Immunopathology and Infectious Diseases

CD11c⁺ Cells Are Required to Prevent Progression from Local Acute Lung Injury to Multiple Organ Failure and Death

Jami E. Milam,^{*,†} John R. Erb-Downward,[†]
Gwo-Hsiao Chen,^{*,†} Marcin F. Osuchowski,[‡]
Roderick McDonald,[†] Stephen W. Chensue,^{*,§}
Galen B. Toews,^{*,†} Gary B. Huffnagle,[¶]
and Michal A. Olszewski^{*,†}

From the VA Ann Arbor Healthcare System,^{*} Ann Arbor, Michigan; the Division of Pulmonary and Critical Care Medicine,[†] Department of Internal Medicine, the Departments of Pathology and Laboratory Medicine,[§] and Microbiology and Immunology,[¶] University of Michigan Medical School, Ann Arbor, Michigan; and the Ludwig Boltzmann Institute for Experimental and Clinical Traumatology,[‡] Vienna, Austria

To investigate the role of CD11c⁺ cells in endotoxin-induced acute lung injury, wild-type or CD11c-diphtheria toxin receptor transgenic mice were treated with intraperitoneal diphtheria toxin (5 ng/g b.wt.) in the presence or absence of intratracheal lipopolysaccharide (51 µg). Lipopolysaccharide treatment resulted in 100% mortality in CD11c-depleted animals but not in control animals. Analysis of local lung tissue revealed no differences in acute lung injury severity; however, analysis of distal tissues revealed severe damage and necrosis to multiple organs (liver, spleen, and kidneys) in CD11c-diphtheria toxin receptor mice but not in wild-type mice. In addition, dramatic increases in systemic levels of liver enzymes (alanine aminotransferase, 657 U/L, aspartate aminotransferase, 1401 U/L), blood urea (53 mg/dl), and 8-iso-prostaglandin F_{2α}, a marker of oxidative stress (350 pg/ml), were observed. These data demonstrate that CD11c⁺ cells play a critical role in protecting the organs from systemic injury caused by a pulmonary endotoxin challenge. (*Am J Pathol* 2010, 176:218–226; DOI: 10.2353/ajpath.2010.081027)

Acute lung injury (ALI) and acute respiratory distress syndrome are conditions that are characterized by diffuse alveolar injury, subsequent impairment of arterial oxygenation associated with protein leakage into the al-

veolar space, and high mortality rates.^{1–3} Interestingly, despite the severe limitation in gas exchange, the mortality associated with this syndrome is not due to hypoxia but rather to a progressive failure in multiple organ systems (frequently referred to as multiple organ dysfunction syndrome [MODS]).²

The majority of injuries precipitating ALI are septic in nature, either due to systemic septicemia or a local pneumonia that leads to the development of a systemic inflammatory condition.^{4,5} Many proinflammatory mediators have been implicated in septic inflammation and MODS including nitric oxide, cytokines, complement factor C5a, and eicosanoids.^{2,5} However, to date, trials targeting many of these mediators have been unsuccessful,⁶ generating interest in interventions directed to the cellular rather than humoral components of the inflammatory response in these syndromes.

Alveolar macrophages and dendritic cells (DCs) are CD11c⁺ antigen-presenting cells, and the main pathogen-sensing cells of the innate immune system in the lungs.^{7–10} These cells rapidly detect and respond to microbial products that are deposited in the lungs.¹⁰ Because of this ability to respond rapidly, these cells are of particular interest. Their responses that can lead to the clearance of the harmful stimuli also can lead to the generation of inflammatory mediators that can promote more profound lung injury.^{5,7,8,11,12}

With the use of CD11c-diphtheria toxin receptor (DTR) mice, transgenic mice that express the diphtheria toxin receptor on CD11c⁺ cells, in the current work we assessed the role of CD11c⁺ cells in controlling a nonlethal endotoxin-induced model of ALI. The CD11c-DTR mice are a robust, well established model that we have previ-

Supported in part by grants from the National Heart, Lung, and Blood Institute (R01-HL51082 to G.B.T. and T32-HL07749 to J.E.M.); The National Institute of Allergy and Infectious Diseases (R01-AI059201 to G.B.H.); and the Department of Veterans Affairs (merit grants to M.A.O. and G.B.T.).

Accepted for publication September 21, 2009.

