Post-BMT lung injury occurs independently of the expression of CCL2 or its receptor, CCR2, on host cells

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Panoskaltsis-Mortari, Angela, John R. Hermanson, Elizabeth Taras, O. Douglas Wangensteen, Israel F. Charo, Barrett J. Rollins, and Bruce R. Blazar. Post-BMT lung injury occurs independently of the expression of CCL2 or its receptor, CCR2, on host cells. Am J Physiol Lung Cell Mol Physiol 286: L284–L292, 2004. First published October 3, 2003; 10.1152/ajplung.00154.2003.—Idiopathic pneumonia syndrome (IPS) is a significant cause of mortality post-bone marrow transplant (BMT) in humans. In our murine model, lethal pre-BMT conditioning and allogeneic T cell transfer result in the recruitment of host antigen-presenting cells (APC) and donor T cells into the lung post-BMT concomitant with development of severe lung dysfunction. CCL2 induction is found in bronchoalveolar lavage fluid (BALF) before host monocyte influx. The major receptor for CCL2 is CCR2 present on monocytes; this interaction can play a crucial role in monocyte recruitment in inflammation. To determine whether blockade of the CCL2/CCR2 pathway could hinder host monocyte influx, lethally conditioned wild-type (WT), CCL2−/−, or CCR2−/− mice were transplanted with allogeneic marrow and spleen cells. WT and CCR2−/− recipients exhibited equivalent lung dysfunction post-BMT. The frequencies of host macrophages as well as donor CD4+ and CD8+ T cells in lungs post-BMT did not differ between WT and CCR2−/− recipients. However, the T cell dependency of the host CD11b+ major histocompatibility complex class II+ cell influx was lost in CCR2−/− recipients. In CCR2−/− mice, this influx was accompanied by elevated levels of CCL20. Post-BMT BALF and sera of CCR2−/− mice did not reveal any decrease in cytokines or chemokines compared with WT mice. CCL2−/− mice had a deficiency of CCL2 in their BALF and sera post-BMT, confirming our hypothesis that CCL2 is predominantly host derived. Therefore, IPS can occur independently of host expression of CCL2 or CCR2, and compensatory mechanisms exist for regulating APC recruitment into the lung during the early post-BMT period.

chemokines; mouse models; monocytes; idiopathic pneumonia syndrome; bone marrow transplant

IDIOPATHIC PNEUMONIA SYNDROME (IPS) remains a major complication after bone marrow transplantation (BMT) (11). Risk factors for developing IPS are related to the intensity of the conditioning regimen used and the degree of allogeneicity of the donor graft (15). We have characterized a murine model of IPS caused by the influx of host monocytes and donor T cells into the lungs early postallogeneic BMT of lethally irradiated mice (30). Intensifying the pre-BMT conditioning with cyclophosphamide potentiates the development of alloreactive T cell-dependent IPS. Lung dysfunction in our model presents as reduced specific compliance, decreased total lung capacity, and increased wet and dry lung weights. Histologically, IPS is associated with injured alveolar type II cells and increased frequencies of cells expressing B7 ligands (costimulatory for T cells) and the cytotoxic protein granzyme B (28, 30). Bronchoalveolar lavage fluid (BALF) of mice with IPS contains elevated levels of inflammatory cytokines as well as other indexes of lung injury as evidenced by increased levels of nitrite, lactate dehydrogenase, and protein (17).

We reported that monocyte- and T cell-attracting chemokines are produced in the lung during the generation of IPS in our model (29). Induction of CCL2 (macrophage chemotactic protein-1 or MCP-1) was found in BALF of these recipient mice before host monocyte recruitment on day 3 post-BMT. CCL2 is produced by numerous cell types including monocytes, epithelial cells, fibroblasts, and tumor cells (33). Endothelial cells are also major producers of CCL2 in response to inflammatory cytokines (32). In murine IPS, the highest levels of CCL2 are produced after allogeneic BMT in a T cell-dependent manner (29). In vitro studies have shown that high amounts of CCL2 are produced by antigen-presenting cells (APC) that are effective inducers of T cell responses (34). The major receptor for CCL2 is CC chemokine receptor 2 (CCR2), present on monocytes, and, in rodent systems, this interaction has been shown to play a crucial role in monocyte/macrophage recruitment in inflammation, autoimmunity, and resistance to infectious organisms (16, 20, 21, 24, 35, 38, 39). In relationship to the lung, reduced recruitment of monocytes, decreased T helper cell type 1 (Th1) cytokine responses, and early death have been seen in rodent models of bacterial-induced allergic airway inflammation, granulomatous lung disease, alloantigen-induced bronchiolitis obliterans, and endotoxin administration, in the presence of neutralizing antibodies to CCL2 and in CCL2−/− or CCR2−/− mice (1, 8, 10, 25, 27, 31).

