

**Cell transfers**

For blood monocyte transfers, ~20 mice were sacrificed and bled to collect an average of 18 ml of blood. Erythrocytes and neutrophils were removed by a Ficoll density gradient, and cells were washed and exposed to biotin-conjugated anti-CD115 Ab (eBioscience), followed by incubation with streptavidin-conjugated MACS beads (Miltenyi Biotec). Cells were then magnetically separated according to manufacturer protocol. The positive fraction was collected and i.v. injected to recipient mice. For mixed BM, recipient mice were irradiated with a lethal dose (950 rad) and 1 fraction was collected and i.v. injected to recipient mice. For mixed BM magnetically separated according to manufacturer protocol. The positive fraction was collected and i.v. injected to recipient mice. The following fluorochrome-labeled mAbs were purchased from eBioscience:

- CD11c
- CD11b
- CX3CR1
- MHC II

**Intratracheal instillation**

PBS (80 μl) containing either DTx (List Biological Laboratories; catalogue 150) or without addition was applied to the tracheae of mice, as previously described, with modifications (32). Briefly, mice were lightly anesthetized using isoflurane and placed vertically, and their tongues were pulled out. Using a long-nasal tip, liquid was placed at the top of trachea and actively aspirated by the mouse. Gasping of treated mice verified liquid application to the alveolar space.

**Results**

**Depletion of lung and alveolar MΦ results in their rapid reconstitution**

Murine pulmonary DC and MΦ, both in the lung parenchyma and the alveolar space, express the β-integrin CD11c. However, whereas pulmonary DC are also positive for MHC II, CD11b, and CX3CR1, MΦ have been defined as low for MHC II and negative for CD11b and CX3CR1 (5, 27, 33, 34).

CD11c–DTR transgenic mice allow for the conditional ablation of pulmonary DC and MΦ (27, 35). This ablation system exploits the fact that murine cells are generally resistant to DTx because they lack a high-affinity DTR required for toxin entry into the cell (36). CD11c–DTR transgenic mice express a primate DTR under the control of the cd11c promoter, resulting in sensitivity of CD11c-expressing cells to DTx-induced cell death (29). Hence, when applied intratracheally i.t.) to CD11c–DTR transgenic mice, DTx causes the depletion of lung and alveolar CD11c⁺ cells, including both DC and MΦ (27, 35) (Fig. 1A).

One day following DTx treatment, lung and alveolar MΦ numbers were dramatically reduced (Fig. 1B). The decrease in alveolar MΦ was followed by their rapid reconstitution, and 2 days after the treatment their amount almost reached initial levels. Lung MΦ, in contrast, continued to decline, reaching their lowest value on day 4, after which also their numbers recovered. By day 9, lung MΦ were restored to their initial levels, whereas at that time the size of the alveolar MΦ population was significantly enlarged over steady state (Fig. 1B).

These results indicate that, following their conditional depletion, both lung and alveolar MΦ populations are rapidly reconstituted, albeit with distinct kinetics.

**Adoptively transferred blood monocytes can give rise to alveolar MΦ**

Elsewhere, we have shown that grafted blood monocytes give rise to lung MΦ in MΦ-depleted, but not untreated, recipients (27). Four days after transfer, monocyte graft-derived MΦ could be readily observed in recipient lungs, whereas we failed to detect graft descendants in the alveolar space (27). This result could support the reported notion that blood monocytes do not give rise to alveolar MΦ (25). Alternatively, the kinetics of monocyte differentiation into alveolar MΦ might vary from their differentiation into lung MΦ, precluding detection of monocyte-derived alveolar
Recipient mice were analyzed for the presence of graft-derived alveolar or with no graft (w/o graft) on day 0. Fourteen days after transfer, BAL of B3 began to be replaced by donor cells only 3 wk after transfer (Fig. 1B). Earlier reported delayed reconstitution (14, 16, 37), alveolar MΦ have been pretreated by i.t. DTx installation. Two weeks after transfer, CD11c+CD11b−CXCR1/GFP+ cells, as indicated by gates. B, Graft-derived alveolar MΦ were further identified as CD45.1+/H9021+ cells, as indicated by gate. Numbers indicate percentage of graft-derived cells (CD45.1+) of total gated population and their absolute numbers (in parentheses). Data show one representative of three independent experiments.

MΦ 4 days after transfer. To distinguish between these two options, we decided to extend our previous study and analyze monocyte recipients 14 days after transfer.

