Severe Sepsis Exacerbates Cell-Mediated Immunity in the Lung Due to an Altered Dendritic Cell Cytokine Profile

Haitao Wen,* Cory M. Hogaboam,* Jack Gauldie,[†] and Steven L. Kunkel*

From the Department of Pathology,* University of Michigan Medical School, Ann Arbor, Michigan; and the Department of Pathology and Molecular Medicine,[†] McMaster University, Hamilton, Ontario, Canada

Severe sepsis leads to long-term alterations in the immune response of surviving individuals. We have modeled this alteration in host immunity by studying the survivors of severe experimental sepsis (murine cecal ligation and puncture), which were subsequently challenged with lung granuloma-inducing *Schistosoma mansoni* eggs. This granulomatous response is a well-studied cell-mediated immune reaction characterized by elevated levels of type-2 cytokines. Pulmonary granulomas induced by *S. mansoni* eggs in cecal ligation and puncture survivors were significantly larger and contained more eosinophils than granulomas in sham-operated mice. Significantly lower interleukin (IL)-12p40 mRNA and IL-12p70 protein levels were observed in the lungs of postseptic mice with developing granulomas, compared with controls. Postseptic mice had significantly fewer dendritic cells in the lungs during the granulomatous response. Isolated lung dendritic cells from postseptic mice at days 8 and 16 after *S. mansoni* egg challenge exhibited defective IL-12 synthesis but enhanced IL-10 synthesis after Toll-like receptor agonist challenge. Pulmonary transfection with an IL-12-expressing adenovirus in postseptic mice reversed the skewing of the pulmonary cytokine profile and normalized the lung granulomatous response. Our data indicate that severe sepsis shifts the pulmonary cytokine environment, presumably via effects on pulmonary dendritic cells, which in turn alters the lung cell-mediated immune response. (*Am J Pathol* 2006, 168:1940–1950; DOI: 10.2353/ajpath.2006.051155)

people annually.^{1–3} The initiation and maintenance of sepsis and acute lung injury are triggered by an initially exaggerated innate immune response leading to a cytokine storm, which has been extensively studied.⁴ In contrast, the long-term consequence of severe sepsis on the host immune response has been poorly characterized. During severe sepsis, dysregulation of the systemic immune response may result in either rapid death due to multiple organ failure or susceptibility to secondary infections with bacteria, viruses, or fungi due to long-term immunosuppression.^{5,6} The dramatic loss of dendritic cells (DCs) in septic patients and in animals with severe sepsis, which may significantly impair B- and T-cell function and contribute to the long-term defective adaptive immunity after the acute phase of sepsis, has been reported.^{7,8} The respiratory system is most susceptible to secondary infections after severe sepsis, and pulmonary complications often lead to mortality in this group of patients.⁹

Previously, our laboratory has shown that mice that have recovered from cecal ligation and puncture (CLP) were susceptible to a pulmonary challenge of *Aspergillus fumigatus*, whereas sham-operated mice were not.^{5,10} This previous study suggested that postseptic mice had impaired innate pulmonary immunity, because *A. fumigatus* normally poses few problems to an intact pulmonary immune system.¹¹ To further investigate the influence of severe sepsis on the pulmonary adaptive immune response, we used granuloma-inducing *Schistosoma mansoni* eggs as a pulmonary challenge in survivors of severe sepsis induced by CLP surgery.

Granulomatous immune responses induced by *S. mansoni* eggs are unique inflammatory sites and offer a rewarding model to study the evolving immune response.¹² The granulomatous response in the lung is not lethal to the host and thereby allows for the long-term

Supported by grants HL31237, HL74024, HL35276, and HL31963 from the National Institutes of Health.

Accepted for publication March 3, 2006.

Address reprint requests to Prof. Steven L. Kunkel, Ph.D., Department of Pathology, University of Michigan Medical School, M5214 Med Sci I, 1301 Catherine St., Ann Arbor, MI 48109-0602. E-mail: slkunkel@umich.edu.

Sepsis is the most common cause of death in intensive care units, afflicting 750,000 people and killing 210,000

study of the immune response. During the pathological development of *S. mansoni* egg-induced granulomas, the host initiates a Th0 immune response, which subsequently switches to a type-2 immune response driven by alternatively activated macrophages, T-lymphocytes, and eosinophils.¹³ In addition, it has been shown that CD11c⁺ DCs are a notable component of *S. mansoni* granulomas and are involved in directing the pulmonary cytokine response.^{14,15}

The purpose of this study was to investigate the long-term influence of severe sepsis on the adaptive immune response reproducibly elicited by *S. mansoni* eggs. We demonstrate that mice, which survived CLP-induced severe sepsis, developed significantly larger granulomas. The greater granulomatous response was associated with a selectively impaired type-1 and enhanced type-2 cytokine profile in the lung. Study of lung DCs revealed a decrease in their recruitment. In addition, pulmonary DCs showed the defective production of interleukin (IL)-12 and the enhanced production of IL-10. After the introduction of an IL-12-producing adenovirus vector, the skewed cytokine profile in the postseptic lung was reversed, and the granulomatous response was normalized, but DC recruitment was not influenced. Thus, this study highlights the adverse consequences of severe sepsis on the adaptive immune response in the lung. *IL-12* gene transfer in the postseptic lung appeared to rebalance the adaptive immune response and diminish the remodeling pathology associated with pulmonary granulomatous responses.

Materials and Methods

Animals

Female C57BL/6 mice (6 to 8 weeks; Taconic Company, Germantown, NY) were housed under specific pathogen-free conditions at The Unit for Laboratory Animal Medicine of the University of Michigan. The animal ethical committee approved the experiments.

Survivors of CLP Subsequently Challenged by *S. mansoni* Eggs

C57BL/6 mice were subjected to sham or CLP surgery as previously described.¹⁶ Before CLP surgery, mice were anesthetized with a combination of 2.25 mg of ketamine HCL (Abbott Laboratories, Chicago, IL) and 150 μ g of xylazine (Lloyd Laboratories, Shenandoah, IA) administered intraperitoneally. Under sterile surgical conditions, a 1-cm midline incision was made to the ventral surface of the abdomen, and the cecum was exposed. The cecum was partially ligated at its base with a 3.0 silk suture and punctured nine times with 21-gauge needle. The cecum was returned to the peritoneal cavity; the abdominal incision was closed using a surgical staple (Stoelting Co., Wood Dale, IL). Mice immediately received 1 ml of saline subcutaneously for fluid resuscitation. Sham-operated mice underwent an identical operation except for cecal ligation and puncture and served as controls. Mice

in both groups were treated with the antibiotic INVANZ (Ertapenem) intraperitoneally administered at 75 mg/kg (Merck & Co., Inc., Whitehouse Station, NJ) beginning at 6 hours after surgery and re-injected every 24 hours until day 3 after surgery. The survival rate of CLP mice was consistently around 60%. At day 3 after surgery, both surviving CLP mice and sham-operated mice were intravenously injected with 5000 viable *S. mansoni* eggs via the tail vein. Live *S. mansoni* eggs were purified from the livers of *S. mansoni* cercariae-infected Swiss-Webster mice, which were kindly provided by Dr. Fred Lewis (Biomedical Research Laboratory, Rockville, MD).