Address reprint requests to Michal Olszewski, D.V.M., Ph.D., VA Ann Arbor Healthcare System, Research Service (11R), 2215 Fuller Rd., Ann Arbor, MI 48105. E-mail: olszewsm@umich.edu.

ously used to study the role of DCs in *Cryptococcus neoformans* infections.^{7,11,13–16} The results presented here demonstrate that the presence of CD11c⁺ cells is critical in preventing oxidative stress and the progression of mild ALI to a MODS-like syndrome, which rapidly results in death.

Materials and Methods

Mice

All animal work for these studies was approved by the University of Michigan Committee on the Use and Care of Animals. The wild-type, BALB/c, and C57BL/6 mice and their transgenic counterparts, transgenic CD11c-DTR BALB/c and transgenic CD11c-DTR C57BL/6 mice, were housed in sterilized, filter-top microisolator cages. The mice were maintained by the Unit for Laboratory Animal Medicine at the University of Michigan (Ann Arbor, MI), with food and water provided *ad libitum*. The generation and screening of CD11c-DTR mice have been reported previously.¹⁷ The offspring mice were generated using standard mouse-cloning procedures and screened for the presence of transgene via Southern blotting. The CD11c-DTR mice were crossed five generations onto the C57BL/6 or the BALB/c backgrounds and subsequently bred in homozygous transgenic lines.¹⁷

Depletion of CD11c⁺ Cells

Mice were injected i.p. with 5 ng/g b.wt. of diphtheria toxin (DT) (D-0564; Sigma-Aldrich, St. Louis, MO) in 0.25 ml of PBS or sterile PBS alone at day –1, as reported previously.^{17,18} The effectiveness of the depletion was assessed by flow cytometry. To assure that the endotoxin levels in DT preparations were negligible, DT aliquots were tested with the E-TOXATE (*Limulus* amebocyte lysate) kit (ET0200; Sigma-Aldrich), according to the manufacturer's protocols (data not shown).

Model of Pulmonary LPS Challenge

To induce an acute lung injury, mice were challenged with 51 μ g of lipopolysaccharides (LPS) (L1519, Sigma-Aldrich) from *Klebsiella* via the intratracheal route, as shown previously.^{3,19} In brief, mice were anesthetized by i.p. injection of ketamine (100 mg/kg, Fort Dodge Laboratories, Fort Dodge, IA) and xylazine (6.8 mg/kg, Lloyd Laboratories, Shenandoah, IA) and restrained on a small surgical board. A small incision was made through the skin over the trachea, and the underlying tissue was separated. The 51 μ g of LPS was dispensed into the trachea in 30 μ l of sterile water via a 30-gauge needle attached to a 1-ml tuberculin syringe. The skin was closed with cyanoacrylate adhesive. The mice recovered from the procedure with minimal visible trauma.

Lung Leukocyte Isolation

The lung leukocyte isolation was performed 1 day after LPS challenge; this time point is consistent with the peak leukocyte recruitment after the low-dose LPS challenge. Individual lungs were excised, minced, and enzymatically digested for 45 minutes in 15 ml of digestion buffer (RPMI 1640, 5% fetal calf serum, antibiotics, 1 mg/ml collagenase, and 30 μ g/ml DNase). The tissues were further dispersed by drawing the suspension up and down 20 times through the bore of a 10-ml syringe. Collagenase solution was removed by centrifugation, and the erythrocytes in the cell pellet were lysed in NH₄Cl buffer (0.83% NH₄Cl, 0.1% KHCO₃, and 0.037% Na₂EDTA, pH 7.4) on ice. Leukocytes were washed with RPMI 1640, filtered through Nitex filters resuspended in complete media, and centrifuged for 30 minutes at 3000 $\times$ g in the presence of 20% Percoll (Sigma-Aldrich) to separate leukocytes from cell debris and epithelial cells. The isolated leukocytes were re-pelleted and resuspended in complete medium. Total live lung leukocyte numbers were enumerated in the presence of trypan blue using a hemocytometer.

