Role of resident alveolar macrophages in leukocyte traffic into the alveolar air space of intact mice

ULRICH A. MAUS, M. AUDREY KOAY, TIM DELBECK, MATTHIAS MACK, MONIKA ERMERT, LEANDER ERMERT, TIMOTHY S. BLACKWELL, JOHN W. CHRISTMAN, DETLEF SCHLÖNDORFF, WERNER SEEGER, AND JÜRGEN LOHMEYER. Role of resident alveolar macrophages in leukocyte traffic into the alveolar air space of intact mice. Am J Physiol Lung Cell Mol Physiol 282: L1245–L1252, 2002. First published January 18, 2002; 10.1152/ajplung.00453.2001.—Intratracheal instillation of the monocyte chemotactrant JE/monocyte chemoattractant protein (MCP)-1 in mice was recently shown to cause increased alveolar monocyte accumulation in the absence of lung inflammation, whereas combined JE/MCP-1/lipopolysaccharide (LPS) challenge provoked acute lung inflammation with early neutrophil and delayed alveolar monocyte influx. We evaluated the role of resident alveolar macrophages (rAM) in these leukocyte recruitment events and related phenomena of lung inflammation. Depletion of rAM by pretreatment of mice with liposomal clodronate did not affect the JE/MCP-1-driven alveolar monocyte accumulation, despite the observation that rAM constitutively expressed the JE/MCP-1 receptor CCR2, as analyzed by flow cytometry and immunohistochemistry. In contrast, depletion of rAM largely suppressed alveolar cytokine release as well as neutrophil and monocyte recruitment profiles upon combined JE/MCP-1/LPS treatment. Despite this strongly attenuated alveolar inflammatory response, increased lung permeability was still observed in rAM-depleted mice undergoing JE/MCP-1/LPS challenge. Lung leakage was abrogated by codepletion of circulating neutrophils or administration of anti-CD18. Collectively, rAM are not involved in JE/MCP-1-driven alveolar monocyte recruitment in noninflamed lungs but largely contribute to the alveolar cytokine response and enhanced early neutrophil and delayed monocyte influx under inflammatory conditions (JE/MCP-1/LPS deposition). Loss of lung barrier function observed under these conditions is rAM independent but involves circulating neutrophils via β2-integrin engagement.

Address for reprint requests and other correspondence: U. A. Maus, Dept. of Internal Medicine, Div. of Pneumology, Justus-Liebig-Univ., Klinikstr. 36, Giessen 35392, Germany (E-mail: Ulrich.A.Maus@med.uni-giessen.de).

http://www.ajplung.org

When formulating immune modulatory strategies targeting the initiation of pulmonary host responses to airborne inflammatory agents, one needs to consider the complex cross-talk among various cellular components of the alveolar microenvironment, their secretory products, and leukocyte populations recruited from the vascular bed on inflammatory challenge. Resident alveolar macrophages (rAM) are strategically located to initiate, propagate, and modulate early lung inflammatory events, and studies employing liposomal clodronate-mediated macrophage depletion in mice have shown that macrophages are important effector cells of pulmonary immune responses, both innate and adaptive (10, 20). In lipopolysaccharide (LPS)-induced pneumonia, depletion of alveolar macrophages is associated with reduced alveolar cytokine liberation and alveolar neutrophil accumulation (1, 19). Contrarily, in Pseudomonas or Klebsiella pneumonia, depletion of alveolar macrophages is associated with reduced alveolar cytokine liberation and alveolar neutrophil accumulation (1, 19). Contrarily, in Pseudomonas or Klebsiella pneumonia, depletion of alveolar macrophages is associated with reduced alveolar cytokine liberation and alveolar neutrophil accumulation (1, 19). Contrarily, in Pseudomonas or Klebsiella pneumonia, depletion of alveolar macrophages is associated with reduced alveolar cytokine liberation and alveolar neutrophil accumulation (1, 19). Contrarily, in Pseudomonas or Klebsiella pneumonia, depletion of alveolar macrophages is associated with reduced alveolar cytokine liberation and alveolar neutrophil accumulation (1, 19). Contrarily, in Pseudomonas or Klebsiella pneumonia, depletion of alveolar macrophages is associated with reduced alveolar cytokine liberation and alveolar neutrophil accumulation (1, 19). Contrarily, in Pseudomonas or Klebsiella pneumonia, depletion of alveolar macrophages is associated with reduced alveolar cytokine liberation and alveolar neutrophil accumulation (1, 19). Contrarily, in Pseudomonas or Klebsiella pneumonia, depletion of alveolar macrophages is associated with reduced alveolar cytokine liberation and alveolar neutrophil accumulation (1, 19). Contrarily, in Pseudomonas or Klebsiella pneumonia, depletion of alveolar macrophages is associated with reduced alveolar cytokine liberation and alveolar neutrophil accumulation (1, 19).
of patients with septic acute respiratory distress syndrome (ARDS), together with heavily increased levels of the monocyte chemoattractant protein (MCP)-1, have recently been correlated with poor patient outcome (17a). Finally, an animal model mimicking key pathophysiological features of ARDS, including early alveolar neutrophil and strongly increased alveolar monocyte recruitment, together with loss of lung barrier integrity, was most recently described in response to combined JE/MCP-1-plus-<i>Escherichia coli</i> LPS challenge in mice (14).