Morphometric Analysis of Pulmonary Granuloma and Eosinophil Count

The left lung lobe from both sham and CLP groups at days 1, 2, 4, 6, 8, 16, and 30 after the intravenous *S. mansoni* egg challenge was fully inflated with 10% formalin, dissected, and placed in fresh formalin for an additional 24 hours. Routine histological techniques were used to paraffin-embedded lung tissue, and 5- μ m sections were stained with hematoxylin and eosin (HE). The structural alternations and eosinophil infiltration were examined around individual (ie, containing a single egg) pulmonary granulomas using light microscopy at a magnification of $\times 200$ and $\times 1000$, respectively. Morphometric analysis of egg granuloma size was performed using the Scion Image 1.63 software program (Scion Corporation, Frederick, MD). The number of eosinophils recruited to the granuloma were counted and normalized to the granuloma size. A minimum of 15 granulomas per lung section was analyzed for granuloma size, and 25 granulomas per mouse were analyzed for the eosinophil infiltrate. The same protocol was applied for the mice transfected with IL-12-expressing adenovirus.

Cytokine and Cytokines ELISA Analysis

IL-4, IL-5, IL-10, IL-12p70, and IL-13 were measured in 50- μ l samples from cell-free supernatants either from lower right lobes homogenates or from tissue culture plates using a standardized sandwich ELISA technique as previously described in detail.¹⁷ Briefly, flat-bottom 96-well microtiter plates (Nunc, Roskilde, Denmark) were coated with 50 μ l/well of antibodies specific for the murine cytokines being measured overnight at 4°C and then washed with phosphate-buffered saline (PBS) and 0.05% Tween 20. All antibodies used for ELISA were purchased from R&D Systems (Minneapolis, MN). Nonspecific binding sites were blocked with 2% bovine serum albumin (BSA) in PBS and incubated for 90 minutes at 37°C. Plates were rinsed four times with wash buffer and cell-free supernatants were loaded and incubated for 1 hour at 37°C. After four washings, a biotinylated secondary polyclonal Ab was added for 30 minutes at 37°C. The plates were washed again, and peroxidase-conjugated streptavidin (Bio-Rad, Richmond, CA) was added to the well for 30 minutes at 37°C. Plates were washed, and a chromogenic substrate (Bio-Rad) was added and incu-

bated at room temperature until fully developed. The reaction was stopped and read at 490 nm using an ELISA plate reader. Each ELISA was screened for antibody specificity. Recombinant murine cytokines were used to generate the standard curves from which the concentrations present in the samples were calculated. The cytokine levels in each sample were normalized to the protein (in milligrams) present in cell-free preparation of each sample measured by the Bradford assay, as described previously.¹⁸

Real-Time TaqMan Polymerase Chain Reaction (PCR) Analysis

Total RNA was isolated from upper right lung lobe from sham and CLP groups of five mice at days 1, 2, 4, 6, 8, 16, and 30 after an intravenous challenge with 5000 *S. mansoni* eggs. A total of 2.0 μ g of RNA was reverse transcribed to yield cDNA in a 25- μ l reaction mixture containing 1 $\times$ first strand (Life Technologies, Gaithersburg, MD), 250 ng of oligo (dT) primer, 1.6 mmol/L dNTPs (Invitrogen), 5 U of RNase inhibitor (Invitrogen), and 100 U of Moloney murine leukemia virus reverse transcriptase (Invitrogen) at 38°C for 60'; and the reaction was stopped by incubating the cDNA at 94°C for 10 minutes. cDNA (1.0 μ g) was then amplified using predeveloped cytokine/chemokine primer and probe pairs in an ABI PRISM 7700 sequence detection system (Applied Biosystems, Foster City, CA). GAPDH was analyzed as an internal control. The fold difference in mRNA expression between treatment groups was determined by software developed by Applied Biosystems.

Flow Cytometry Analysis

Whole lungs were dispersed in 0.2% collagenase (Sigma-Aldrich) in RPMI 1640 (Mediateck, Inc.) and 5% fetal bovine serum (Atlas, Fort Collins, CO) at 37°C for 45 minutes. After lysing red blood cells with ammonium chloride buffer (4.01 g of NH_4Cl , 0.42 g of NaHCO_3 , and 0.185 g of tetrasodium EDTA in 500 ml of dH_2O), Fc binding was blocked via a 10-minute incubation with purified rat anti-mouse CD16/CD32 (Fc γ III/II receptor). Then the cells were stained with either phycoerythrin (PE)-labeled anti-CD11c (HL3) and fluorescein isothiocyanate (FITC)-anti-CD11b (M1/70) or PE-anti-CD11c and FITC-anti-CD45R/B220 (RA3-6B2) in Dulbecco's PBS, 0.2% BSA, and 0.1% NaN_3 for 30 minutes at 4°C in the dark. The appropriate IgG isotypes were used as controls. All antibodies and IgG isotypes were purchased from BD PharMingen (San Diego, CA). The cells were fixed in 1% paraformaldehyde and kept in the dark at 4°C until analysis with a FACSCaliber (CELLQuest software; Becton and Dickinson, Mountain View, CA).