Our previous findings in murine IPS demonstrated the association of early CCL2 production in the lung followed by monocyte influx and inflammatory cytokine production (29). We hypothesized that prevention of this initial monocyte recruitment might blunt the subsequent inflammatory cascade. Therefore, we tested whether CCL2 or CCR2 might be critical mediators of IPS and whether blockade of the CCL2/CCR2 recruitment pathway could hinder the influx of host monocytes. Contrary to what we anticipated, CCL2−/− and CCR2−/− mice still developed IPS as severe as wild-type (WT) BMT recipi-
ents, and recruitment of host major histocompatibility complex (MHC) class II+ cells was unimpaired.

MATERIALS AND METHODS

Mice. C57BL/6 (H2b) mice were purchased from the National Institutes of Health (Bethesda, MD). B10.BR (H2b) mice were purchased from Jackson Laboratories (Bar Harbor, ME). CCL2−/− and CCR2−/− mice backcrossed onto the C57BL/6 background (>10 generations) have been described previously (10, 24). Mice were housed in microisolator cages in the specific pathogen-free facility of the University of Minnesota and cared for according to the Research Animal Resources guidelines of our institution. Experiments were approved by the Institutional Animal Care and Use Committee of the University of Minnesota. Sentinel mice were found to be negative for infectious microorganisms known to cause pulmonary pathology such as pneumonia virus, K virus, Sendai, etc. For BMT, donors were 8–12 wk of age, and recipients were used at 8–10 wk of age.

Pre-BMT treatment and conditioning. C57BL/6 WT or CCL2−/− or CCR2−/− mice received PBS or cyclophosphamide (Cytoxan; Bristol Myers Squibb, Seattle, WA), 120 mg/kg “day −1” ip, as a conditioning regimen pre-BMT on days −3 and −2. All mice were lethally irradiated on the day before BMT (7.5 Gy TBI) by X-ray at a dose rate of 0.41 Gy/min as described previously (3). BMT. Our BMT protocol has been described previously (2). Briefly, donor B10.BR bone marrow was T cell depleted (TCD) by X-ray with anti-Thy 1.2 monoclonal antibody (MAb; clone 30-H-12, rat IgG2a, kindly provided by Dr. David Sachs, Charlestown, MA) plus complement (Nieffenegger, Woodland, CA). C57BL/6 WT or CCL2−/− or CCR2−/− recipient mice were transplanted via caudal vein with 20 × 106 TCD B10.BR (H2b) marrow or with or without 15 × 106 natural killer (NK) cell-depleted (PK136, anti-NK1.1 + complement) spleen cells as a source of IPS-causing T cells.

Lung weights. Mice were euthanized with pentobarbital sodium, and the thoracic cavity was partially dissected. Lungs were excised and perfused with 1 ml of saline via the right ventricle of the heart. To minimize the number of mice needed for the study without compromising the data, the right lung (bilobed) was used for weight determinations while the left lung was processed for histopathology. For each mouse, the wet weight was taken immediately after right lung removal from the thorax. Lungs were dried overnight to a constant weight at 80°C followed by determination of dry weights. The wet-to-dry weight ratio was calculated and taken as a measure of the severity of lung injury (37). No correction for extravascular blood content was used in the calculations.

Pressure-volume curves. After full heart-lung excision, the lungs were suspended via the trachea and kept moist with saline. Pressure-volume (P-V) curves of air-filled lungs were obtained as previously described (30). Air was delivered into the lungs via a tracheal cannula in 0.05-ml increments with a syringe while measuring intratracheal pressure with a transducer until 30 cmH2O pressure was reached. The volume at this pressure was denoted as the total lung capacity (TLC), assuming the volume of air in the collapsed lungs before inflation was negligible (compared with the TLC). Air was then withdrawn in 0.05-ml increments until pressure was atmospheric. This was done three times, and data were procured from the third series. Specific lung compliance was calculated from the slope of the deflation curve from points flanking 5 cmH2O pressure, considered normal breathing range, by the formula (∆volume/∆pressure)/Av volume, where volume is in milliliters, pressure is in cmH2O, and Av volume is average volume over the pressure range used to generate the slope of the P-V curve (i.e., ∆volume/∆pressure).

Bronchoalveolar lavage. The trachea was cannulated with a 19-gauge needle, infused with 0.5 ml of PBS, and the fluid was withdrawn. This was repeated twice, and a total of 1.5 ml of BALF were collected per mouse, centrifuged (1,000 g) at 4°C for 10 min to pellet the cells, and stored at −80°C.

Serum collection. At the time of death, blood was collected by cardiac puncture, placed immediately at 4°C, and the serum was separated at 4°C and stored at −80°C.