Bronchoalveolar Lavage

After euthanasia, tracheas were exposed and cannulated with polyethylene tubing (PE50). Catheters were secured with surgical silk and mounted on a 23-gauge needle/tuberculin syringe. The lungs were lavaged twice with 0.75 ml of ice-cold PBS. The fluid, recovered by aspiration with gentle chest massage (~0.5 ml total), was spun at 1000 rpm for 10 minutes, and the supernatant was transferred to a new tube and stored at –20°C for further analysis. Protein levels in the bronchoalveolar lavage fluid were measured using the Bradford assay (Bio-Rad Protein Assay, Bio-Rad Laboratories, Hercules, CA).

Analysis of Lung Leukocyte Subsets

Isolated lung cells (10⁵) in complete medium were cyto-spun onto glass slides (Shandon Cytospin, Pittsburgh, PA). The slides were fixed for 2 minutes during a one-step, methanol-based Wright-Giemsa stain (Harleco, EM Diagnostics, Gibbstown, NJ), followed by steps 2 and 3 of the Diff-Quik whole-blood stain kit (Diff-Quik, Baxter Scientific, Miami, FL). Neutrophils, mononuclear cells, and eosinophils were visually counted under a microscope from randomly chosen high-power fields, for a total of 200 cells. The percentage of a specific leukocyte subset was multiplied by the total number of leukocytes to calculate the absolute number of the subset in the sample.

RT-PCR Analysis

Total RNA from whole lung samples harvested on day 3 after LPS challenge was isolated using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's directions. The purified RNA was subse-

quently reverse-transcribed, and DNA was amplified using a SuperScript One-Step RT-PCR kit (Invitrogen). The following murine oligonucleotide primers were used for RT-PCR analysis: tumor necrosis factor (TNF)- α , 5'-CCTGTAGCCCACGTCGTAGC-3' (sense) and 5'-AGCAATGACTCCAAAGTAGACC-3' (antisense); interleukin (IL)-6, 5'-GACAAAGCCAGAGTCCTTCAGAGAG-3' (sense) and 5'-CTAGGTTTGCCGAGTAGATCTC-3' (antisense); interferon (IFN)- γ , 5'-TCTACCTCAGACTCTTTGAAGTCT-3' (sense) and 5'-CAGCGACTCCTTTTCCGCTT-3' (antisense); IL-10, 5'-CTATGCTGCCTGCTCTTACTG-3' (sense) and 5'-GATGGCCTTGTAGACACCTTGG-3' (antisense); granulocyte-macrophage colony-stimulating factor (GM-CSF), 5'-GTGGCTGCAGAATTTACTTTTCCTGGGCATTGTG-3' (sense) and 5'-TTGCATTCAAAGGGGATATCAGTCAGAAAG-3' (antisense); IL-23p19, 5'-GTTCCAAGATCCTTCGAAGCCTC-3' (sense) and 5'-GCCTGGGCTCACTTTTCTGATG-3' (antisense); MIP-2, 5'-GCTGGCCACCAACCACCAGG-3' (sense) and 5'-AGCGAGGCACATCAGGTACG-3' (antisense); KC, 5'-TGAGCTGCGCTGTCACTGCCT-3' (sense) and 5'-AGAAGCCAGCGTTCACCAGA-3' (antisense); and β -actin, 5'-TGGAATCCTGTGGCATCCATGAAAC-3' (sense) and 5'-TAAACGCAGCTCAGTAACAGTCCG-3' (antisense).

The reverse transcription was conducted at 45°C for 45 minutes and the RT enzyme was denatured at 94°C for 2 minutes. The PCR steps were cycled 25 or 30 times (denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and elongation at 68°C for 1 minute). The final elongation step was performed at 72°C for 7 minutes. The β -actin (housekeeping gene) was used as the mRNA loading control. Samples were separated by electrophoresis in a 2% agarose gel. Bands were visualized by ethidium bromide/UV luminescence and photographed.

Flow Cytometry Analysis

To establish the rate of CD11c⁺ cell depletion, a lung cell suspension (5×10^5) was Fc-blocked with anti-murine CD16/CD32, rat IgG2b and labeled with either a labeled isotype control or with the following anti-mouse monoclonal antibodies/fluorescent dye conjugates: CD11c-fluorescein isothiocyanate (553801, BD Biosciences, San Jose, CA); and Gr-1-PE (553128, BD Biosciences). Cells were incubated for 30 minutes on ice, centrifuged, and washed with staining buffer, fixed in 2.5% paraformaldehyde in buffered saline, and analyzed by flow cytometry.