Monocytes, defined as positive for CD115 (also known as M-CSFR), were isolated from blood of CD11c+CXCR1−/−,CD45.1 mice and transferred into CD11c−DTR,CD45.2 transgenic mice, which had been pretreated by i.t. DTx installation. Two weeks after transfer, the BAL content of recipient mice was analyzed for graft-derived alveolar MΦ. The latter were identified as CD45.1+CD11c+CD11b−CXCR1/GFP+ cells to discriminate them from CD45.1+CD11c+CD11b+CXCR1/GFP− graft-derived DC and CD45.2−recipient’s cells (27) (Fig. 2A). As shown in Fig. 2A, 2 wk after transfer, we could detect graft-derived alveolar MΦ. This indicates that blood monocytes do have the capacity to give rise to alveolar MΦ.

Lung and alveolar MΦ show distinct reconstitution kinetics following irradiation and BM transfer

Together with our previous study (27), the above result shows that blood monocytes can give rise to both lung and alveolar MΦ, albeit with distinct kinetics. To further investigate the precursor/progeny relation of blood monocytes and pulmonary MΦ, we investigated the kinetics of their reconstitution after irradiation and total BM engraftment. To this end, we transferred syngeneic wild-type (wt) BM cells (CD45.1) into lethally irradiated recipients (CD45.2) and followed the regeneration of the blood monocyte compartment, as well as lung and alveolar MΦ, by donor-derived cells (Fig. 3A).

As expected from the literature (20), 1 wk following BM transfer, all blood monocytes were fully replaced by donor-derived (CD45.1+) cells (Fig. 3B). In contrast, and in agreement with their earlier reported delayed reconstitution (14, 16, 37), alveolar MΦ began to be replaced by donor cells only 3 wk after transfer (Fig. 3B). Interestingly, the exchange of lung MΦ by donor BM-derived cells significantly preceded this event and showed intermediate kinetics, beginning at day 10, with full reconstitution being reached 3 wk after transfer (Fig. 3B).

In conclusion, all three cell types studied are reconstituted after irradiation by BM-derived cells, albeit with significantly distinct kinetics. Interestingly, our data suggest that lung MΦ begin to be replaced by donor BM cells only after exchange of the blood monocyte compartment, and alveolar MΦ start to be replaced only after full reconstitution of parenchymal lung MΦ (Fig. 3B).

Alveolar MΦ do not originate directly from blood monocytes

The DTx-induced ablation of alveolar MΦ in CD11c−DTR transgenic mice was followed by their rapid reconstitution (Fig. 1B). To investigate the origin of these newly generated alveolar MΦ, we transferred CD45.1 wt BM into irradiated CD11c−DTR transgenic recipients (CD45.2). The resulting wt→CD11c−DTR BM chimeras allow the conditional depletion of host lung and alveolar MΦ. On day 5 after BM transfer, i.e., at a time when some blood monocytes are of donor BM origin, but all lung and alveolar MΦ are still host derived (Fig. 3B), chimeras were either treated i.t. with DTx or left untreated. Mice were analyzed on day 8, 3 days after DTx treatment, when >90% of blood monocytes are donor derived (Figs. 3B and 4A). Whereas parenchymal lung MΦ of untreated chimeras were still host derived, in DTx-treated mice one-half of them were replaced by donor cells, most likely of blood monocyte origin (27) (Fig. 4A). Interestingly, in the same mice, >90% of newly generated alveolar MΦ were of host origin (CD45.1−) (Fig. 4A). Hence, in our experimental setting, newly generated alveolar MΦ originated from a host-derived precursor, although all blood monocytes were donor derived (Figs. 3B and 4A). This result argues that alveolar MΦ do not originate directly from blood monocytes.

Lung MΦ serve as alveolar MΦ precursor

Depletion of both CD11c−DTR transgenic lung and alveolar MΦ by i.t. DTx treatment was followed by their rapid reconstitution (Fig. 1B). The kinetics of these processes was, however, different. Although alveolar MΦ numbers arose after 1 day, the number of parenchymal MΦ continuously declined for the first 4 days following the DTx treatment (Fig. 1B). The mean human serum 1/12 of DTx has been estimated to be 18 h (38). Accordingly, the DTx treatment of CD11c−DTR transgenic mice generally induces a transient, short-term depletion of CD11c+ cells (29). It is therefore unlikely that the persistent decline of lung MΦ from day 1 to day 4 (Fig. 1B) results from ongoing toxin-induced apoptosis, but rather suggests MΦ emigration from the lung parenchyma to the alveolar space. In addition, on day 5 after BM transfer, newly generated alveolar MΦ originated from host-derived precursors, whereas at that time lung MΦ were of host origin (Fig. 4A). Taken together, our results suggest that parenchymal lung MΦ act as immediate alveolar MΦ precursors.

