ACUTE PULMONARY INFLAMMATION is characterized by successive waves of early alveolar neutrophil and relatively delayed alveolar monocyte recruitment. The monocyte chemoattractant CCL2 [JE, monocyte chemoattractant protein (MCP)-1] and its receptor CCR2 are critical determinants for recruitment of monocytes to inflamed lungs. Recently, reports have identified monocytes as an active participant in the pathogenesis of acute lung inflammation and may contribute to respiratory failure (14). We have shown that bone marrow-derived blood-borne monocytes play a role in amplification of neutrophilic alveolitis in response to treatment with endotoxin (9, 10, 16). Also, CCL2-driven monocyte recruitment has been suggested to contribute to development of other inflammatory lung diseases, including idiopathic pneumonia syndrome (2).

Previous studies have characterized the sequence of recruited leukocyte populations during inflammatory responses, and a mechanism has been proposed that regulates the switch from neutrophilic to monocytic recruitment in response to treatment with endotoxin (13, 19). Although some recent studies have addressed the mechanism by which recruited macrophages are being cleared from inflamed sites (1), the mechanism(s) that determine the intensity and duration of monocyte recruitment to the lung along CCL2 gradients is not yet clear. A better understanding of this process seems important for developing strategies to restrict the leukocyte burden in inflamed organs without substantially interfering with host defense capacities.

In the present study, we tested the hypothesis that monocytes regulate their accumulation in the lungs by disrupting locally established chemokine CCL2 gradients. Our data show that alveolar recruited monocytes contribute to CCR2-mediated consumption of LPS-induced CCL2 gradients in inflamed lungs. This mechanism may serve as negative feedback loop, limiting the accumulation of monocytes into inflamed lungs and promoting the maintenance of homeostasis.

METHODS

Animals. CCR2-deficient mice were generated on a mixed C57BL/6 × 129/Ola genetic background by targeted disruption of the CCR2 gene as described previously (3). The disrupted CCR2 gene was back-crossed for six generations to wild-type BALB/c mice. Parent and offspring CCR2−/− mice on the BALB/c background were bred under specific pathogen-free (SPF) conditions. Wild-type control animals were purchased from Charles River Laboratories (Sulzfeld, Germany). Animals 8–12 wk old and between 18–21 g were used for the described experiments. This animal study was approved by our local government committee of Giessen.
deficient mice received 12 Gy of total body irradiation using a 60Co (GIBCO) before transplantation. Recipient wild-type and CCR2− cell aggregates. The cells were washed in Leibovitz L15 medium 70–m nylon meshes (BD Biosciences) to remove residual carefree prepared from the bone marrow isolates and filtered through CCR2 infused via the lateral tail veins with donor marrow cells suspended in in two doses separated by a 3-h interval. For transplantation, the recipient chimeric animals were then housed under SPF conditions for at least 3–4 wk with free access to autoclaved food and water. The described protocol has been shown to yield engraftment efficiencies of >97% (10).

Treatment protocols. Alveolar neutrophil and monocyte recruitment profiles were evaluated in wild-type mice and CCR2-deficient mice and, in selected experiments, also in chimeric wild-type mice (lethally irradiated wild-type mice reconstituted with bone marrow cells from CCR2-deficient mice) and chimeric CCR2-deficient mice (lethally irradiated CCR2-deficient mice reconstituted with wild-type bone marrow cells). Mice received intratracheal instillations of LPS (0.1, 1, 10, and 20 μg/mouse) for various times (0, 12, 24, 48, 72, 96, 120, and 168 h), as previously described in detail (5–10). In some inhibition experiments, wild-type mice received an injection of 250–500 μg/mouse ip of anti-CCR2 MAb MC21 6 h before inflammatory challenge. Selected mice were also treated with intratracheal instillations of E. coli LPS (20 μg/mouse) plus MC21 (100 μg/mouse) for various time points. Subsequently, mice were killed with an overdose of isoflurane. Blood samples and bronchoalveolar lavage (BAL) fluid (BALF) were collected as described earlier (5–10). Quantitation of alveolar recruited neutrophils and monocytes was done on differential cell counts of Pappenheim-stained cytocentrifuge preparations (Shandon), using overall morphological criteria, including differences in cell size and shape of nuclei and subsequent multiplication of those values with the respective absolute BAL cell counts, as recently described (8).