In the current study, we evaluated the role of rAM in the process of alveolar monocyte recruitment in response to the monocyte chemoattractant JE/MCP-1 in either the absence (noninflammatory conditions) or the presence of <i>E. coli</i> LPS (acute lung inflammatory conditions). In response to sole JE/MCP-1 challenge, rAM were not found to be involved in the alveolar monocyte recruitment process. In the presence of JE/MCP-1 plus <i>E. coli</i> LPS, however, selective depletion of alveolar macrophages dramatically affected alveolar inflammatory responses reflected by profoundly reduced bronchoalveolar lavage (BAL) levels of tumor necrosis factor (TNF)-α and macrophage inflammatory protein (MIP)-2 and suppression of alveolar neutrophil and monocyte recruitment. In contrast, induction of lung barrier failure was not influenced by rAM depletion but was blocked by interfering with neutrophil migration. Thus distinct functions during lung inflammatory sequences, but not during monocyte recruitment in noninflamed lung tissue, are attributable to rAM.

**MATERIALS AND METHODS**

**Animals.** Female BALB/c mice (18–21 g) were purchased from Charles River (Sulzfeld, Germany) and used throughout the study between 8 and 12 wk of age.

**Reagents.** JE, the murine homolog to the human MCP-1 gene product (JE/MCP-1), was purchased from R&D Systems (Wiesbaden, Germany) as a recombinant protein preparation. The recombinant JE/MCP-1 was LPS-free as analyzed by amebocyte lysate assay (detection limit <10 pg/ml, COATEST; Chromogenix, Mölndal, Sweden). Clodronate (dichloromethylene-diphosphonate, C2,2MDP) was obtained from Sigma (St. Louis, MO). The red fluorescent dye PKH26, as well as diluent B solution, was obtained from Invitrogen (Carlsbad, CA) using a 29-gauge Abbocath, which was inserted into the trachea. For inhibition experiments using function-blocking antibodies, mice were anesthetized with inhaled isoflurane until a white film remained. Further removal of the white film was achieved under low vacuum in a Speedvac Savant concentrator. The clodronate solution was made by dissolving 1.2 g of dichloromethylene diphosphonic acid in 5 ml of sterile phosphate-buffered saline (PBS). Five milliliters of the clodronate solution were added to the liposomes and mixed thoroughly. Empty liposomes were made by the addition of sterile PBS alone. This solution was sonicated and ultracentrifuged at 10,000 g for 1 h at 4°C. The liposomal pellets were then removed and resuspended in PBS, followed by ultracentrifugation at 10,000 g for 1 h at 4°C. Subsequently, liposomes were resuspended in 5 ml of sterile PBS, stored at 4°C, and used within 48 h. The final concentration of the liposomal clodronate suspension was 5 mg/ml.

**Treatment of animals.** A single dose of liposomal clodronate was administered to mice via intratracheal instillation (IT) routes. Control mice received empty (PBS containing) liposomes. Briefly, mice were anesthetized with tetrazenol hydrochloride and ketamine, and tracheas were exposed by surgical resection. IT of liposomal clodronate (100 μl) was performed under stereomicroscopic control (L10; Leica, Wetzlar, Germany) using a 29-gauge Abbocath, which was inserted into the trachea. After instillation, the neck wound was closed with sterile sutures. After 24–36 h of liposomal clodronate pretreatment of mice for efficient depletion of the rAM pool, mice were challenged with IT of rJEMCP-1 (50 μg/mouse) in either the absence or presence of <i>E. coli</i> LPS (10 μg/mouse), using the same instillation technique as described for the liposomal clodronate, corresponding to most recent reports (12, 14).