Pulmonary Dendritic Cell Isolation and in Vitro Stimulation

Dendritic cells were isolated from the pooled lungs from sham and CLP groups ($n = 5$ mice/group) at days 8 and

16 after the *S. mansoni* egg challenge, as described in Flow Cytometry Analysis. Then, cell suspensions were enriched with anti-CD11c magnetic beads and positive selection MS^+ columns according to the manufacturer's instructions (Miltenyi Biotec, Auburn, CA). Briefly, the cells, resuspended in 10-ml RPMI 1640, were incubated in a 200-mm cell culture dish (Corning Inc., Acton, MA) for 1 hour at 37°C to remove the adherent macrophages. Nonadherent cells were collected and resuspended in 400 μ l of buffer (1 $\times$ PBS/0.5% BSA) containing 100 μ l of CD11c microbeads. After a 15-minute incubation at 4°C, free beads were washed away, and the cells conjugated with beads were passed through MS^+ columns for positive selection. The viability of the purified DCs was estimated by trypan blue staining. The purity of these DCs was determined by either cell morphology using cyto-spin (Shandon Inc.) followed by hematoxylin-eosin staining or stained by PE-labeled CD11c and FITC-labeled-MHCII followed by flow cytometry analysis. Then the purified DCs were counted on a hemocytometer and subsequently diluted at 5×10^6 /ml. The aliquots of 100 μ l containing these cells were added to 96-well plates. A group of stimuli was added to the plated DCs including 100 μ g/ml *S. mansoni* egg antigen (SEA), 1 μ g/ml lipopolysaccharide (LPS), or 2.5 μ g/ml Pam3cys. After 6 hours of stimulation, total RNA was isolated using Trizol reagent, and quantitative real-time PCR (Taqman) was performed to measure IL-10 and IL-12 gene expression. After 48 hours of stimulation, cell-free supernatant from each sample was collected and stored in -80°C until IL-12 and IL-10 protein levels were measured by ELISA.

IL-12-Expressing Adenovirus (AdmIL-12) Intrapulmonary Infection

A recombinant human adenovirus expressing a functional heterodimeric mouse IL-12 protein was generated as previously described.¹⁹ The AdmIL-12 construct contains the p35 and p40 cDNA fragment inserted into the E1 and E3 regions of human type-5 adenoviral genome. Expression of both p35 and p40 cDNAs is driven by the human CMV immediate early promoter and terminated by the SV40 polyadenylation signal. Transfection with this replication-defective construct results in the expression of active IL-12 both *in vitro* and *in vivo*.¹⁹ As a control, a previously described replication-deficient, E1-deleted Ad70 construct was used.²⁰ At day 3 after CLP or sham surgery, mice were anesthetized with a combination of ketamine and xylazine, as described for the CLP procedure (see above). The tracheas of mice were exposed by making 0.5-cm midline incision on the ventral surface of the neck. Mice were intratracheally injected with 30 μ l of saline containing 3×10^8 plaque-forming units of either AdmIL-12 or Ad70 and then immediately injected intravenously with 5000 *S. mansoni* eggs. The adenoviral vector results in very efficient infectivity of the airway epithelium and produces high levels of IL-12 mRNA and protein in lung.²¹ At 6, 8, and 16 days, the mice were sacrificed, and the whole lungs were collected for histology, gene

expression analysis (Taqman), and protein measurement (ELISA) (see above).

Statistical Analysis

All results are expressed as mean $\pm$ SEM. The means between sham and CLP groups at different time points were compared by two-way analysis of variance. Individual differences were further analyzed using the Bonferroni posttests. For the *in vitro* studies with lung DCs, the Mann-Whitney *U*-test was used to compare the means between sham and CLP group. $P < 0.05$ was considered statistically significant.

Results

Severe Sepsis Augmented the Pulmonary Granulomatous Response

To investigate the long-term consequence of severe sepsis on the adaptive immune response in the lung, mice that survived 3 days after severe experimental sepsis induced by CLP were subsequently challenged intravenously with *S. mansoni* eggs. Sham-operated mice were used as controls. The formation and development of pulmonary granulomas induced by *S. mansoni* eggs have been previously well characterized in nonsurgically manipulated mice,^{22,23} thereby providing a paradigm for the study of this response in postseptic mice. Histological examination of lung tissue revealed that *S. mansoni* egg-induced granulomas were similar in size in both sham and postseptic mice up to day 8 after egg injection (Figure 1A). However, at day 16, postseptic mice exhibited significantly ($P < 0.001$) larger granulomas ($28,498.3 \pm 1152.6 \mu\text{m}^2$, $n = 5$) compared with the sham mice ($18,882.2 \pm 814.1 \mu\text{m}^2$, $n = 5$). This difference was also present at day 30 after egg challenge ($28,232.7 \pm 1607.7 \mu\text{m}^2$, $n = 4$ versus $14,310.6 \pm 644.7 \mu\text{m}^2$, $n = 5$; $P < 0.001$) (Figure 1B). Quantification of eosinophil content in lung granulomas revealed that eosinophil numbers in postseptic mice at day 8 ($0.525 \pm 0.026/100 \mu\text{m}^2$) and day 16 ($0.999 \pm 0.052/100 \mu\text{m}^2$) were significantly greater than those in the sham mice at day 8 ($0.370 \pm 0.025/100 \mu\text{m}^2$; $P < 0.01$) and day 16 ($0.612 \pm 0.040/100 \mu\text{m}^2$; $P < 0.001$), respectively (Figure 1C).

Severe Sepsis Altered the Cytokine Profile in Lung Tissue

The changes in pulmonary granuloma size and cellular content in postseptic mice suggested that an altered immune status was present at this tissue site, therefore we hypothesized that this was associated with an altered cytokine profile. The expression of a series of cytokines in lung tissue was measured both at the transcript level by quantitative real-time PCR (Taqman) and at the protein level by ELISA. IL-12 is a heterodimeric cytokine, which consists of covalently bound p40 and p35 subunits and has a central role in type-1 immune response.²⁴ In the present study,

postseptic mice had significantly lower whole-lung transcript level of *IL-12p40* (Figure 2) and protein level of IL-12p70 (Figure 3) compared with sham mice at several time points up to day 8 after *S. mansoni* egg challenge. For the type-2 related cytokines, no differences were observed at the transcript level for *IL-4*, *IL-5*, *IL-10*, and *IL-13* between two groups (Figure 2). However, postseptic mice exhibited significantly higher whole-lung IL-4 and IL-13 protein levels at day 1 and IL-10 protein level at day 2 after egg challenge compared with sham group (Figure 3).

S. mansoni Egg-Induced DC Recruitment to Lung Was Impaired in Postseptic Mice

Given its accessory role during immune responses, DCs are recognized as one of the key initiators of adaptive immunity and have a pivotal role in directing the type-1/type-2 cytokine response.^{25–28} Because the cytokine profile in the lung revealed an altered adaptive immune response in postseptic mice, we used flow cytometry to study the influence of severe sepsis on the presence of DC subpopulations in the lungs. In sham mice, peak numbers of two subpopulations of DCs, namely CD11c⁺CD11b⁺ DCs and CD11c⁺B220⁺ DCs, were observed in the lung at day 2 after egg challenge (Figure 4). However, lungs from postseptic mice contained significantly fewer numbers of these two subpopulations of DCs at days 2 and 8 after egg challenge when compared with similar samples from sham mice. At day 16 after egg challenge, DC numbers were similar in both groups of mice.