To further investigate this option, we next studied the origin of alveolar MΦ at a time when parenchymal lung MΦ were reconstituted by donor-derived cells. To this end, wt→CD11c−DTR chimeras were treated 12 days after BM transfer with DTx i.t., and control chimeras were left untreated. At this time, all blood monocytes and a fraction of the parenchymal lung MΦ are of donor origin, but alveolar MΦ are still host derived (Fig. 3B). We analyzed the recipient mice 3 days later, i.e., on day 15, when all lung MΦ are donor derived (Figs. 3B and 4B). Although in untreated mice most alveolar MΦ were of host origin, following their DTx-induced depletion all newly generated alveolar MΦ were donor derived (Fig. 4B).

To conclude, the origin of newly generated alveolar MΦ correlated with the donor/host origin of lung MΦ (Fig. 4). Taken together with their differential reconstitution kinetics following deple tion, these data support the notion that lung MΦ act as alveolar MΦ precursors.
Proliferation of lung and alveolar MΦ

All blood monocytes are CX3CR1 positive, and therefore express cytoplasmatic GFP in cx3cr1 gfp+/− knockin mice (30), whereas pulmonary MΦ are CX3CR1 negative (27) (Fig. 5A). Because GFP is stable for several days (39), all monocyte descendants inherit the cytoplasmic GFP label, even though expression of CX3CR1/GFP might have ceased. Although they are monocyte derived and therefore expected to be GFP labeled, pulmonary MΦ of cx3cr1 gfp+/− mice are GFP negative (27) (Fig. 5A). Because MΦ are long-lived cells (40), they most likely lost the GFP label over time. Alternatively, the rapid loss of the GFP label can be explained by cell divisions, which in the absence of GFP de novo synthesis will result in the dilution of the label between daughter cells. The latter is in agreement with previous studies suggesting that murine alveolar MΦ originate from proliferating precursors in the lung parenchyma (13, 23, 25).

To distinguish between those two options, we analyzed the GFP label of newly coming lung and alveolar MΦ. To this end, we

FIGURE 3. Different reconstitution kinetics of blood monocytes, lung MΦ, and alveolar MΦ follow irradiation and BM transfer. CD45.1 BM cells were transferred to CD45.2 wt recipient mice on day 0, and recipients’ blood, digested lungs, and BAL fluids were analyzed on days 1, 5, 7, 9, 12, 14, 16, 19, and 26 for donor-derived monocytes and MΦ. A, Identification of donor BM-derived cells. Blood of recipient mice was stained with PE-coupled anti CD115 Ab and biotin-coupled anti CD45.1 Ab, followed by allophycocyanin-conjugated streptavidin. Cells isolated from lung parenchyma and BAL fluids were stained with PE-coupled anti-CD11c Ab, PerCP-coupled anti-CD11b Ab, and biotin-coupled anti-CD45.1 Ab, followed by allophycocyanin-conjugated streptavidin. R1 gate indicates CD45.1+ donor-derived cells of total blood monocytes (CD115+ cells). R2 and R3 gates indicate CD45.1+ donor-derived cells of total lung and alveolar MΦ, respectively (identified as CD11c−CD11b− cells, as indicated by gates). B, Kinetics of blood monocytes (▲), lung MΦ (●), and alveolar MΦ (■) reconstitution by donor BM-derived cells. Graph shows the percentage of donor-derived cells (CD45.1+) of total blood monocytes (cells gated in R1, as indicated in A), lung MΦ (cells gated in R2, as indicated in A), and BAL MΦ (cells gated in R3, as indicated in A). n = 2 for each time point.