Depletion of circulating neutrophils was achieved by intra-peritoneal injections of carefully titrated Gr-1 MAb (7.5 μg/mouse, diluted in 100 μl PBS/0.1% mouse serum) using aseptic conditions. Successful selective depletion of circulating neutrophils was determined by evaluation of differential cell counts of Pappenheim-stained blood smears and ranged between ~85 and 90%.

For inhibition experiments using function-blocking anti-CD18 MAb, mice were anesthetized and received intravenous injections of 100 μg of MAb in a volume of 100 μl PBS/0.1% mouse serum) via lateral tail veins 15 min before IT procedures, as recently outlined in detail (13, 14).

Analysis of lung barrier function was performed by assessment of FITC-albumin leakage into the alveolar space, as described in detail (14). Briefly, mice received intravenous injections of FITC-labeled albumin (1.5 mg/mouse, Sigma) via lateral tail veins 1 h before death. Undiluted BAL fluid and serum samples (diluted 1:10 and 1:100 in PBS) were measured with a spectrometer (FL 600; Bio-Tek, Winooski, VT; absorbance, 488 nm; emission, 520 ± 20 nm). The lung permeability index is defined as the ratio of fluorescence signals of undiluted BAL fluid samples to fluorescence signals of 1:100 diluted serum samples.

**Isolation of peripheral blood leukocytes and alveolar macrophages.** Mice were killed with an overdose of isoflurane (Forene; Abbott, Wiesbaden, Germany). Isolation of peripheral blood leukocytes from EDTA-anticoagulated blood and BAL for isolation of rAM or alveolar-recruited neutrophils
and monocytes from macrophage-depleted and JE/MCP-1 with or without LPS-challenged mice was performed as recently described in detail (12, 14). Quantitation of alveolar-recruited monocytes recovered from the lungs of macrophage-depleted, JE/MCP-1-challenged mice and alveolar-recruited neutrophils and monocytes recovered from the lungs of macrophage-depleted and JE/MCP-1 plus LPS-challenged mice was done on differential cell counts of Pappenheim-stained cytocentrifuge preparations, 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.

Immunofluorescence analysis. Single-color immunofluorescence staining was used to analyze expression of the JE/MCP-1 receptor CCR2 and the monocyte/macrophage marker F4/80 (7) on the cell surface of rAM from untreated mice. Briefly, ~5 × 10^6 cells were preincubated with Fc-Block (10 μl; BD Biosciences) for blockade of FcγRII receptors in flexible microtiter plates (BD Biosciences) on ice. Negative controls were incubated with isotype-matched control IgG (PharMingen, Wiesbaden, Germany). Cells were incubated with either anti-CCR2 MAb (11) or anti-F4/80 MAb (Serotec) for 30 min on ice, washed three times in PBS (supplemented with 5% mouse serum/0.2% Na azide), followed by incubation with biotinylated F(ab')2 fragments for 30 min on ice. Subsequently, cells were washed three times, and phycoerythrine (PE)-conjugated streptavidin (BD Biosciences) was added to the wells for 15 min on ice in the dark.

Dual-color immunofluorescence staining was used to simultaneously analyze CCR2 expression on F4/80-positive peripheral blood monocytes. Briefly, blood leukocytes were incubated with anti-CCR2 MAb, washed, and incubated with secondary biotinylated F(ab')2 fragments for 30 min on ice, followed by incubation of cells with PE-conjugated streptavidin and FITC-conjugated anti-F4/80 MAb. After 15 min, cells were washed twice and subjected to flow cytometric analysis.

Flow cytometry. All samples were analyzed on a FACStar plus flow cytometer (BD Biosciences) equipped with an argon ion laser operating at an 488-nm excitation wavelength and a laser output of 200 mW. The optical system of the flow cytometer was adjusted daily, using standardized fluorescent Calibrite beads (BD Biosciences).