Determination of BALF cytokine levels. BALF cytokine levels of TNF-α, macrophage inflammatory protein (MIP)-2, CCL2 (MCP-1, JE), and CCL12 (MCP-5) collected from mice of the various treatment groups were determined with commercially available ELISA kits (R&D Systems).

Lung histology. Mice were killed with a lethal dose of isoflurane, the chest was rapidly opened, and the thoracic organs were carefully removed. We fixed lungs by instilling PBS-buffered formaldehyde solution (4.5%, pH 7.2) through the trachea at a constant pressure of 20 cmH2O, as described recently (5). Fixation was allowed to proceed overnight at room temperature; subsequently tissue samples were paraffin embedded. Sections of 5 μm from all lungs were stained with hematoxylin-eosin and evaluated for evidence of cellular infiltrates at a ×400 original magnification. In selected experiments, lungs of mice were subjected to BAL (PBS, pH 7.2) before fixation in paraformaldehyde solution, as described above.

In vitro CCL2 consumption experiments. We used both human and mouse blood monocytes for CCL2 consumption experiments. Peripheral blood from healthy donors was subjected to combined Ficoll density gradient centrifugation and centrifugal elutriation to obtain highly purified monocyte and neutrophil preparations, as recently described (14). Peripheral blood monocytes (PB-Mo) of both wild-type and CCR2-deficient mice were isolated according to recently described gating and sort protocols using a DIVA-assisted FACSVantage SE flow cytometer (5). Monocytes (1 × 105 cells/ml) were cultured at 37°C in an atmosphere of 5% CO2 in RPMI 1640 supplemented with 5% FCS in the absence or presence of defined amounts of recombinant human or murine CCL2 (600 and 500 pg/ml, respectively). In selected experiments, neutrophils were tested for their ability to consume CCL2. In addition, PB-Mo collected from wild-type mice were incubated with the lysosomotropic agent ammonium chloride [20 mM, 30 min (17, 20)] before addition of recombinant murine CCL2 (500 pg/ml, 24 h) to block CCR2 receptor recycling to the cell surface. Subsequently, cell-free culture supernatants were collected and stored at −80°C until CCL2 analysis with commercial ELISA (R&D Systems).

Statistics. The data are expressed as means ± SD. Significant differences between treatment groups were estimated by Mann-Whitney U-test. Differences were assumed to be significant when P values were <0.05.

**RESULTS**

Alveolar neutrophil and monocyte recruitment and cytokine production induced by intratracheal LPS treatment in wild-type and CCR2-deficient mice. In BALF of both untreated wild-type and CCR2-deficient mice, alveolar macrophages represented the main cellular component, with alveolar neutrophils rarely exceeding 1–2% of the total BAL cell counts. Instillation of endotoxin into the lungs of wild-type and CCR2-deficient mice elicited a dose- and time-dependent recruitment of alveolar neutrophils with similar kinetics between these groups peaking at −24 to 48 h posttreatment (Fig. 1, left). In contrast, LPS treatment induced alveolar monocyte accumulation, peaking at −48–72 h posttreatment in wild-type mice but not CCR2-deficient mice. Alveolar monocyte recruitment was highly significantly reduced in CCR2-deficient mice (Fig. 1, right), demonstrating that alveolar monocyte traffic in LPS-induced pulmonary inflammation is predominantly mediated via CCR2.