Alteration in the Cytokine Production in Pulmonary DCs Induced by Severe Sepsis

The cytokine profile in the whole-lung samples revealed a significant decrease in IL-12 level in postseptic mice compared with sham-operated mice. These findings indicated that pulmonary type-1 immune response might have been impaired by severe sepsis. Given the importance of DCs in the initiation of the adaptive immune response and polarizing type-1 and type-2 response, we hypothesized that, in addition to impairing the recruitment of DCs into the lungs, severe sepsis also altered cytokine production by lung DCs. After positive selection by CD11c microbeads, 90% of the purified DCs were alive established by trypan blue staining. Given that lung macrophages have been removed by 1 hour of incubation at 37°C, HE staining revealed that the purified cells homogeneously matched DC morphology (Figure 5A). Ninety percent of these cells were CD11c⁺MHCII⁺ (data not shown). When compared with DCs from sham mice, lung DCs from postseptic mice showed decreases of 4- and 7-fold in the baseline mRNA level of *IL-12p40* at days 8 and 16, respectively (Figure 5, B and D, medium). In response to the stimulation with different Toll-like receptor (TLR) agonists (SEA [TLR2], LPS [TLR4], and Pam3Cys [TLR2]), lung DCs from postseptic mice contained significantly lower *IL-12p40* mRNA levels compared with DCs from sham mice (Figure 5, B and D). Conversely,

lung DCs from postseptic mice exhibited a twofold increase in baseline mRNA level of *IL-10* at both time points after egg challenge (Figure 5, C and E, medium). Significantly higher *IL-10* mRNA level in lung DCs was observed in postseptic mice compared with sham mice in response to LPS and Pam3Cys at both time points (Figure 5, C and E). The stimulation with SEA only induced a significant increase in *IL-10* mRNA level in DCs from postseptic mice at day 8 after egg challenge.

The protein levels of IL-12p70 and IL-10 in DC cultures were consistent with mRNA levels measured in DCs from sham and postseptic mice. Specifically, DCs from postseptic mice produced lower amounts of IL-12p70 and higher amounts of IL-10 constitutively and after TLR activation compared with DCs from sham mice (Figure 6).

IL-12 Adenoviral Vector Restored the Type-1 Cytokine Profile in Postseptic Mice

Having observed that postseptic mice had larger pulmonary granulomas and greater eosinophil recruitment when challenged with *S. mansoni* eggs, we next sought to determine whether this amplified granulomatous response could be altered by exogenous IL-12. To increase IL-12 expression level in the lung, we used a transient *IL-12* transgene expression system whereby mice were administrated intratracheally an adenoviral vector containing the p35 and p40 cDNAs inserted into the E1 and E3 region of the viral genome, respectively. Given that the E1 region of the viral genome has been deleted, this recombinant viral vector is replication deficient, resulting in transient expression of IL-12 in lung. The peak concentration of IL-12 within the bronchoalveolar lavage fluid was observed at day 1 after intratracheal administration of AdmIL-12, followed by a gradual decrease through day 7.²¹ As shown in Figure 7, there were lower IL-12p-70 protein levels in the whole lungs from postseptic mice compared with sham mice in the control virus group at days 6 and 8, which is consistent with the observation shown in Figure 3. However, compared with Ad70 group, postseptic mice receiving the IL-12 vector showed a significant increase in the expression of IL-12p70 at day 6 (0.272 ± 0.008 versus 0.5 ± 0.029 ng/mg total protein, $n = 4$; $P < 0.001$) and day 8 (0.274 ± 0.029 versus 0.556 ± 0.021 ng/mg total protein, $n = 6$; $P < 0.001$). Although the IL-12 vector did not affect all of the type-2 related cytokines, it did reduce IL-4 protein levels significantly at day 6 (0.141 ± 0.009 versus 0.032 ± 0.004 ng/mg total protein, $n = 4$; $P < 0.001$). No changes in IL-5, IL-10, and IL-13 expression were observed in postseptic mice that received the IL-12 vector.

Figure 1. The histology of pulmonary granulomas induced by *S. mansoni* eggs. **A:** Sham and CLP groups were challenged with 5000 live *S. mansoni* eggs at day 3 after surgery. The left lung lobe from both groups was collected at different time points for histological analysis. HE-stained lung sections from sham or CLP mice are shown. **B:** Quantification of granuloma size at a series of indicated time points after egg challenge. *, $P \leq 0.05$ compared with granuloma size measured in sham mice. **C:** Quantification of eosinophil number in pulmonary granulomas. Each group: $n = 5-6$ /time point. The results shown are representative of three experiments and are expressed as mean $\pm$ SEM. τ , $P \leq 0.05$ compared with eosinophil number in the pulmonary granulomas from sham mice.

Figure 2. Cytokine transcript expression in the lungs from sham and CLP mice. After egg challenge at day 3 after surgery, mice from the sham or CLP groups were anesthetized, and the upper right lobe was collected to investigate the gene expression level of IL-4, IL-5, IL-10, IL-12p40, and IL-13 in the whole lungs at days 1, 2, 4, 6, 8, 16, and 30 after egg challenge. Total RNA was isolated and reverse transcribed to cDNA. Quantitative real-time PCR (Taqman) was performed to measure the transcript level of each cytokine. The mRNA level of each sample was normalized by mRNA level in naïve mice, and the data are shown as the fold of increase [$2^{(-\Delta\Delta CT)}$]. * $P \leq 0.05$ compared with IL-12p40 mRNA level measured in the lungs of sham mice, $n = 4-6$.

Figure 3. Cytokine protein level in the whole lungs from mice of sham and CLP group. To investigate the production of IL-4, IL-5, IL-10, IL-12p40, and IL-13 in the whole lungs of sham and CLP mice, the lower right lobes from sham or CLP mice were analyzed by ELISA and normalized to the total protein level. *, $P \leq 0.05$ compared with IL-12p70 protein level in the lungs of sham mice; ϕ , $P \leq 0.05$ compared with IL-4 level in the lungs of sham mice at day-1; τ , $P \leq 0.05$ compared with IL-10 level in the lungs of sham mice at day 2; γ , $P \leq 0.05$ compared with IL-13 level in the lungs of sham mice at day 1.

Figure 4. Severe sepsis impaired DC recruitment to pulmonary granulomas induced by *S. mansoni* eggs. Mice from sham or CLP group ($n = 5-7$ /group/time point) were sacrificed at days 2, 8, and 16. Mice killed at day 0 did not receive eggs and were used as baseline values. Lungs were digested by collagenase IV, and the dispersed cells were stained as follows: CD11c⁺CD11b⁺ and CD11c⁺B220⁺. These cell surface markers were assessed by flow cytometry analysis. The results shown are representative of two experiments and are expressed as mean $\pm$ SEM. * and τ , $P \leq 0.05$ compared with CD11c⁺CD11b⁺ DCs and CD11c⁺B220⁺ DCs in the lungs of sham mice at days 2 and 8, respectively.