The FL1 (CD11c) versus FL2 (Gr-1) scatter plots were analyzed, following initial selection of intact leukocytes using forward versus side scatter plots. This previously published gating strategy for defining CD11c⁺ DCs and CD11c⁺ alveolar macrophages in lung leukocyte isolates was used to quantify the percentage of CD11c⁺ cells and to confirm their depletion.²⁰ Isotype control antibodies were used to subtract the background.

8-iso-Prostaglandin $F_{2\alpha}$ Assay

On day 2, post-LPS challenge mice were euthanized, and blood was collected via orbital bleeding and instantly

centrifuged to obtain plasma. Plasma was diluted 1:1 with 15% KOH for 1 hour at 40°C. The free lipids were extracted from the samples twice using the 1:3 dilution of ethyl acetate and dried down under grade 5 nitrogen. Samples were then resuspended in electroimmunoassay buffer, and the 8-iso-prostaglandin (PG) $F_{2\alpha}$ levels were measured by an 8-iso-PG $F_{2\alpha}$ -specific electroimmunoassay (Cayman Chemical, Ann Arbor, MI) according to the manufacturer's protocols.

Organ Function Parameters

On day 2, post-LPS challenge mice were euthanized, and blood was collected via orbital bleeding into sero-separator mini tubes and allowed to clot at room temperature. Serum was separated via centrifugation and assayed using diagnostic tests. Activities of alanine aminotransferase and aspartate aminotransferase and levels of blood urea nitrogen and creatine were assayed and evaluated. All diagnostic tests were performed in the University of Michigan's Unit for Laboratory Animal Medicine Animal Diagnostic Laboratory.

Pathology and Histological Examination

Dead animals underwent autopsy to evaluate gross pathology. To evaluate the lesions, organs sampled for histological examination were fixed by submersion in 10% neutral buffered formalin and subsequently dehydrated/processed for paraffin embedding. The 5- μ m sections were cut from paraffin blocks, de-paraffinized, stained with H&E and examined by light microscopy. For comparative histopathological analysis, mice from parallel groups were euthanized at 3 days postinfection, lungs were inflated with formalin and processed for histology as described above. Other organs and tissues for comparative histopathological analysis were processed as described above.

Statistical Analysis

Although all experiments were performed in both C57BL/6 and BALB/c strains, the results presented were calculated on the basis of data obtained from the C57BL/6 mice, unless noted otherwise. Survival data were analyzed by the Kaplan-Maier (% of surviving animals) method, and comparison of multiple survival curves was performed by log-rank analysis. All remaining quantitative data are reported as means $\pm$ SE. Statistical significance was determined using one-way analysis of variance and the unpaired two-tailed Student's *t*-test corrected for multiple comparisons, when appropriate; $P < 0.05$ was considered statistically significant. All analyses were performed using the Prism 3.0 program for Windows (GraphPad Prism, San Diego, CA).

Figure 1. Effect of DT on CD11c depletion in the lungs of wild-type (WT) and CD11c-DTR mice. This representative graph is a gray-scale pseudo-color plot of lung leukocytes from wild-type and C57BL/6 CD11c-DTR mice harvested 24 hours after DT treatment and then stained with control antibodies (**left panel**), or antibodies against CD11c and Gr-1 (**middle and right panels**).

Results

Depletion of CD11c⁺ Cells

To test the efficacy of our experimental model, the extent of CD11c⁺ cell depletion in the CD11c-DTR mice was evaluated after DT treatment. Lung leukocytes were isolated and analyzed by flow cytometry (Figure 1). The myeloid DC and lung alveolar macrophages were selected based on previously identified gate positions in Gr-1 versus CD11c⁺ scatter plots.²⁰ Before DT treatment both wild-type and CD11c-DTR mice had similar numbers of CD11c⁺ cells in the lungs (2.0 ± 0.5 and $2.3 \pm 0.2 \times 10^6/\text{lung}$, respectively). After the DT treatment, the lung population of CD11c⁺ cells in CD11c-DTR mice decreased by 94% (down to $0.08 \pm 0.06 \times 10^6/\text{lung}$) but remained unaltered ($2.1 \pm 0.2 \times 10^6/\text{lung}$) in the wild-type mice. Our results showed that the DT-induced depletion of CD11c⁺ cells in CD11c-DTR mice is highly effective, producing a relevant experimental model.