We additionally performed lung histology to exclude the possibility that differences in monocyte numbers observed in BALF of LPS-challenged wild-type vs. CCR2-deficient mice were due to a selective, LPS-induced retention of monocytes in the lungs of CCR2-deficient mice. As shown in Fig. 2, instillation of LPS into the lungs of wild-type (Fig. 2C) and CCR2-deficient mice (Fig. 2D) induced a similar pattern of interstitial and alveolar accumulating leukocytes (48 h posttreatment), associated with a thickening of the alveolar septae, compared with untreated controls (Fig. 2, A and B, respectively). However, when the lungs of LPS-treated wild-type and CCR2-deficient mice were subjected to BAL before preparation for lung histology, post-BAL histological examination revealed no selective retention of alveolar recruited monocytes in either of the investigated treatment groups (Fig. 2, E and F), demonstrating that different numbers of alveolar monocytes observed in BALF of the two treatment groups were not due to
a selective monocytic sticking in the lungs of CCR2-deficient mice.

We next compared BALF CCL2, CCL12, TNF-α, and MIP-2 concentrations in wild-type and CCR2-deficient mice challenged with intratracheal application of LPS. TNF-α and MIP-2 levels were similar in wild-type mice and CCR2-deficient mice under baseline conditions and showed similar time- and dose-dependent induction patterns with peak values observed at ~12 h postchallenge (Fig. 3, left and middle). In contrast, CCR2-deficient mice showed baseline BALF CCL2 levels in the same range as wild-type mice but demonstrated ~10-fold elevated LPS-induced BALF CCL2 levels with peak values observed at ~48–72 h posttreatment (Fig. 3, right). In contrast to the strongly different BALF CCL2 levels observed in LPS-challenged wild-type vs. CCR2-deficient mice, BALF CCL12 (MCP-5) levels of the same mice did not show significant differences between both treatment groups [wild type (24 h), 5 ± 8 pg/ml vs. CCR2 knockout (24 h), 19 ± 14 pg/ml; wild type (48 h), 109 ± 42 pg/ml vs. CCR2 knockout (48 h), 150 ± 30 pg/ml; wild type (72 h), 114 ± 37 pg/ml vs. CCR2 knockout (72 h), 148 ± 67 pg/ml; mean ± SD, n = 5 each]. These data again support the role of CCR2 as primary receptor involved in CCL2 turnover in vivo.

Effect of systemic or intra-alveolar administration of neutralizing CCR2 antibodies on LPS-induced alveolar CCL2 levels in wild-type mice. The inverse relationship between alveolar monocyte accumulation and BALF CCL2 levels observed in wild-type mice vs. CCR2-deficient mice prompted us to evaluate whether systemic treatment of wild-type mice with the anti-CCR2 antibody MC21 would influence alveolar monocyte trafficking and BALF CCL2 levels in response to LPS. We observed that wild-type mice pretreated with the anti-CCR2 antibody MC21 before intratracheal LPS application showed a selectively reduced alveolar monocyte but not neutrophil accumulation, which was associated with increased BALF CCL2 levels peaking at 48–72 h posttreatment (Fig. 4, A and C). Interestingly, this systemic (MC21-mediated) blockade of CCR2, resulting in decreased LPS-induced alveolar
monocyte recruitment, significantly decreased alveolar TNF-α levels at 24 h posttreatment, indicating that alveolar recruited monocytes contribute to LPS-induced intra-alveolar liberation of proinflammatory mediators such as TNF-α (Fig. 4D).

To further specify whether CCR2 expressed by recruited or resident cells within the alveolar compartment is directly involved in regulating alveolar space CCL2 levels, wild-type mice received intratracheal coapplication of LPS (20 μg/mouse) and anti-CCR2 antibody MC21 (100 μg/mouse). In fact, this coapplication resulted in a marked increase of BALF CCL2 levels compared with control antibody-treated mice (Fig. 5A). At the same time, BALF monocyte counts were not significantly different from those observed in wild-type mice treated with LPS alone (data not shown). In contrast to CCL2, wild-type mice cochallenged with LPS plus MC21 had similar BALF TNF-α levels as in wild-type controls. (Fig. 5B).