Single-color flow cytometry of CCR2 or F4/80 expression by rAM was done by gating on forward-scatter vs. side-scatter characteristics, followed by analysis of F4/80 or CCR2 cell surface expression in the fluorescence 2 channel (F488/575).

Dual-color flow cytometry of CCR2 expression by F4/80-positive peripheral blood monocytes was done by gating F4/80-positive leukocytes on forward-scatter vs. F4/80 fluorescence 1 (FITC) characteristics, followed by analysis of CCR2 cell surface expression in the fluorescence 2 channel (F488/575).

Histology and immunohistochemistry. Mice were killed with an overdose of isoflurane, and cryomicrotome of mouse lungs (10 μm sections) was done as recently described (4). Briefly, after a short fixation, sections were preincubated to block nonspecific binding. Incubation with the anti-CCR2 antibody was performed overnight at 4°C. After incubation with the secondary alkaline phosphatase-conjugated antibody, the color reaction was developed with a VectorRed substrate kit. Levamisole (2.5 mM) was added to inhibit endogenous alkaline phosphatase activity. Counterstaining of the sections was performed with methyl green. Control staining was done by incubation with nonspecific serum instead of primary antibody.

Statistics. All data are given as means ± SE. Statistical significance between treatment groups was estimated by Mann-Whitney’s U-test. Differences were assumed to be statistically significant when P values were < 0.05.

RESULTS

Effect of alveolar macrophage depletion on alveolar monocyte recruitment elicited by intratracheal JE/MCP-1 instillation. Both flow cytometric (Fig. 1) as well as immunohistochemical analysis (Fig. 2) clearly demonstrated CCR2 expression on alveolar macrophages, albeit at lower levels than on peripheral blood monocytes, indicating the potential of alveolar macrophages for specifically binding the CCR2 ligand JE/MCP-1. This agent is known to elicit alveolar monocyte recruitment upon deposition within the alveolar space (12). To determine whether alveolar macrophages are indeed involved in this JE/MCP-1-induced alveolar monocyte recruitment, we specifically depleted alveolar macrophages by IT of liposomal clodronate. In mice pretreated for 24–36 h with liposomal clodronate (100 μl), the pool of rAM was found to be depleted by 85 ± 5% compared with alveolar macrophage counts in BAL fluids recovered from control mice receiving PBS-containing (empty) liposomes (100 μl) (means ± SE; n = 7; P < 0.05 vs. control). Importantly, peak alveolar monocyte recruitment rates at 48 h post-JE/MCP-1 challenge in mice either pretreated with liposomal clodronate or receiving PBS-containing empty liposomes were in the same order of magnitude (Fig. 3). Thus rAM depletion by pretreatment with liposomal clodronate...
nate did not affect JE/MCP-1-driven alveolar monocyte recruitment.

Effect of alveolar macrophage depletion on intralveolar cytokine liberation and alveolar neutrophil and monocyte recruitment profiles in response to intralveolar deposition of JE/MCP-1 plus E. coli LPS. We next questioned whether selective depletion of alveolar macrophages affects the liberation of proinflammatory cytokines TNF-α and MIP-2 and subsequent alveolar neutrophil and monocyte traffic after combined JE/MCP-1/LPS challenge. IT of liposomal clodronate into the lungs of mice per se did not provoke TNF-α or MIP-2 release, as shown in Fig. 4 (values at 0 h). Alveolar macrophage-depleted mice challenged intratracheally with JE/MCP-1/LPS for various time periods had drastically reduced peak TNF-α and MIP-2 BAL fluid levels, which was particularly evident after 6 h, compared with nonmacrophage-depleted mice (Fig. 4). Moreover, as shown in Fig. 5A, macrophage-depleted mice challenged intratracheally with combined JE/MCP-1/LPS demonstrated significantly reduced alveolar neutrophil recruitment at 12–48 h posttreatment compared with nonmacrophage-depleted mice undergoing combined JE/MCP-1/LPS challenge (Fig. 5A, P < 0.05 vs. control). In parallel, alveolar macrophage-depleted mice challenged intratracheally with combined JE/MCP-1/LPS also demonstrated significantly attenuated alveolar monocyte recruitment at 12–48 h posttreatment (Fig. 5B; P < 0.05 vs. control).