Chemokine/cytokine level determination. Bronchoalveolar lavage (BAL) and serum levels of predominant monocyte/macrophage attractants CCL2 (MCP-1), CCL7 (MCP-3), and CCL20 (macrophage inflammatory protein-3α or MIP-3α); predominant T cell attractants CCL3 (MIP-1α), CCL4 (MIP-1β), CCL5 (RANTES), CCL11 (eotaxin), CCL17 (TARC), CXCL1 (lymphotoxin), CCL6 (C10), CCL21 (Exodus-2), and CXCL10 (IP-10); neutrophil attractants CXCL1 (KC) and CXCL2 (MIP-2); proinflammatory Th1-type cytokines IFN-γ, TNF-α, IL-1β, and IL-6; and anti-inflammatory Th2-type cytokines IL-13 and IL-10 were determined by sandwich ELISA using mouse-specific commercial kits (R&D Systems, Minneapolis, MN; sensitivity 1.5–3.0 pg/ml) or by sandwich ELISA (sensitivity 1 pg/ml) empirically developed using specific MAbs and results interpolated from standard curves of the relevant recombinant proteins (R&D Systems).

Frozen tissue preparation. A mixture of 0.5 ml of optimal cutting temperature compound (OCT; Miles, Elkhart, IN)-PBS (3:1) was infused via the trachea into the lungs. Lung tissue was embedded in OCT, frozen in liquid nitrogen, and stored at −80°C. Histology was scored using a previously documented scoring system (4).

Immunohistochemistry. Cryosections (6 µm) were fixed in acetone and immunoperoxidase stained using biotinylated MAbs as described previously (5) with avidin-biotin blocking reagents, ABC-peroxidase conjugate, and 3,3-diaminobenzidine chromogen purchased from Vector Laboratories (Burlingame, CA). The biotinylated MAbs used were as follows: anti-CD4 (clone GK1.5), anti-CD8 (clone 2.43), anti-CD11b (Mac-1, clone M1/70), and anti-Gr-1 (clone RB6-8C5), all purchased from BD Pharmingen (San Diego, CA). The number of positive cells in the lung was quantitated as the percent of nucleated cells under ×200 magnification (×20 objective lens). Four fields per lung were evaluated. For immunofluorescent costaining, FITC-labeled CD11b was used with Cy3-labeled CD11c (BD Pharmingen), and images were obtained using an Olympus FV500 confocal laser scanning fluorescence microscope with Fluoview software (Olympus America, Melville, NY).

Flow cytometry. Phenotyping of BAL cells was evaluated on days 3 and 7 post-BMT by quantitation of donor cells using biotin-labeled anti-H2b MAb (clone 11–4.1, BD Pharmingen) with SA-PertCp and host cells using phycoerythrin (PE)-labeled anti-H2b (clone E1H144, BD Pharmingen). T cells and monocytes/macrophages were quantitated using fluoresceochrome-labeled MAbs (FITC or PE, Pharmingen) directed to CD3 (clone 1452C11), CD4 (clone GK1.5), CD8 (clone 53-6.72), and CD11b (clone M1/70). Flow cytometry was done on a FACS Calibur (BD Biosciences, Mountain View, CA) with 10,000 events analyzed (determined by forward and side scatter).

Statistical analysis. Survival data were analyzed by life table methods using the Mantel-Peto-Cox summary of chi-square. Other data were analyzed by ANOVA or Student’s t-test. P values ≤ 0.05 were considered statistically significant.

RESULTS

Post-BMT lung dysfunction and inflammatory cell recruitment are not decreased in CCL2−/− or CCR2−/− recipients. In our initial description of mouse IPS post-BMT (30), lung injury was highly correlated with decreased total lung capacity and inflammatory cell influx. Therefore, we wanted to know whether these parameters were affected by loss of CCL2 or CCR2. Lethally irradiated C57BL/6, CCL2−/−, or CCR2−/− mice were transplanted with B10.BR bone marrow with or without 15 × 106 splenocytes. CCL2−/− and CCR2−/− mice