Figure 5. Alteration of gene expression of IL-12p40 and IL-10 in DCs from postseptic mice. Sham and CLP-operated mice ($n = 6-8$ /group/time point) were subsequently intravenously challenged with 5000 live *S. mansoni* eggs. Eight and 16 days later, mice were sacrificed, and lung DCs were purified by positive selection with CD11c⁺ microbeads. After overnight culture, purified DCs (as shown in **A**) were stimulated with medium, 80 μ g/ml SEA (TLR2 agonist), 1 μ g/ml LPS (TLR4 agonist), or 2.5 μ g/ml Pam3Cys (TLR2 agonist). Quantitative real-time PCR was performed to measure the mRNA levels of IL-10 and IL-12p40 in cultured DCs after 6 hours of stimulation. * and α , $P \leq 0.05$ compared with IL-12p40 mRNA level in the DCs from sham mice at days 8 and 16, respectively. τ and β , $P \leq 0.05$ compared with IL-10 mRNA level in the DCs from sham mice at days 8 and 16, respectively, after egg challenge.

Figure 6. DCs from postseptic mice showed the defective IL-12p70 production and enhanced IL-10 production. Sham and CLP-operated mice were sacrificed at days 8 and 16 after egg challenge. Lung DCs were purified by positive selection with CD11c⁺ microbeads from each animal. After overnight culture, purified DCs were stimulated with medium, 80 μ g/ml SEA (TLR2 agonist), 1 μ g/ml LPS (TLR4 agonist), or 2.5 μ g/ml Pam3Cys (TLR2 agonist). ELISA was performed to measure the protein levels of IL-10 and IL-12p70 in the supernatant of cultured DCs after 48 hours of stimulation. * and α , $P \leq 0.05$ compared with IL-12p70 level in the DCs from sham mice at days 8 and 16, respectively. τ and β , $P \leq 0.05$ compared with IL-10 level in the DCs from sham mice at days 8 and 16, respectively.

IL-12 Adenoviral Vector Restored the Altered Pulmonary Granulomatous Response in Postseptic Mice but Did Not Influence DC Recruitment to Lung

We sought to determine whether the compartmentalized overexpression of IL-12 influenced the alteration in granuloma size and eosinophil content in postseptic mice. Histological analysis showed that in Ad70 control groups, postseptic mice exhibited significantly larger pulmonary granulomas than sham mice ($30,838.0 \pm 1166.4 \mu\text{m}^2$ versus $20,713.6 \pm 1125.9 \mu\text{m}^2$, $n = 5$; $P < 0.001$) at day 16. IL-12 vector significantly ($P < 0.001$) decreased the granuloma size in postseptic mice compared with postseptic mice receiving Ad70 control virus ($21,792.3 \pm 1133.0 \mu\text{m}^2$ versus $30,838.0 \pm 1166.4 \mu\text{m}^2$, $n = 5$; $P < 0.001$) (Figure 8A). Interestingly, the significant reduction in granuloma size in postseptic mice induced by the IL-12 vector was also observed at days 6 and 8. Quantification of eosinophil showed that the IL-12 vector also significantly reduced eosinophil recruitment to postseptic lung granulomas at day 8 (0.322 ± 0.023 versus $0.488 \pm 0.027/100 \mu\text{m}^2$, $n = 25$; $P < 0.001$) and day 16 (0.610 ± 0.025 versus $0.909 \pm 0.036/100 \mu\text{m}^2$, $n = 25$; $P < 0.001$).

Finally, to determine whether the attenuation of granulomatous response induced by the administration of AdmIL-12 was due to alteration in DC recruitment into the lungs of postseptic mice, we investigated the influence of IL-12 overexpression on pulmonary DC numbers. Compared with postseptic mice receiving Ad70, postseptic mice receiving AdmIL-12 contained similar numbers of both DC subpopulations in the lung at day 8 after egg challenge, indicating that IL-12 overexpression did not

Figure 7. Introduction of IL-12-expressing adenovirus reversed the type-2-biased cytokine profile in the postseptic lung. Sham or postseptic mice ($n = 4-6$ /group/time point) were intratracheally administered with 3×10^8 colony-forming unit (CFU) IL-12-expressing adenovirus (AdmIL-12) or control adenovirus (Ad70) immediately followed by 5000 *S. mansoni* eggs given intravenously. At 6, 8, and 16 days after virus and egg challenge, mice were killed, and the lower right lobes were collected from each mouse for cytokine analysis by ELISA. The results shown are representative of two experiments and are expressed as mean $\pm$ SEM. *, $P \leq 0.05$ compared with IL-12p70 level in the lungs of sham mice receiving control virus at days 6 and 8. α , $P \leq 0.05$ compared with IL-12p70 level in the lungs of CLP mice receiving Ad70 at days 6 and 8. β , $P \leq 0.05$ compared with IL-4 protein level in the lungs of CLP mice receiving control virus at day 6.

influence the recruitment of DCs to lung of postseptic mice (Figure 9).

Discussion

Although the diagnosis and treatment of sepsis during the acute phase of this disease remain clinically chal-

lenging, recent attention has been directed toward studying the long-term consequence of severe sepsis on the host immune activity. It has been shown that patients who survive severe sepsis are at a higher risk for secondary infections, cancer, and other complications for up to 8 years after the septic episode.^{9,29} This suggests that the initial sepsis episode results in a profound alteration to the host innate and adaptive immune responses. Previously, our lab observed that postseptic mice were more susceptible to a pulmonary infection initiated by the fungal pathogen *A. fumigatus*.⁵ In the present study, we

Figure 8. Introduction of IL-12-expressing adenovirus attenuates pulmonary granuloma pathology. Sham or postseptic mice ($n = 4-6$ /group/time point) were intratracheally administered with 3×10^8 PFU IL-12-expressing adenovirus (AdmIL-12) or control adenovirus (Ad70) immediately followed by 5000 *S. mansoni* eggs given intravenously. At 6, 8, and 16 days after virus and egg challenge, mice were killed, and the left lobe was collected from each mouse for histological analysis. **A:** Quantification of granuloma size. *, $P \leq 0.05$ compared with granuloma size measured in sham mice receiving Ad70 at day 16 after egg challenge. α , $P \leq 0.05$ compared with CLP mice receiving Ad70 at all of three time points. **B:** Eosinophil number. **, $P \leq 0.05$ compared with intragranulomatous eosinophil count in sham mice receiving Ad70 at days 8 and 16 after egg challenge. β , $P \leq 0.05$ compared with intragranulomatous eosinophil count in CLP mice receiving Ad70.