Effect of alveolar macrophage depletion on induction of lung barrier dysfunction in JE/MCP-1/LPS-challenged mice. To evaluate whether reduced BAL fluid levels of proinflammatory cytokines such as TNF-α and MIP-2, as well as heavily reduced alveolar leukocyte traffic achieved by macrophage depletion, also prevent a lung microvascular permeability increase from occurring in mice challenged with combined JE/MCP-1/LPS (Fig. 6A), we assessed lung barrier function in clodronate- and empty liposome-treated mice. Of note, depletion of rAM by liposome-encapsulated clodronate per se did not provoke induction of lung permeability compared with sham-treated control mice (0-h time points in Fig. 6, A and B). Surprisingly, alveolar macrophage depletion before inflammatory challenge did not prevent lung barrier dysfunction within the 48-h observation period (Fig. 6B). Notably, however, when mice were made neutropenic in parallel to depletion of alveolar macrophages or received intravenous injections of function-blocking anti-CD18 MAb before the challenge with JE/MCP-1 plus LPS, their lung barrier function was nearly fully preserved (Fig. 6, C and D), suggesting an alveolar macrophage-independent but CD18- and PMN-mediated process promoting increased microvascular permeability.

DISCUSSION

In the present study, we evaluated the contribution of rAM to pulmonary responses elicited by IT of JE/MCP-1 in either the absence or presence of E. coli LPS. Although alveolar macrophages were found, by both flow cytometry and immunohistochemistry, to constitutively express the JE/MCP-1 receptor CCR2 and thus might represent a potential target for JE/MCP-1, liposomal clodronate-mediated depletion of alveolar macrophages did not affect solely JE/MCP-1-driven alveolar monocyte accumulation. In contrast, depletion of alveolar macrophages strongly
attenuated the intra-alveolar release of TNF-α and MIP-2 and also significantly suppressed the alveolar neutrophil and monocyte recruitment profiles elicited by combined JE/MCP-1/LPS challenge. However, this attenuated inflammatory response after alveolar macrophage depletion did not protect mice from an increase in lung vascular permeability, whereas the latter was drastically reduced by either simultaneous depletion of circulating neutrophils or systemic administration of anti-CD18 MAb before combined JE/MCP-1/LPS challenge.

Alveolar macrophages as resident phagocytes located at the air-tissue interface of the lung are strategically positioned to respond to the local appearance of microbial or inflammatory agents, thus contributing to the initiation and modulation of lung host defense and inflammation. Interestingly, using a highly sensitive flow cytometric approach for detection of low-level chemokine receptor expression, we found that rAM collected from untreated mice express the major JE/MCP-1 receptor CCR2, albeit at lower levels than circulating monocytes collected from the same animals. Moreover, our immunohistochemical studies also identified rAM along with bronchial epithelial cells of large and small airways as CCR2-expressing cells within the alveolar compartment in situ. Thus we speculated that the constitutive expression of CCR2 by rAM might indicate direct involvement of these phagocytes in the JE/MCP-1-driven alveolar monocyte recruitment process. Alternatively, alveolar deposited or locally liberated low-molecular-weight chemokine JE/MCP-1 might directly pass the epi-/endothelial barrier to bind to and chemoattract circulating CCR2-expressing monocytes from the vascular into the alveolar compartment.

Evaluation of the role of CCR2-expressing alveolar macrophages in the pulmonary responses to JE/MCP-1 in either the absence or presence of E. coli LPS was accomplished by IT of a single dose of liposomal clodronate. This way, a depletion of the rAM pool of ~85% was achieved, corresponding well to previously published data (3, 8, 20, 23). However, this substantial rAM depletion did not affect the JE/MCP-1-driven alveolar monocyte accumulation, supporting the view that, despite their CCR2 expression, these phagocytes are not directly involved in the monocyte recruitment process in noninflamed lungs. This finding does not exclude the possibility that the constitutive CCR2 expression of the alveolar macrophages may well enable these cells to respond with directed migration to locally released JE/MCP-1, as may be secreted by alveolar epithelial cells and/or alveolar-recruited neutrophils during, e.g., pulmonary inflammation (18, 22), thus promoting the accumulation of rAM at the focus of alveolar inflammation.