Effect of intratracheal LPS application on alveolar monocyte recruitment and alveolar CCL2 levels in chimeric wild-type and chimeric CCR2-deficient mice. To further distinguish the respective role of CCR2 expressed on recruited monocytes vs. resident alveolar cells in regulating alveolar space CCL2 levels, we compared LPS-induced CCL2 levels in reciprocally transplanted chimeric wild-type and chimeric CCR2-deficient mice. Chimeric wild-type mice treated with intratracheal application of LPS showed a significantly reduced alveolar monocyte accumulation together with drastically increased BALF CCL2 levels, comparable to those observed in CCR2-deficient mice (Fig. 6, A and B). On the other hand, LPS instillation into the lungs of chimeric CCR2-deficient mice provoked a significantly increased alveolar monocyte traffic compared with CCR2-deficient mice, with peak values observed at ~48 h posttreatment. In parallel, chimeric CCR2-deficient mice exhibited significantly reduced intra-alveolar CCL2 levels (Fig. 6, C and D). These data suggest a major role of CCR2-positive alveolar recruited monocytes in regulating alveolar space CCL2 levels in lung inflammation.

Monocytes are capable of lowering CCL2 levels in vitro. We additionally performed cell culture experiments to confirm the proposed mechanism of monocyte-dependent CCL2 consumption in vitro. We used both highly purified human (Fig. 7A) and mouse PB-Mo (Fig. 7B) (purities >95%) for CCL2 consump-
tion assays. In supernatants of unstimulated monocytes, CCL2 protein was nearly undetectable (Fig. 7, A and B, bar 2). Also, no spontaneous degradation of recombinant CCL2 protein was observed during the 24-h time period in the absence of monocytes (Fig. 7, A and B, bars 3 and 4). Addition of human monocytes (1 × 10^5 cells/ml) to culture media containing defined concentrations of recombinant CCL2 led to a time-dependent decrease of CCL2 protein, peaking at 24 h postinduction (Fig. 7A, bars 5–7). Similarly, highly purified PB-Mo collected from wild-type mice (1 × 10^5 cells/ml) also consumed CCL2 protein levels within the 24-h observation period (P < 0.001, Fig. 7B, bar 5). Addition of function-blocking anti-CCR2 antibodies to the monocyte cultures strongly inhibited this CCL2 consumption process (Fig. 7A, bars 8–10; Fig. 7B, bar 7). Similarly, blockade of receptor recycling by the lysosomotropic agent ammonium chloride significantly inhibited CCL2 consumption by wild-type PB-Mo (Fig. 7B, bar 6). Lack of CCL2 consumption was also observed in cultures of PB-MO collected from CCR2-deficient mice incubated with CCL2 for 24 h (Fig. 7B, bar 8). Notably, addition of neutrophils instead of monocytes to cell cultures did not induce any detectable CCL2 consumption (data not shown). These data strongly support the concept that the consumption of CCL2 protein observed in vivo is monocyte and CCR2 dependent.