Figure 9. Administration of IL-12-expressing adenovirus fails to enhance DC recruitment to postseptic lung. Postseptic mice ($n = 5$ /group) were intratracheally administered with 3×10^8 PFU AdmIL-12 or Ad70 immediately followed by 5000 *S. mansoni* eggs given intravenously. Eight days later, lungs were harvested from both group and digested by collagenase IV. The dispersed lung cells were stained as follows: CD11c⁺ in combination with CD11b⁺ or B220⁺, followed by flow cytometry analysis.

explored the pulmonary adaptive immune response after severe sepsis using freshly isolated *S. mansoni* eggs to induce a pulmonary granulomatous response. *S. mansoni* eggs promote the development of a type-2 biased pulmonary granuloma,^{30,31} but this response is not lethal, even under an immunodeficient condition. In the present study, postseptic mice exhibited significantly larger granulomas with a higher eosinophil content. These histological findings in postseptic mice coincided with significantly decreased whole-lung IL-12 levels and significantly increased whole-lung levels of IL-4, IL-10, and IL-13 compared with sham mice during the formation and maintenance of the granulomatous response. Because DCs play a central role during adaptive immunity and can polarize a type-1 immune response through the production of IL-12,^{32,33} we hypothesized that the skewed adaptive response might have been a consequence of alterations in DC recruitment and/or DC phenotype. Post-septic mice had lower numbers of two DC subpopulations: CD11c⁺CD11b⁺ DCs and CD11c⁺B220⁺ DCs in the lungs. Also associated with changes in DC numbers, lung DC production of IL-12 was decreased, whereas these cells produced much greater IL-10 in postseptic mice compared with DCs from sham mice. The importance of IL-12 to the pulmonary immune response was highlighted in subsequent experiments in which an IL-12-expressing adenovirus modulated the pulmonary granulomatous response to that typically observed in nonsurgically manipulated mice. Furthermore, the mechanism whereby IL-12 overexpression attenuated the granulomatous response in lung seemed to be downstream of DC function rather than DC recruitment, because the impaired DC recruitment induced by severe sepsis was not reversed by AdmIL-12 administration.

The polymicrobial peritonitis induced by CLP surgery closely mimics the clinical course of abdominal sepsis, resulting in a systemic inflammatory response syndrome.¹⁶ Although the severity of sepsis depends on the number of punctures and the gauge (size) of needle, the CLP operation may lead to the formation of an intra-abdominal abscess,³⁴ which is considered to be a protective "walling-off" response of the host against bacterial.³⁵ In our system, we found that the localized inflammatory response in the peritoneal cavity was significantly decreased 3 days after CLP, evidenced by the normalized levels of inflammatory cytokines/chemokines (data not shown). This suggested the termination of the acute phase of the peritonitis; however, there is the possibility of a chronic inflammatory response continuing at the original puncture lesion.

S. mansoni egg-induced granulomas require the efficient and sequential recruitment of a series of immune effector cells, including macrophages,³⁶ CD4⁺ T cells,³⁷ and eosinophils.³⁸ These specific immune cells form a strong cellular barrier and sequester the *S. mansoni* eggs from the surrounding healthy tissue. We and others have shown that the cytokine/chemokine network plays a central role in the recruitment and activation of immune cells around the eggs.^{22,39} IL-4 operates as the key cytokine driving the type-2 response.^{31,40} IL-5 induces the recruitment of eosinophil.³¹ IL-13 is a key mediator in the fibrotic

process during schistosomiasis.⁴¹ Conversely, mice receiving exogenous IL-12 protein develop smaller pulmonary and hepatic granulomas and exhibit less severe tissue fibrosis.^{42,43} In the present study, the defect in IL-12 expression in the lungs of postseptic mice was associated with the development of a larger pulmonary granuloma characterized by significantly greater eosinophil numbers. In contrast, the administration of an IL-12-expressing gene vector attenuated the aggressive eosinophilic granulomatous response in postseptic mice. This supports the concept that IL-12 is a negative regulator of granulomatous responses induced by *S. mansoni* eggs, probably by altering the type-1/type2 cytokine profile, as shown in the present study. Although the increased expression of type-2 related cytokines IL-4, IL-5, IL-10, and IL-13 in postseptic mice was observed early and not later during the granulomatous response, it appears that this early alteration in the cytokine profile has profound effects in progression of the granulomatous response. The development of a type-1 immune response requires the dominance of type-1 over type-2 cytokines, however, the persistent impairment in IL-12 production in postseptic lungs appears to have resulted in the imbalance between the type-1 and type-2 response.

Several studies have focused on the recruitment and function of DCs in the granulomatous response, because the DC is a critical cell type linking the innate and adaptive immune response and the DC has the capacity to modulate type-1 or type-2 immune responses.^{14,15,44} Immature DCs capture antigen in the peripheral tissue and migrate to the draining lymph nodes, where they activate T cells, and initiate the type-1 or type-2 immune response depending on the presence of IL-12^{32,33} or IL-4,³² respectively. Controversy surrounds the origin of DCs, subsets, and functional differences between subsets, despite considerable recent focus on DC biology. In the present study, two general DC subsets, myeloid (CD11c⁺CD11b⁺) and plasmacytoid (CD11c⁺B220⁺) DCs were differentially measured,⁴⁵ which have been reported to differ in TLR expression⁴⁶ and cytokine production in response to viral or bacterial infection.⁴⁵ We observed the decreased recruitment of both DC subpopulations and a defect in IL-12 production by a mixed population of DCs. With the development of pulmonary granulomas, local immunity typically proceeds to a type-2 biased response from a Th0 lung environment.¹³ However, in the postseptic lungs, the alteration in the recruitment of DCs combined with the alteration in their cytokine generation appeared to have accelerated the appearance and magnitude of the type-2 cytokine profile. This may be explained in part by the enhanced IL-10 production and impaired IL-12 production in pulmonary DCs from postseptic mice. IL-10 is a pleiotropic immunomodulatory cytokine that regulates the development and function of immune cells. IL-10 induces anergy in T cells by inhibiting the proliferation and cytokine production of these cells⁴⁷ and promotes the development of regulatory T cells.⁴⁸ In addition to its effect on suppressing inflammatory and type-1 response, IL-10 strongly inhibits DC maturation and down-regulates MHC class II expression and the capacity to produce IL-12.^{49–51} Also

DC-derived IL-10 was required for the optimal development of type-2 cells in a DC transfer study.⁵² The ability of IL-10 to inhibit the type-1 response and IL-12 production is well established, although the manner in which IL-10 achieves its regulatory function has not been identified yet.²⁴

Several studies have reported the loss of DCs in the spleen and lymph nodes of septic patients or animals with severe sepsis.^{7,8,53} In the present study, we found a similar loss of DCs in the lung, supporting the concept that severe sepsis results in the systemic loss of DCs, probably due to detrimental influence on DC development or increased DC apoptosis. We observed an altered cytokine production profile by lung DCs in postseptic mice, which was directly related to the exacerbation of *S. mansoni* egg-induced granulomatous response. Although we did not investigate other functions of lung DCs, it is highly possible that severe sepsis also impairs antigen presentation by DCs. The present study suggested that the altered cytokine production in pulmonary DCs induced by severe sepsis was due to an alteration in DC phenotype rather than differential recruitment of different DC subtypes,⁵⁴ but further study of the role of DC subsets in the altered adaptive immune response in the postseptic lung is warranted. Accordingly, the modulation of DC recruitment and/or function could be a potential therapeutic modality to restore normal adaptive immune response in postseptic hosts.