Although depletion of alveolar macrophages did not affect alveolar monocyte recruitment under noninflammatory conditions, depletion of the resident phagocytes dramatically altered the overall pulmonary inflammatory response developing on combined JE/MCP-1/LPS treatment of mice (14). We observed strongly reduced BAL fluid TNF-α and MIP-2 levels in macrophage-depleted mice undergoing this challenge, which suggests that the alveolar macrophages are centrally involved in the cytokine release response elicited by alveolar LPS. Moreover, depletion of alveolar macrophages also greatly attenuated the early alveolar neutrophil accumulation reported recently to peak at ~12 h after combined JE/MCP-1/LPS challenge (14). These data are well in line with previous observations in models of LPS only-induced lung inflammation, where depletion of alveolar macrophages was also associated with strongly reduced lung neutrophil recruitment profiles (1, 19). Thus alveolar macrophages apparently function to promote rapid neutrophil influx into the alveolar compartment under conditions of acute inflammation.

Interestingly, the alveolar macrophage-depleted mice also demonstrated a significant reduction of the alveolar monocyte recruitment response to JE/MCP-1 plus E. coli LPS challenge. As discussed, this contrasts to the finding that clodronate-mediated depletion of macrophages did not affect the peak alveolar monocyte accumulation provoked by sole JE/MCP-1 instillation, mimicking noninflammatory conditions. Thus it ap-

---

**Fig. 4.** BAL fluid cytokine levels in AM-depleted and nondepleted mice challenged with combined JE/MCP-1 plus *Escherichia coli* lipopolysaccharide (LPS). Mice were pretreated with liposomal clodronate (open bars) or empty liposomes (solid bars). After 24 h, mice were either left untreated to control for effects of liposomes per se (0 h) or were challenged for 6, 12, or 24 h with combined JE/MCP-1 (50 μg/mouse) plus *E. coli* LPS (10 ng/mouse), followed by BAL. BAL values of tumor necrosis factor (TNF)-α and macrophage inflammatory protein (MIP)-2 are given as means ± SE (*n = 9/time point). *P < 0.05 vs. nonmacrophage-depleted mice.
pears that rAM largely contribute to the overall inflammatory response to LPS that underlies the monocyte recruitment-enhancing function of the resident phagocytes under these conditions. The rAM-dependent pathways orchestrating the local cytokine response and the strong promotion of early neutrophil and delayed monocyte influx in response to alveolar JE/MCP-1/LPS deposition do, however, still need to be defined in detail. In this context, several previous observations are of interest. Previous studies in vitro showed that alveolar macrophage-derived secretory products like TNF-α can activate alveolar epithelial cells to release chemokines such as interleukin-8 and MCP-1, which may then promote both alveolar neutrophil and monocyte recruitment (18). Recent studies from our laboratory showed that stimulation of primary isolates of human alveolar epithelial cells with TNF-α provoked a directed apical MCP-1 secretion together with a directed migratory response of monocytes across the alveolar epithelial layer (17). These data support the concept that rAM-dependent alveolar cytokine release may employ local epithelial cells to promote directed alveolar leukocyte traffic under conditions of inflammation. This may hold true for both neutrophil and monocyte recruitment being considered as independent events. However, interdependencies

Fig. 5. Effect of depletion of AM on the alveolar recruitment of neutrophils (A) and monocytes (B) in mice challenged with JE/MCP-1 plus E. coli LPS. AM-depleted (open bars) or nondepleted mice (solid bars) were challenged for 6, 12, 24, 48, or 72 h with combined JE/MCP-1 plus E. coli LPS. After indicated time points, mice were killed and subjected to BAL, followed by quantitation of leukocytes, as outlined in MATERIALS AND METHODS. Values are given as means ± SE (n = 9/time point). *P < 0.05 vs. nonmacrophage-depleted mice. PMN, polymorphonuclear neutrophils.