Effect of CD11c⁺ Cell Depletion on Mouse Survival in LPS-Induced ALI Model

Wild-type and CD11c-DTR mice, which had received DT or PBS on day -1, were given an intratracheal inoculation of LPS or sterile PBS on day 0 and monitored. CD11c-DTR mice that had been given both DT and LPS very rapidly began to display symptoms of illness (hunched posture and lethargy), and by day 2 50% of the mice had died. None of these animals survived beyond day 3 (Figure 2). In contrast, 100% survival of wild-type mice was observed for all of the treatment groups past the end of the 10-day observation period. In the CD11c-DTR groups not given DT and LPS, 100% of the CD11c-DTR mice that received LPS alone and 80% of the CD11c-DTR mice that received DT alone survived. The 20% mortality seen in the CD11c-DTR mice treated with DT alone was unexpected but reproducible. The DT was tested for LPS contamination using the E-TOXATE (*Limulus* amebocyte lysate) kit, but levels were found to be below the limit of detection of the assay. Previous studies have shown that the portal circulation carries a significant load of bacterial products derived from the gut microbiota into the liver.²¹ It is likely that the hypersensitivity to LPS that these CD11c-DTR exhibit after DT treatment extends to microbiota-derived bacterial products as well. To exclude the possibility of a strain-specific phenomenon, a similar ex-

periment was performed using CD11c-DTR mice generated on the BALB/c background. Consistently, combined administration of DT + LPS in BALB/c CD11c-DTR mice produced 100% lethality with a slightly accelerated time frame: 100% mortality of BALB/c CD11c-DTR by day 2 versus 50% in C57BL/6 (data not shown). These results show that mice depleted of CD11c cells become extremely vulnerable to LPS challenge and that this effect was independent of their genetic backgrounds.

Characterization of ALI in DT-Treated Wild-Type and CD11c-DTR Mice

To determine whether the 100% mortality of CD11c⁺-depleted ALI mice was associated with an exaggerated inflammatory response in the lungs, we quantified two major hallmarks of inflammatory response. First, pulmonary leukocyte populations were analyzed in the wild-type and CD11c-DTR mice at the peak of the inflammatory response after LPS (day 1). Total and differential leukocyte counts were performed in enzymatically dispersed lungs of mice treated with or without LPS and with or without DT. In all LPS-treated mice the total numbers of eosinophils, neutrophils, and mononuclear cells were higher than those in the saline group. However, there was no significant difference in the composition of the cellular influx of wild-type and CD11c-DTR mice (Figure 3, A–D). This result indicates that the depletion of CD11c⁺ cells

Figure 2. Effect of DT and LPS on survival in C57BL/6 wild-type (WT) versus CD11c-DTR Tg mice of the same background. The mice were treated i.p. with 5 ng/g b.wt. DT on day -1 and/or intratracheally inoculated with (51 μg) of LPS on day 0. The number in parentheses indicates the number of animals/treatment group.

Figure 3. Comparison of lung leukocyte recruitment in response to LPS challenge in wild-type (WT) C57BL/6 and Tg CD11c-DTR mice. Samples of individual lung leukocyte suspensions were spun onto slides and stained with Wright-Giemsa stain for visual quantification of leukocyte subsets as described in *Materials and Methods*. **A:** Total cells. **B:** Neutrophils. **C:** Eosinophils. **D:** Mononuclear cells. * $P < 0.05$ compared with the untreated mice. $n = 3$ to 11 mice/group.

did not alter either the magnitude or character of the inflammatory response in the lungs exposed to the LPS challenge.

Second, to determine whether the CD11c⁺ cell depletion affected the LPS-induced protein leak into the alveolar space, total protein concentration in the bronchoalveolar lavage fluid was measured in the wild-type and CD11c-DTR mice. No significant difference in protein concentration was observed in either wild-type or CD11c-DTR mice treated with DT only compared with their saline-treated controls (data not shown). Significant increases in the concentration of protein in the bronchoalveolar lavage fluid of LPS-treated mice were observed; however, the magnitude of the protein increase was similar in both wild-type and CD11c-DTR, whether or