In summary, we examined the alteration in pulmonary adaptive immunity after a severe innate immune response (due to sepsis). After sepsis, a type-2-biased cytokine environment was observed in the lung, leading to an accelerated granulomatous response characterized by augmented eosinophilia. Severe sepsis resulted in decreased DC recruitment and alterations in IL-12 and IL-10 synthesis by these cells. Interestingly, this response was reversed by the artificial elevation of IL-12 in the postseptic lung. This study supports the concept that a biased type-2 cytokine profile in the lung is a major consequence of severe sepsis, which may account for the altered host immunity seen in postseptic patients. Thus, restoring the cytokine balance in the postseptic lung may be a novel therapy in this highly susceptible group of patients.

Acknowledgments

We thank Robin Kunkel for her assistance in image processing. We thank Holly Evanoff and Pam Lincoln for their technical assistance.

References

- Hotchkiss RS, Karl IE: The pathophysiology and treatment of sepsis. *N Engl J Med* 2003, 348:138–150
- Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR: Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med* 2001, 29:1303–1310
- Martin GS, Mannino DM, Eaton S, Moss M: The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003, 348:1546–1554
- Riedemann NC, Guo RF, Ward PA: The enigma of sepsis. *J Clin Invest* 2003, 112:460–467
- Benjamin CF, Hogaboam CM, Lukacs NW, Kunkel SL: Septic mice are susceptible to pulmonary aspergillosis. *Am J Pathol* 2003, 163:2605–2617
- Cohen J: The immunopathogenesis of sepsis. *Nature* 2002, 420:885–891
- Efron PA, Martins A, Minnich D, Tinsley K, Ungaro R, Bahjat FR, Hotchkiss R, Clare-Salzler M, Moldawer LL: Characterization of the systemic loss of dendritic cells in murine lymph nodes during polymicrobial sepsis. *J Immunol* 2004, 173:3035–3043
- Hotchkiss RS, Tinsley KW, Swanson PE, Grayson MH, Osborne DF, Wagner TH, Cobb JP, Coopersmith C, Karl IE: Depletion of dendritic cells, but not macrophages, in patients with sepsis. *J Immunol* 2002, 168:2493–2500
- Perl TM, Dvorak L, Hwang T, Wenzel RP: Long-term survival and function after suspected gram-negative sepsis. *JAMA* 1995, 274:338–345
- Benjamin CF, Lundy SK, Lukacs NW, Hogaboam CM, Kunkel SL: Reversal of long-term sepsis-induced immunosuppression by dendritic cells. *Blood* 2005, 105:3588–3595
- Romani L: Immunity to fungal infections. *Nat Rev Immunol* 2004, 4:1–23
- Sandor M, Weinstock JV, Wynn TA: Granulomas in schistosome and mycobacterial infections: a model of local immune responses. *Trends Immunol* 2003, 24:44–52
- Vella AT, Pearce EJ: CD4+ Th2 response induced by *Schistosoma mansoni* eggs develops rapidly, through an early, transient, Th0-like stage. *J Immunol* 1992, 148:2283–2290
- Iyonaga K, McCarthy KM, Schneeberger EE: Dendritic cells and the regulation of a granulomatous immune response in the lung. *Am J Respir Cell Mol Biol* 2002, 26:671–679
- Chiu BC, Freeman CM, Stolberg VR, Hu JS, Komuniecki E, Chensue SW: The innate pulmonary granuloma: characterization and demonstration of dendritic cell recruitment and function. *Am J Pathol* 2004, 164:1021–1030
- Baker CC, Chaudry IH, Gaines HO, Baue AE: Evaluation of factors affecting mortality rate after sepsis in a murine cecal ligation and puncture model. *Surgery* 1983, 94:331–335
- Evanoff HL, Burdick MD, Moore SA, Kunkel SL, Strieter RM: A sensitive ELISA for the detection of human monocyte chemoattractant protein-1 (MCP-1). *Immunol Invest* 1992, 21:39–45
- Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976, 72:248–254
- Bramson J, Hitt M, Gallichan WS, Rosenthal KL, Gaudie J, Graham FL: Construction of a double recombinant adenovirus vector expressing a heterodimeric cytokine: in vitro and in vivo production of biologically active interleukin-12. *Hum Gene Ther* 1996, 7:333–342
- Angiolillo AL, Sgadari C, Taub DD, Liao F, Farber JM, Maheshwari S, Kleinman HK, Reaman GH, Tosato G: Human interferon-inducible protein 10 is a potent inhibitor of angiogenesis in vivo. *J Exp Med* 1995, 182:155–162
- Greenberger MJ, Kunkel SL, Strieter RM, Lukacs NW, Bramson J, Gaudie J, Graham FL, Hitt M, Danforth JM, Standiford TJ: IL-12 gene therapy protects mice in lethal *Klebsiella pneumoniae*. *J Immunol* 1996, 157:3006–3012
- Qiu B, Frai KA, Reich F, Komuniecki E, Chensue SW: Chemokine expression dynamics in mycobacterial (type-1) and schistosomal (type-2) antigen-elicited pulmonary granuloma formation. *Am J Pathol* 2001, 158:1503–1515
- Chensue SW, Warmington KS, Lukacs NW, Lincoln PM, Burdick MD, Strieter RM, Kunkel SL: Monocyte chemotactic protein expression during schistosome egg granuloma formation. Sequence of production, localization, contribution, and regulation. *Am J Pathol* 1995, 146:130–138
- Trinchieri G: Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat Rev Immunol* 2003, 3:133–146
- Banchereau J, Steinman RM: Dendritic cells and the control of immunity. *Nature* 1998, 392:245–252
- Moser M, Murphy KM: Dendritic cell regulation of TH1-TH2 development. *Nat Immunol* 2000, 1:199–205