**DISCUSSION**

In the current study, we tested the hypothesis that CCR2-positive alveolar recruited monocytes limit their further accumulation in LPS-challenged mouse lungs by downregulating the alveolar levels of CCL2. We found that LPS-treated wild-type mice with intact CCR2 expression showed a robust...
CCR2-dependent alveolar monocyte accumulation, associated with relatively low BALF CCL2 levels. In contrast, CCR2-deficient and chimeric wild-type mice reconstituted with the hematopoietic system of CCR2-deficient mice lacking monocyctic CCR2 expression demonstrated a heavily reduced alveolar monocyte traffic in response to LPS together with highly elevated alveolar CCL2 levels. Conversely, reconstitution of CCR2-deficient mice with the hematopoietic system of wild-type mice raised their LPS-induced alveolar monocyte accumulation to wild-type values, again accompanied with a sharp drop in BALF CCL2 levels. Furthermore, systemic anti-CCR2 antibodies to block LPS-induced alveolar monocyte traffic in wild-type mice significantly increased alveolar CCL2 levels. Moreover, intra-alveolar blockade of CCR2 receptor function by intratracheal antibody administration in LPS-challenged mice did not affect the alveolar monocyte accumulation but again significantly increased alveolar CCL2 levels. Finally, addition of human or mouse monocytes to culture media containing defined amounts of recombinant CCL2 protein caused a sharp and time-dependent drop of CCL2 levels in vitro, which was not observed upon addition of anti-CCR2 antibodies to the cultures or when highly purified CCR2-deficient monocytes were employed. Together, the data suggest that CCR2-positive monocytes regulate alveolar CCL2 levels in inflamed lungs. As alveolar CCL2 generation represents the main driving force of monocyte accumulation in this compartment, the consumption of alveolar CCL2 by CCR2-positive monocytes appearing in the alveolar space may represent an important negative feedback mechanism, limiting excess monocyte accumulation in this compartment and promoting a return to homeostasis.

It has been proposed that early recruited neutrophils elicit subsequent alveolar monocyte recruitment by local release of CCL2 (21). In addition, resident alveolar epithelial cells, due to their polarized CCL2 release upon inflammatory challenge, also appear to be involved (14). Both mechanisms may be operative in the described system, given that neutrophil accumulation preceded alveolar monocyte accumulation in LPS-inflamed lungs of both wild-type and CCR2-deficient mice. The strikingly different BALF CCL2 levels noted in LPS-treated wild-type and CCR2-deficient mice, however, were not due to different neutrophil recruitment profiles in these two treatment groups. Moreover, we found that primary isolates of type II epithelial cells collected from wild-type and CCR2-deficient mice responded with the same amount of CCL2 release upon LPS stimulation in vitro, thus making differential CCL2 releasing capacities of wild-type vs. CCR2 knockout epithelial cells unlikely (M. Srivastava, unpublished observation). In addition, because neutrophils were recently shown to lack CCR2 expression, it is rather unlikely that neutrophils are

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**Fig. 4.** Effect of systemic anti-CCR2 antibody application on alveolar CCL2 and TNF-α levels and alveolar leukocyte traffic in LPS-treated WT mice. WT mice either received IT application of E. coli LPS (20 µg/mouse, solid bars) for various time points or were pretreated systemically with the blocking anti-CCR2 MAb MC21 (crosshatched bars) before LPS instillation procedures. At 24, 48, or 72 h post-LPS treatment, mice were killed and subjected to BAL for determination of differential BAL cell counts and BALF cytokine levels. Data are presented as means ± SD (n = 6 animals per time point). *P < 0.01 vs. LPS-treated animals (A, D) and P < 0.05 vs. LPS-treated animals (C).

**Fig. 5.** Effect of IT anti-CCR2 antibody application on LPS-induced alveolar CCL2 and TNF-α levels. Mice received co-instillations of either control antibodies plus E. coli LPS (20 µg/mouse, solid bars) or blocking anti-CCR2 Ab MC21 (100 µg/mouse) plus E. coli LPS (20 µg/mouse, hatched bars) for various time points. Subsequently, mice were killed and subjected to BAL for determination of differential BAL cell counts and BALF cytokine levels. Data are presented as means ± SD (n = 5 animals/time point). *P < 0.01 vs. animals treated with coapplications of control Ab plus LPS.
CCR2 KO mice. Compared with WT mice; means differ.