Figure 4. The comparison of severity in alveolar-capillary leak as reflected by the protein concentration of bronchoalveolar lavage fluid in wild-type (WT) C57BL/6 and Tg CD11c-DTR mice with or without DT and LPS treatment compared with LPS alone. * $P < 0.05$ compared with the untreated mice of the same group. $n = 3$ to 12 mice/group; the specific number of animals per group is described in *Materials and Methods*.

not DT had been administered (Figure 4). The histological analysis of lungs at day 3 post-LPS displayed characteristics of acute lung injury, ranging from the moderate (multifocal acute neutrophilic bronchopneumonia) to the severe (widespread acute neutrophilic bronchopneumonia [data not shown]). Again, whether the mice had been treated with DT made no difference in the histological findings. These results show that the CD11c depletion did not result in the enhanced alveolar-capillary protein leak, differences in leukocyte subsets at the peak of inflammation, or increased histological evidence of lung injury. Together, these data demonstrate that despite a marked difference in mortality between CD11c-depleted mice and nondepleted mice, there was no difference in the severity of lung injury or degree of local response.

Characterization of Soluble Mediators After ALI in Wild-Type and CD11c-DTR Mice

Induction of cytokines and chemokines during ALI can either contribute to tissue injury or play a protective role in the lungs.⁵ In addition, as soluble mediators, cytokines and chemokines have systemic effects and contribute to the systemic response resulting from local injury. Our next objective was to determine whether CD11c⁺ cell depletion affected pulmonary cytokine responses after the LPS challenge. Cytokines exhibiting either injurious (IFN- γ , TNF- α , IL-6, IL-23p19, MIP-2, and KC) or protective (GM-CSF and IL-10) properties in ALI were analyzed (Figure 5). The LPS inhalation alone caused a similar increase of mRNA expression for all cytokines analyzed, regardless of the strain (data not shown). However, the depletion of CD11c⁺ cells did not have a uniform effect on LPS-induced cytokine expression in the lungs of transgenic (Tg) mice. Whereas the expression of proinflammatory IFN- γ and TNF- α cytokines was reduced in comparison with that in the wild-type mice, the expression of proinflammatory KC and MIP-2 chemokines was notably up-regulated. A similar level of increase was apparent for anti-inflammatory cytokine (IL-10 and IL-23p19), but no evident change was observed in the expression of GM-CSF and IL-6 between wild-type and Tg mice administered DT + LPS. Thus, CD11c⁺ cell depletion caused some changes in pulmonary cytokine responses; how-

Figure 5. RT-PCR analysis of interferon- γ (IFN- γ), tumor necrosis factor- α (TNF α), IL-10, IL-17, IL-23p19, granulocyte-macrophage colony-stimulating factor (GM-CSF), MIP-2, KC, and β -actin in the whole lung homogenates from wild-type (WT) C57BL/6 and Tg CD11c-DTR mice of the same background. Each lane is a representative of at least three animals per group.

ever, these changes were not consistent with a massive inflammatory response and/or increased lung injury in CD11c⁺-depleted mice challenged with LPS.

Widespread cell injury associated with membrane lipid peroxidation and oxidative stress is implicated in the pathogenesis of ALI and the progression to MODS. To evaluate the magnitude of systemic oxidative stress, a marker of lipid peroxidation, 8-iso-PGF_{2 α} was measured in plasma 3 days after LPS challenge. After the DT + LPS treatment, the circulating level of 8-iso-PGF_{2 α} in the CD11c-DTR mice increased to 340 pg/ml and was three-fold greater than the 110 pg/ml measured in the wild-type mice (Figure 6). This finding indicates that the depletion of CD11c⁺ cells in ALI mice resulted in a dramatic increase of systemic oxidative stress and injury.

Figure 6. Markers of oxidative stress/injury in wild-type (WT) and BALB/c CD11c-DTR mice. The total 8-isoprostane-prostaglandin F_{2 α} (8-iso-PGF_{2 α}) level, a marker of oxidative stress and injury, was measured on day 2 after LPS administration in the fresh plasma of mice treated with DT + LPS. * P < 0.05. n = 5 mice.