27. Liu YJ: Dendritic cell subsets and lineages, and their functions in innate and adaptive immunity. *Cell* 2001, 106:259–262
28. Macatonia SE, Hosken NA, Litton M, Vieira P, Hsieh CS, Culppepper JA, Wysocka M, Trinchieri G, Murphy KM, O'Garra A: Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4+ T cells. *J Immunol* 1995, 154:5071–5079
29. Quartin AA, Schein RM, Kett DH, Peduzzi PN: Magnitude and duration of the effect of sepsis on survival: Department of Veterans Affairs Systemic Sepsis Cooperative Studies Group. *JAMA* 1997, 277:1058–1063
30. Kunkel SL, Lukacs NW, Strieter RM, Chensue SW: Th1 and Th2 responses regulate experimental lung granuloma development. *Sarcoidosis Vasc Diffuse Lung Dis* 1996, 13:120–128
31. Cheever AW, Yap GS: Immunologic basis of disease and disease regulation in schistosomiasis. *Chem Immunol* 1997, 66:159–176
32. Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM: Development of Th1 CD4+ T cells through IL-12 produced by *Listeria*-induced macrophages. *Science* 1993, 260:547–549
33. Manetti R, Parronchi P, Giudizi MG, Piccinni MP, Maggi E, Trinchieri G, Romagnani S: Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. *J Exp Med* 1993, 177:1199–1204
34. Maier S, Traeger T, Entleutner M, Westerholt A, Kleist B, Huser N, Holzmann B, Stier A, Pfeffer K, Heidecke CD: Cecal ligation and puncture versus colon ascendens stent peritonitis: two distinct animal models for polymicrobial sepsis. *Shock* 2004, 21:505–511
35. Wittmann DH, Schein M, Condon RE: Management of secondary peritonitis. *Ann Surg* 1996, 224:10–18
36. Chensue SW, Ruth JH, Warmington K, Lincoln P, Kunkel SL: In vivo regulation of macrophage IL-12 production during type 1 and type 2 cytokine-mediated granuloma formation. *J Immunol* 1995, 155:3546–3551
37. Hogan LH, Wang M, Suresh M, Co DO, Weinstock JV, Sandor M: CD4+ TCR repertoire heterogeneity in *Schistosoma mansoni*-induced granulomas. *J Immunol* 2002, 169:6386–6393
38. Jakubzick C, Wen H, Matsukawa A, Keller M, Kunkel SL, Hogaboam CM: Role of CCR4 ligands, CCL17 and CCL22, during *Schistosoma mansoni* egg-induced pulmonary granuloma formation in mice. *Am J Pathol* 2004, 165:1211–1221
39. Lukacs NW, Boros DL: Lymphokine regulation of granuloma formation in murine schistosomiasis *mansoni*. *Clin Immunol Immunopathol* 1993, 68:57–63
40. Brunet LR, Finkelman FD, Cheever AW, Kopf MA, Pearce EJ: IL-4 protects against TNF-alpha-mediated cachexia and death during acute schistosomiasis. *J Immunol* 1997, 159:777–785
41. Chiaramonte MG, Donaldson DD, Cheever AW, Wynn TA: An IL-13 inhibitor blocks the development of hepatic fibrosis during a T-helper type 2-dominated inflammatory response. *J Clin Invest* 1999, 104:777–785
42. Wynn TA, Eltoun I, Oswald IP, Cheever AW, Sher A: Endogenous interleukin 12 (IL-12) regulates granuloma formation induced by eggs of *Schistosoma mansoni* and exogenous IL-12 both inhibits and prophylactically immunizes against egg pathology. *J Exp Med* 1994, 179:1551–1561
43. Wynn TA, Cheever AW, Jankovic D, Poindexter RW, Caspar P, Lewis FA, Sher A: An IL-12-based vaccination method for preventing fibrosis induced by schistosome infection. *Nature* 1995, 376:594–596
44. Chiu BC, Freeman CM, Stolberg VR, Hu JS, Zeibecoglou K, Lu B, Gerard C, Charo IF, Lira SA, Chensue SW: Impaired lung dendritic cell activation in CCR2 knockout mice. *Am J Pathol* 2004, 165:1199–1209
45. Steinman RM: Some interfaces of dendritic cell biology. *APMIS* 2003, 111:675–697
46. Boonstra MK, Asselin-Paturel C, Gilliet M, Crain C, Trinchieri G, Liu YJ, O'Garra A: Flexibility of mouse classical and plasmacytoid-derived dendritic cells in directing T helper type 1 and 2 cell development: dependency on antigen dose and differential toll-like receptor ligation. *J Exp Med* 2003, 197:101–109
47. Joss A, Akdis M, Faith A, Blaser K, Akdis CA: IL-10 directly acts on T cells by specifically altering the CD28 co-stimulation pathway. *Eur J Immunol* 2000, 30:1683–1690
48. Levings MK, Sangregorio R, Galbiati F, Squadrone S, de Waal Malefyt R, Roncarolo MG: IFN-alpha and IL-10 induce the differentiation of human type 1 T regulatory cells. *J Immunol* 2001, 166:5530–5539
49. De Smedt T, Van Mechelen M, De Becker G, Urbain J, Leo O, Moser M: Effect of interleukin-10 on dendritic cell maturation and function. *Eur J Immunol* 1997, 27:1229–1235
50. Morel AS, Quarantino S, Douek DC, Londei M: Split activity of interleukin-10 on antigen capture and antigen presentation by human dendritic cells: definition of a maturative step. *Eur J Immunol* 1997, 27:26–34
51. Steinbrink K, Wolff M, Jonuleit H, Knop J, Enk AH: Induction of tolerance by IL-10-treated dendritic cells. *J Immunol* 1997, 159:4772–4780
52. Maldonado-Lopez R, Maliszewski C, Urbain J, Moser M: Cytokines regulate the capacity of CD8alpha(+) and CD8alpha(-) dendritic cells to prime Th1/Th2 cells in vivo. *J Immunol* 2001, 167:4345–4350
53. Ding Y, Chung CS, Newton S, Chen Y, Carlton S, Albina JE, Ayala A: Polymicrobial sepsis induces divergent effects on splenic and peritoneal dendritic cell function in mice. *Shock* 2004, 22:137–144
54. Edwards AD, Manickasingham SP, Sporri R, Diebold SS, Schulz O, Sher A, Kaisho T, Akira S, Reis e Sousa C: Microbial recognition via Toll-like receptor-dependent and -independent pathways determines the cytokine response of murine dendritic cell subsets to CD40 triggering. *J Immunol* 2002, 169:3652–3660