Fig. 6. Effect of depletion of AM on induction of lung barrier failure in mice challenged with combined JE/MCP-1/LPS. Nonpretreated mice received intratracheal (it) instillations of combined JE/MCP-1/LPS and were analyzed after 6, 12, 24, or 48 h as indicated (A, n = 5/time point). AM depletion was performed with liposomal clodronate (100 μl/mouse, 24 h), again followed by intratracheal instillation of combined JE/MCP-1/LPS for 6, 12, 24, or 48 h (B–D, n = 9/time point). Note that clodronate-mediated depletion of rAM per se did not provoke lung barrier dysfunction (0-h time point in B). In selected experiments, mice were additionally depleted of circulating neutrophils by intraperitoneal injection of anti-Gr-1 monoclonal antibody (C) or were depleted of AM and received intravenous injections of anti-CD18 monoclonal antibody (100 μg/mouse in PBS/0.1% mouse serum) before combined JE/MCP-1/LPS challenge (D). All mice received intravenous injections of FITC-conjugated human serum albumin 1 h before death for measurement of lung permeability. Values are given as means ± SE. *P < 0.05 vs. the respective 0-h time points. +P < 0.05 compared with the respective time points given in B.
between these two types of alveolar leukocyte flux may also be relevant, and two observations may support this assertion. First, alveolar-recruited and -activated neutrophils are well known to be capable of releasing MCP-1 within the alveolar air space (22). Second, transient neutropenia, as well as selective inhibition of neutrophil chemotaxis in BALB/c mice, was most recently shown to drastically attenuate the alveolar monocyte influx in response to JE/MCP-1 plus E. coli LPS, implying a potential role of corecruited neutrophils in the alveolar monocyte traffic (U. Maus, K. v. Grote, W. A. Kuziel, M. Mack, E. J. Miller, J. Cihak, M. Stangassinger, R. Maus, D. Schlöndorff, W. Seeger, and J. Lohmeyer; unpublished observations).

An unexpected finding of the present study was the observation that, despite heavily reduced alveolar cytokine release, as well as strongly suppressed leukocyte recruitment patterns, rAM-depleted mice still exhibited a marked loss of lung barrier function at 6 and 12 h post-JE/MCP-1/LPS challenge. Importantly, alveolar macrophage-depleted control mice did not exhibit such an increase in lung permeability, thus excluding a direct effect of liposomal clodronate on lung barrier integrity. However, when macrophage-depleted mice were additionally made neutropenic before intrabronchial instillation of combined JE/MCP-1/LPS, lung barrier failure was largely prevented, which was also true when mice were systemically treated with function-blocking anti-CD18 MAb, which were most recently shown to be relevant for the alveolar recruitment of both neutrophils and monocytes (13). These data clearly demonstrate that 1) induction of lung barrier failure is not necessarily dependent on rAM; 2) a β2-integrin-dependent step is obviously required for the loss of barrier integrity, probably involving leukocyte-endothelial interactions. This finding corresponds well to recently published observations from Gao and coworkers (5), who demonstrated that pulmonary microvascular permeability induced by E. coli in mice was solely the result of neutrophil engagement of CD18 integrins; and 3) most probably the circulating neutrophil is a major culprit in provoking increased lung vascular permeability in response to alveolar JE/MCP-1/LPS challenge, even when its migratory response to the alveolar compartment is largely suppressed due to rAM depletion. In this context, it is important to note that Gautam et al. (6) have most recently proposed neutrophil-derived heparin-binding protein (CAP37, azurocidin) as a missing link in neutrophil-evoked alteration of vascular permeability, which notably exerts its effects on endothelial cell permeability on engagement of neutrophil β2-integrins. Certainly, whether this mode of action is also operative in the described model of acute lung inflammation still deserves further investigation.

The IT route used here as an experimental approach to deliver clodronate into the lungs of mice yielded an ~85% depletion of alveolar macrophages and was less effective than the inhalative route reported earlier in other studies with depletion rates amounting to 95% (8, 9). Although we cannot fully exclude the possibility that residual alveolar macrophages still might have been participating in the inflammatory response to combined JE/MCP-1 plus LPS, the lack of any attenuation of lung barrier dysfunction after depletion of ~85% alveolar macrophages argues strongly against a major contribution of residual alveolar macrophages to the lung barrier failure observed in the present study.

In conclusion, rAM are not involved in JE/MCP-1-driven alveolar monocyte recruitment in noninflamed lungs. They are, however, centrally enrolled in the alveolar cytokine response and the enhanced early neutrophil and delayed monocyte traffic into the alveolar compartment occurring under inflammatory conditions due to alveolar JE/MCP-1/LPS deposition. Interestingly, the loss of lung barrier function observed under these conditions is rAM independent but involves circulating neutrophils in a CD18-dependent fashion.

REFERENCES