Figure 7. Photomicrographs of liver sections showing necrosis in LPS- and DT + LPS-treated Tg (CD11c-DTR) mice that were harvested on day 1. Wild-type BALB/c mice (A, C, E, and G), labeled (WT), and the Tg (CD11c-DTR) mice of the same background (B, D, F, and H), labeled Tg (CD11c-DTR): untreated (A and B), labeled (UN); or treated i.p. with 5 ng/g b.wt. with DT (C and D) day -1, labeled (DT); or intratracheally inoculated with (51 μ g) of LPS (E and F) day 0, labeled (LPS); or treated with both DT and LPS (G and H) and harvested on day 1, labeled (DT + LPS). The liver sections were stained with H&E and examined under a microscope ($\times 40$). Livers from the Tg (CD11c-DTR) mice treated with DT (D) show liver necrosis including fatty/vacuolar dystrophy and red blood cell infiltration. Livers from the Tg (CD11c-DTR) mice treated with DT + LPS (H) show extensive liver necrosis including fatty/vacuolar dystrophy, red blood cell infiltration, and hepatocyte disintegration.

Characterization of Systemic MODS-Like Pathology in LPS-Treated CD11c-Deficient Mice

We evaluated the effect of CD11c⁺ cell depletion on liver pathology in mice with ALI. No macro- or micropathological changes were noted in livers of wild-type animals, regardless of the treatment (Figure 7, A, C, E, and G). In contrast, extensive liver pathology was evident in CD11c-DTR mice treated with DT + LPS (Figure 7F). Macroscopic-

Figure 8. Photomicrographs of kidney sections showing necrosis in DT- and DT + LPS-treated Tg (CD11c-DTR) mice that were harvested on day 1. The wild-type BALB/c mice (**A**, **D**, **G**, and **J**), labeled (WT), and the Tg (CD11c-DTR) mice of the same background (**B**, **E**, **H**, and **K**), labeled Tg (CD11c-DTR), and also a enlarged multicellular tubule unit (**C**, **F**, **I**, and **L**): untreated (**A**, **B**, and **C**), labeled (UN); or treated i.p. with 5 ng/g b.wt. with DT (**D**, **E**, and **F**) day -1, labeled (DT); or intratracheally inoculated with (51 μ g) of LPS (**G**, **H**, and **I**) on day 0, labeled (LPS); or treated with both DT and LPS (**J**, **K**, and **L**) and harvested on day 1, labeled (DT + LPS). The kidney sections were stained with H&E and examined under a microscope ($\times 40$). The **arrows with dots on the ends** show where the enlarged groups of cells were located. Kidney from the Tg (CD11c-DTR) mice treated with DT (**E** and **F**) shows necrosis (NE) of the tubules with some normal (N) cells. Kidney from the Tg (CD11c-DTR) mice treated with DT + LPS (**K** and **L**) shows extensive necrosis (NE) of the tubules and renal cell disintegration. Tg (CD11c-DTR) figure sets with DT (**E** and **F**) and DT + LPS (**K** and **L**) have a brown color compared with the normal pink/purple. Scale bar = 40 μ m.

cally all livers from the CD11c-DTR DT + LPS mice were very pale and bruised (not shown), consistent with extensive necrosis. The microscopic examination revealed severe damage of hepatic tissue, featuring centrilobular vacuolar fatty change, congestion, and extensive necrosis of hepatocytes and epithelium (Figure 7, B, D, F, and H). Additional micropathology included widespread hepatocellular microlipidosis with periportal lymph node nodules containing scattered necrotic cells and granulocyte infiltrates.

The effect of CD11c⁺ cell depletion on kidney pathology was also evaluated. No macro- or micropathological changes were noted in kidneys of wild-type animals, regardless of the treatment (Figure 8, A, D, G, J, C, F, I, and L). In contrast, microscopic examination of the tubular compartment in CD11c-DTR mice treated with DT + LPS revealed the presence of numerous necrotic epithelial cells and tubular dilatation (Figure 8, B, E, H, and K), consistent with acute tubular necrosis.

This extensive organ damage and necrotic changes observed in the CD11c-DTR DT + LPS group corresponded with the dramatic elevation of circulating liver enzymes (Figure 9, A and B) and significant increases in blood urea nitrogen (Figure 9C). The same treatment (DT + LPS) in the wild-type mice and LPS alone in either strain did not cause elevation in any of these markers.

These data indicate that a mild lung injury in the absence of CD11c⁺ cells causes mice to rapidly progress to a MODS-like condition consisting of massive liver damage and necrosis and kidney failure and ultimately leads to the death of the animal.