FIGURE 4. Alveolar MΦ do not originate directly from blood monocytes. BM cells of CD45.1 wt mice were adoptively transferred into irradiated CD11c-DTR CD45.2 transgenic recipients on day 0. Recipient mice were either treated i.t. with DTx on indicated days or left untreated and sacrificed 3 days later. Cells were then analyzed for CD45.1 expression, as described in Fig. 3A. Donor-derived (CD45.1+) blood monocytes, lung MΦ, and alveolar MΦ are cells gated in R1, R2, and R3, respectively, whereas host-derived (CD45.1−) cells are cells outside those gates. A, Recipient mice were either treated i.t. with DTx on day 5 (+) or left untreated (−) and were sacrificed on day 8. Bar diagram shows percentage of donor (CD45.1+, ▲)- and host (CD45.1−, □)-derived blood monocytes, lung MΦ, and alveolar MΦ of the total population. n = 5 for each group. One representative of three independent experiments. B, Recipient mice were either treated i.t. with DTx on day 12 (+) or left untreated (−) and were sacrificed on day 15. Bar diagram shows percentage of donor (CD45.1+, ▲)- and host (CD45.1−, □)-derived blood monocytes, lung MΦ, and alveolar MΦ of the total population. n = 5 for each group. One representative of three independent experiments.
generated a \(cx_{cr1}^{gfp+/+};CD11c\):DTR double-transgenic mouse line, treated the mice with DTx i.t., and investigated the green fluorescence intensities of their lung and alveolar MΦ on the following days (Fig. 5B). During the first 5 days after depletion, newly coming lung MΦ showed various green fluorescence intensities, ranging from high to low (Fig. 5B). This may result from differentiation of CX2CR1/GFP-positive monocytes into CX2CR1-negative MΦ and the division of the latter. From day 7 and on, lung MΦ fluorescence intensity was low and comparable to that of lung MΦ from PBS-treated mice (Fig. 5, A and B). This suggests that there is no more input of monocytes at this stage, but because lung MΦ population increases (Fig. 1B), proliferation of differentiated lung MΦ most likely persists.

Throughout the experiment, alveolar MΦ showed similar green fluorescence intensities to those of PBS-treated mice (Fig. 5, compare A and B). This supports the notions that the differentiation of blood monocyte into alveolar MΦ is indirect and requires a lung MΦ intermediate. Furthermore, these data also suggest that the proliferation of lung MΦ (which allows the loss of the GFP label) preceded their migration into the alveolar space.

Next, we decided to directly study the proliferation status of lung and alveolar MΦ by determining their PCNA expression levels. The amount of PCNA increases during late G1 phase at sites of DNA replication, reaches a maximum in S phase, and declines during G2 phase (41, 42).

We therefore stained lung, BAL, and blood cells of \(CD11c\):DTR mice 3 days after i.t. treatment with either DTx or PBS for PCNA. In PBS-treated mice, CD11c\(^+\) lung cells were PCNA\(^{-}\), indicating that they were in G1 phase, and upon DTx treatment a majority of these cells became PCNA\(^{high}\) (Fig. 5C), documenting S phase entry and proliferation (41). Because CD11c is expressed by both lung MΦ and DC (33, 34), the analyzed population includes both cell types. In contrast, MHC II is highly expressed by lung DC, but not by MΦ, and therefore allows the discrimination of the two cell types (34). To exclude a potential contamination with MHC II-expressing B cells, we crossed \(CD11c\):DTR mice with B cell-deficient mice (\(\mu\)MT mice) (31), allowing the exclusive definition of lung MHC II\(^+\) cells as DC. The comparison of PCNA expression levels of MHC II- and CD11c-positive lung cells of DTx-treated \(CD11c\):DTR;\(\mu\)MT mice confirmed that the PCNA\(^{high}\) cells were

![Image](http://www.jimmunol.org/)
lung MΦ (CD11c+MHC II+), whereas lung DC (CD11c+MHC II-) were PCNAlow-int (Fig. 5D). This indicates that lung MΦ, but not lung DC, undergo proliferation.

Blood monocytes were PCNAint (Fig. 5C), in agreement with other studies showing that monocytes do not proliferate (28, 43). Interestingly, following the DTx treatment, alveolar MΦ became PCNAhigh (Fig. 5C), supporting the notion that they can proliferate, as previously reported in humans (44).

To conclude, both lung and alveolar MΦ have proliferative potential, which manifests itself upon their DTx-induced depletion and reconstitution. In addition, our results show that lung MΦ proliferation precedes their migration into the alveolar space.

**Discussion**

In this study, we provide direct evidence that alveolar MΦ originate from blood monocytes, and show that this process requires a lung-resident intermediate. We proposed that generation of alveolar MΦ involves the differentiation of blood monocytes into MΦ in the lung parenchyma, proliferative expansion of these cells, and their emigration into the alveolar space. In such a scenario, lung MΦ may serve as a local reservoir, from which alveolar MΦ can be generated whenever needed.