Fig. 6. Effect of CCR2 chimerism in WT and CCR2 KO mice on E. coli LPS-induced alveolar monocyte accumulation and alveolar CCL2 levels. WT mice (solid bars in A and B) and chimeric WT mice (open bars in A and B) or CCR2 KO mice (solid bars in C and D) and chimeric CCR2 KO mice (open bars in C and D) were treated with IT application of E. coli LPS for various time points, as indicated. Subsequently, mice were killed and subjected to BAL for determination of BAL cell differentials and BALF cytokine levels. Values are shown as means ± SD (n = 5–9 animals/time point). A and B: *P < 0.05 compared with WT mice; C and D: *P < 0.05 compared with CCR2 KO mice.

Directly involved in local CCL2 depletion processes (9, 10). Accordingly, neutrophils failed to lower recombinant CCL2 levels in vitro (data not shown). Thus the significant differences in BALF CCL2 levels between wild-type and CCR2-deficient mice support the hypothesis that CCR2-positive monocytes themselves are actively involved in regulating local CCL2 levels within inflamed lungs. Indeed, in addition to CCR2 deletion, blockade of CCR2 function by systemic MC21 administration in wild-type mice attenuated the alveolar monocyte but not neutrophil traffic with a concomitant increase in alveolar CCL2 levels. From the presented data, we cannot exclude the notion that other mechanisms may also contribute to CCL2 removal from inflamed lungs such as proteolytic degradation in alveolar lining fluids. In this regard, Tylaska et al. (18) recently reported that CCL2 levels in allogeneic sponges implanted subcutaneously in CCR2-deficient mice remain elevated for up to 6–8 days after implant compared with wild-type controls, which was prolonged compared with the CCL2 kinetics (up to 3 days) reported to occur in LPS-challenged CCR2-deficient mice in the current study. However, both different experimental models (acute vs. chronic) and different body compartments (lung vs. systemic) investigated may contribute to the differences in CCL2 removal kinetics reported in this study compared with that of Tylaska et al. (18).

Type II alveolar epithelial cells were recently shown to express CCR2 mRNA and respond to CCL2 stimulation in vitro, presumably via CCR2 (11). In addition, resident alveolar macrophages were also demonstrated to exhibit some CCR2 expression, albeit at much lower levels than on monocytic precursors (8). Tylaska et al. (18) recently demonstrated that peritoneal macrophages from wild-type mice are capable of integrating CCL2 gradients in a CCR2-dependent manner in vitro, and this CCR2-dependent control of locally released CCL2 levels at inflammatory sites was also reported to occur in response to allogeneic sponge implants in wild-type but not CCR2-deficient mice. However, in the present study, a major role of resident alveolar macrophages in consuming locally released CCL2 levels within the alveolar compartment is unlikely due to the findings in the chimeric mice. Chimeric wild-type mice, recently shown to exhibit a CCR2-negative circulating monocyte but CCR2-positive alveolar macrophage phenotype (10), demonstrated similar alveolar CCL2 levels as did CCR2-deficient mice, strongly supporting the predominant role of the mononuclear cells in regulating alveolar CCL2 levels. This view is further supported by the fact that consumption of alveolar CCL2 along with strong monocyte traffic into the alveolar space was noted in chimeric CCR2-deficient mice. In this line, highly elevated BALF CCL2 levels observed in LPS-treated wild-type mice upon intratracheal MC21 application are considered to primarily reflect MC21 interference with CCR2 function on newly recruited monocytes rather than resident lung cells, including alveolar macrophages and epithelial cells. However, the MC21-induced increase in alveolar CCL2 levels noted in wild-type mice was not associated with increased numbers of alveolar recruited monocytes. The most probable explanation for this observation may be that diffusion of the antibody toward the interstitial compartment has hampered additional monocytes from further accumulating within the alveolar compartment of MC21-treated mice.