Discussion

Although many advances have been made in the understanding of ALI, the mechanisms that prevent the escalation

Figure 9. Activity of circulating alanine aminotransferase (ALT) (A) and aspartate aminotransferase (AST) (B) and blood urea nitrogen (BUN) (C) in LPS- and DT + LPS-treated BALB/c CD11c-DTR and wild-type (WT) mice. Blood was collected via orbital bleeding on day 2 after LPS challenge. Data are means $\pm$ SE. * $P < 0.05$.

of the injury and subsequent progression to MODS are not understood.¹ In the work presented we have used a model of self-resolving ALI, induced by local pulmonary LPS challenge, and CD11c-DTR transgenic mice to study the involvement of CD11c⁺ cells in this process. This study has shown for the first time that the presence of CD11c⁺ cells during local ALI is required to prevent its progression to MODS. Depletion of CD11c⁺ cells rendered mice dramatically more susceptible to progression from ALI to systemic distress and death. Interestingly, the ALI itself was not more severe in mice that had been depleted of their CD11c⁺ cells, but LPS-treated CD11c⁺ depleted mice displayed symptoms consistent with a MODS-like syndrome: systemic oxidative stress as well as soluble markers of severe liver and kidney damage.²² Histological analysis of these tissues supported these findings, revealing pronounced organ damage and tissue necrosis. Together these data indicate

that CD11c⁺ cells are critical for controlling the progression of ALI to multiple organ dysfunction.

Although the role of CD11c⁺ cells in ALI has not been extensively studied, there have been studies on the role of CD11c⁺ cells in multimicrobial sepsis,^{14,23,24} a syndrome that frequently results in ALI, as well as organ failure and death.^{1,22,25} Here it has been observed that CD11c⁺ cells also play a critical role in the progression of sepsis. One such study, using a model of cecal ligation and puncture-induced sepsis and the CD11c-DTR mice, showed that depletion of CD11c⁺ cells similarly caused a significant increase in sepsis-associated mortality. Furthermore, this increase was eliminated on adoptive transfer of wild-type CD11c⁺ cells to a mouse that had been previously depleted of CD11c⁺ cells.¹⁴ However, cecal ligation and puncture has a more severe impact on the systemic homeostasis, as it involves surgical manipulation to cause necrotizing lesions in a portion of intestine and septic insult that spans over extended periods of time. In contrast, the inhaled LPS model used in this study is viewed as a mild, temporary, and self-limited lung injury that is predominantly contained within the lungs. Our data demonstrate that even such limited injury is tightly controlled and that CD11c⁺ cells play the major role in its pulmonary containment.

The mechanism by which the CD11c⁺ cells are preventing organ damage remains an open question. We considered whether tissue-specific CD11c⁺ DCs function to filter out dangerous bacterial products (eg, LPS), and thus systemic depletion of CD11c⁺ cells renders the organs vulnerable to damage. This mechanism could explain how CD11c⁺ cells protected mice from cecal ligation and puncture-induced sepsis; however, in the current study, neither the degree of injury (Figure 9) nor the amount of inflammation (Figures 5 and 9) is different in the lungs (both control and CD11c⁺-depleted) despite being the site of LPS treatment. One would expect that if the CD11c⁺ cells were directly protecting the tissue from damage, then greater damage would be visible at the site of LPS challenge in their absence. Alternatively, the mechanism by which the CD11c⁺ cells could be protecting the organs would be the production of a factor(s) necessary to facilitate proper immune functioning and that the loss of this signal results in a feed-forward inflammatory response. One line of evidence that supports this hypothesis is that transfection with an IL-10-expressing adenovirus caused the DCs to adopt a regulatory phenotype and when transferred to a cecal ligation and puncture-challenged animal conferred greater survival and resistance to sepsis.^{26,27} Although our present study has not addressed this mechanism(s), it is clear that CD11c⁺ cells are required to prevent a self-resolving ALI from progressing to systemic organ dysfunction and ultimately death. Further studies are needed to address the mechanistic questions that cannot be answered by the current study.

Acknowledgments

We thank Drs. Steffen Jung, Leonie S. van Rij, and Bart N. Lambrecht for consultation of data analysis. We also thank Dr. John Osterholzer and Nicole Falkowski for their

assistance in parts of experimental efforts and Drs. Rashida Horton and Kathryn Eaton for their help in pathological data analysis.

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