This model is in accordance with results of most previous studies on the alveolar MΦ origin. Our finding, suggesting an indirect connection of blood monocytes and alveolar MΦ, agrees, for example, with the finding of Sawyer et al. (25), showing that the alveolar MΦ population is unaffected by experimentally induced blood monocyte depletion. Furthermore, the derivation of alveolar MΦ from blood monocytes is in agreement with the previously reported replacement of these MΦ by donor cells upon BM transfer (11, 12), and the need for a lung intermediate can further explain the observed delayed reconstitution (13, 14). Bowden and Adamson (23) suggested a dual origin for alveolar MΦ, either directly from blood-borne precursor or from local proliferation. Our results suggest the steady-state derivation of alveolar MΦ from local proliferating precursor, i.e., lung MΦ, and therefore partially agree with the mentioned study. Alveolar DC had not been identified by that time Bowden’s study was conducted (24), and our current data and previous reports suggest that they are derived from blood monocytes without local proliferation (27). We would therefore like to propose that the cells shown by Bowden and Adamson (23) to directly derive from blood monocyte are alveolar DC, potentially mistaken for alveolar MΦ. To conclude, our model offers a connection between works reporting local precursor (13, 23, 25, 45) and those indicating blood-borne precursor (11, 12, 40) for alveolar MΦ, previously thought to be contradictory.

We show that the pulmonary MΦ reservoir is maintained by MΦ proliferation in situ, in both lung parenchyma and alveolar space. In situ proliferating precursors were also suggested for other MΦ population, such as splenic MΦ and liver Kupffer cells (46, 47). Therefore, dependency on a local self-renewal capacity seems to be a general feature of MΦ populations, rather than unique to alveolar MΦ.

Because the main role of alveolar MΦ is the maintenance of the alveolar space by clearing dying cells, particles, and pathogens, there is a constant need for their presence in adequate numbers. By abolishing the dependency on recruitment of blood precursors, the existence of local precursor as reservoir for alveolar MΦ allows a tight control and prompt adjustment of their numbers. Consequently, the rate of monocyte differentiation into lung MΦ under steady state is likely to be low, precluding the detection of monocyte-derived alveolar MΦ (25, 45). In this study, we use a noninflammatory, experimentally induced MΦ depletion protocol to accelerate the MΦ turnover rate, allowing us to detect lung MΦ-derived alveolar MΦ in resting state.

Under conditions other than steady state, the dependency of alveolar MΦ population on input from the blood might differ. Inflammation is, for example, associated with an increase in alveolar MΦ levels (16, 37, 48), and i.t. LPS treatment of BM recipient mice accelerates the replacement of alveolar MΦ by donor cells (16). Interestingly, the rate of lung MΦ replacement by donor-derived cells was also promoted in these studies (16). In agreement with these findings, we have previously shown the differentiation of blood monocytes into lung MΦ in i.t. LPS-treated recipients, but not in untreated mice (27). We, however, had failed to detect graft-derived alveolar MΦ in these studies, which included the analysis of recipient mice 4 days following monocyte transfer (27). In addition, Adamson and Bowden’s (48) kinetic studies (when interpreted as discussed above) suggest the existence of a lung intermediate for alveolar MΦ also during inflammation. We therefore suggest the kinetics of both monocyte differentiation into lung MΦ and the migration of the latter to the alveolar space to accelerate under inflammation. It thus remains possible that the differentiation of alveolar MΦ under inflammation also requires a lung MΦ intermediate.

The irradiation protocol used for the depletion of host BM may also affect the kinetic of alveolar MΦ differentiation from blood monocyte. In this respect, it is of note that different irradiation protocols before BM transfer resulted in distinct alveolar MΦ reconstitution kinetics (11, 13–15). Because irradiation primarily affects dividing cells, proliferating alveolar MΦ might become sensitive to irradiation. Therefore, the turnover of alveolar MΦ can be promoted by the stress caused by tissue damage (49), by a direct effect on pulmonary MΦ (13), or by a combination of the two.

One of the serious side effects of BM transplantation in humans is the increased susceptibility of patients to pulmonary infections (17, 18). It has been proposed that in addition to their lower number, alveolar MΦ of BM transplantation patients might be functionally impaired and therefore less reactive (37). The proliferative capacity of both lung parenchymal and alveolar MΦ might make both these cell types sensitive to irradiation. This can affect both the functionality of alveolar MΦ and the size of their reservoir in the lung parenchyma, reducing their capacity to efficiently combat pathogens. The indirect route from monocytes to alveolar MΦ might result in a delayed replacement of damaged alveolar MΦ with new and unaffected cells, maintaining the patient susceptibility to pathogen. Therefore, our results suggest that a strategy aiming at the active depletion of pulmonary MΦ might trigger an advantageous renewal from donor BM-derived monocytes, and could potentially reduce the period of patient vulnerability to pulmonary pathogens.

**Acknowledgments**

We thank our laboratory members for critical reading of the manuscript, and are grateful to Y. Chermesh and Y. Melamed for animal husbandry.

**Disclosures**

The authors have no financial conflict of interest.

**References**