We and others recently showed that monocyte recruitment to the lungs in response to CCL2 or CCL2 plus LPS (5, 7) or during idiopathic pneumonia syndrome (2) and septic acute respiratory distress syndrome (14) was accompanied by increased monocytic CD14 cell surface expression. These CD14-positive alveolar monocytes exhibited increased TNF-α mRNA levels and responded with greater TNF-α release upon LPS stimulation in vitro than circulating CD14-negative monocytes, suggesting that transmigration across the highly specialized endothelial/alveolar epithelial barrier primes but not fully
activates monocytes, irrespective of the event eliciting the migratory response (5). Similar to these reported results, here we report that reduced BALF TNF-α levels were observed in wild-type mice pretreated with MC21 to effectively block the alveolar monocyte but not neutrophil appearance in response to LPS. Another explanation for the significantly reduced BALF TNF-α levels observed in MC21-pretreated, LPS-challenged wild-type mice has been offered by a recent report, showing that CCL2-elicited monocyte recruitment. However, two aspects argue against the idea that this mechanism plays also a significant role in the described mouse system in vivo: First, we found that LPS-treated wild-type mice receiving intratracheal applications of MC21 to block local CCR2 receptor function on newly recruited monocytes showed heavily increased BALF CCL2 levels without a notable decrease in BALF TNF-α levels compared with LPS-treated controls, suggesting that the contribution of alveolar recruited monocytes to intra-alveolar liberated TNF-α levels is independent from the CCR2/CCL2 axis but may involve other signaling pathways such as CD14. Second, LPS-treated CCR2-deficient mice not capable of responding to CCL2 showed similar BALF TNF-α levels as detected in LPS-treated wild-type mice, again indicating that the CCR2/CCL2 axis appears to play a minor role in the local TNF-α release in the described mouse model.

Our data suggest that consumption of alveolar CCL2 levels by monocyte CCR2 receptors is a possible mechanism that accounts for the reciprocal relationship between monocyte recruitment and CCL2 concentrations in BALF, although a role of monocytes in cell-cell signaling events initiated by the CCL2-CCR2 axis to downregulate CCL2 in the major cellular sources of this chemokine in the alveolar compartment (presumably alveolar epithelial cells) cannot be fully excluded. The concept of CCL2 scavenging by CCR2-positive monocytes is further supported by the CCL2 consumption assays in vitro: a sharp drop in CCL2 levels was observed in cultures employing human or wild-type mouse monocytes, but not in cultures of wild-type monocytes with blocked CCR2 receptor function (i.e., impaired receptor recycling using ammonium chloride or antibody blockade of CCR2) or CCR2-deficient monocytes. Notably, similar to these findings regarding the role of monocytes in CC chemokine consumption, a recent report demonstrated that G-CSF-enhanced neutrophil recruitment to LPS-inflamed rat lungs was associated with strongly reduced BALF MIP-2 and cytokine-induced neutrophil chemoattractant levels due to scavenging of soluble CXC chemoattractants by recruited neutrophils as a mechanism of lowering chemokine gradients in the surrounding alveolar space (22). It is important to add that similar to the chemokine receptors’ lowering local chemokine levels, local chemokine levels may in turn affect chemokine receptor levels, reflecting the complex regulatory interplay of receptor-ligand interactions. In addition, other parameters are known to regulate chemokine receptor expression levels at inflammatory sites, such as the pathogen-associated molecular patterns themselves (17a) and leukocytic differentiation processes taking place during leukocyte migration towards inflamed tissues, as recently demonstrated for the CCR2 expression profiles on circulating monocytes vs. alveolar macrophages (8).

In summary, the current study presents data that support the role of a mechanism shaping the intensity and duration of alveolar monocyte accumulation in acute lung inflammation. CCR2 expression by circulating monocytes is a prerequisite to mount an appropriate alveolar monocyte accumulation in LPS-challenged wild-type mice. The appearance of CCR2-positive monocytes in the alveolar space is the main determinant causing consumption of alveolar CCL2 levels. This negative feedback mechanism may limit excess alveolar monocyte accumulation and promote a return to homeostasis. It is possible that this mechanism of self-regulating alveolar monocyte traf-
fac may offer new perspectives for developing effective therapeutic interventions to selectively modulate monocyte recruitment to the lung in critically ill patients.

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