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exhibited similar decreases in total lung capacity and specific compliance on day 7 post-BMT equivalent to WT recipients (total lung capacities shown in Fig. 1). Increases in both wet and dry lung weights, indicative of cellular inflammation and fluid leakage, were equally elevated among the WT, CCL2−/−, and CCR2−/− recipients (not shown). Histological examination of the lungs procured on day 7 post-BMT showed equivalent degrees of perivascular and peribronchiolar cuffing in WT, CCL2−/−, and CCR2−/− recipients of allogeneic bone marrow and IPS-causing splenocytes (Fig. 2) with equivalent histological scores (4) (not shown). Lungs were examined by immunohistochemistry to determine whether accumulation of T cells or monocytes/macrophages in the interstitial parenchyma of the lungs had been altered. No decreases in donor CD4+ or CD8+ T cell or CD11b+IAb+ (host haplotype) macrophage numbers were seen (CD11b data shown in Fig. 3; T cell data not shown). Flow cytometric analysis of BAL cells on day 7 post-BMT revealed no differences in the percentages of macrophages, MHC class II+ cells (predominantly of host haplotype), or donor CD4+ or CD8+ T cells that had accumulated in the alveolar airways (not shown). These data are consistent with the similar degrees of decreased lung capacity and specific compliance seen at this time point in WT, CCL2−/−, and CCR2−/− recipients. Therefore, contrary to what we expected, the frequencies of monocytes/macrophages, the cells whose migration is presumed to be dictated by the CCL2/CCR2 pathway, did not decrease, indicating that the
Recipients deficient in CCR2 have elevated levels of the myeloid dendritic cell chemokine MIP-3α (CCL20). Because CCR2^{−/−} mice exhibited an accelerated early T cell-independent influx of CD11b^{+} cells (Fig. 4A), we analyzed the lungs for changes in the levels of chemokine receptors at different time points in the peri-BMT period. Of the panel of chemokines we were able to measure (listed in MATERIALS AND METHODS), a dramatic difference was seen for CCL20/MIP-3α (and moderately so for CCL2). Compared with WT, CCR2^{−/−} mice (but not CCL2^{−/−} mice) have inherently higher levels of CCL20 in their lungs before conditioning (day −3), and these levels remain elevated post-BMT (Fig. 4B). Interestingly, the highest levels of CCL20 were seen on day 3 post-BMT in the lungs of CCR2^{−/−} mice receiving allogeneic bone marrow alone (i.e., in the absence of IPS-causing splenocytes). Cells bearing the CD11b^{+} MHC class II^{+} APC phenotype are consistent with those of the myeloid dendritic cell lineage that express CCR6, the only known receptor for CCL20 (MIP-3α). Myeloid dendritic cells also coexpress CD11c along with CD11b, and immunofluorescent staining for these markers also showed increased numbers of cells coexpressing CD11b and CD11c in CCR2^{−/−} recipient mouse lungs on day 3 post-BMT as shown in Fig. 4C. As expected, there was a lack of CCL2 in the lungs and sera of the CCL2^{−/−} mice post-BMT (Fig. 5), confirming our previous hypothesis that CCL2 is host and not donor derived post-BMT (29). These same CCL2^{−/−} recipients of allogeneic bone marrow and spleen had elevated lung (but not systemic) levels of CCL21 (predominant T cell attractant) compared with WT or CCR2^{−/−} recipients (not shown). However, this was only seen at the later day 7 post-BMT time point and did not lead to increased numbers of donor T cells that were elevated compared with WT or CCR2^{−/−}. Thus elevated levels of CCL20 may be compensatory for the lack of CCL2 in facilitating host APC recruitment into the lung post-BMT.

Higher levels of both pro- and anti-inflammatory T cell mediators in CCL2^{−/−} and CCR2^{−/−} recipients. Because differences in some chemokine levels were found in the lungs of CCL2^{−/−} and CCR2^{−/−} recipients, levels of Th1- and Th2-related inflammatory cytokine mediators in BALF and lung protein extracts were measured to ascertain whether changes in cytokines may have been affected. We found an elevated level of IFN-γ in the BALF of CCL2^{−/−} recipients of allogeneic bone marrow and spleen compared with WT B6 recipients (Fig. 5). Therefore, this elevated level of IFN-γ coexisted, in these mice, with an elevated level of CCL21 but was not associated with elevated T cell numbers compared with either WT or CCR2^{−/−} recipients of allogeneic bone marrow and spleen.

Systemic levels of cytokines and chemokines may also influence the pulmonary vasculature, the ensuing inflammatory milieu, chemokine gradients, and the extravasation events post-BMT. Of the panel of cytokines and chemokines measured (listed in MATERIALS AND METHODS) in the sera, most were not affected by the lack of CCL2 or CCR2 in the recipient mice on day 7 post-BMT. Besides the expected paucity of CCL2 in the CCL2^{−/−} mice (as stated above), the exception was IL-10 that was elevated, compared with WT, in the sera of CCL2^{−/−} and CCR2^{−/−} mice given bone marrow and allogeneic spleen cells (Fig. 5, P < 0.05). Other Th2 cytokines, such as IL-4 and IL-13, were present at equivalent levels in knockout (KO) mice compared with WT mice post-BMT, despite the fact that CCL2 is considered to be critical for Th2 responses. The

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**Fig. 3.** Influx of host CD11b^{+} cells into the lungs of allogeneic BMT recipients is not altered by absence of either CCL2 or CCR2 in the host, but T cell dependency is lost in CCR2^{−/−} recipients. Expression of CD11b and host IA^{b} on the indicated days relative to BMT was determined by immunoperoxidase staining with biotinylated monoclonal antibodies. Data are presented as percent of nucleated cells. Mean values ± SE are indicated for 6 mice/group pooled from 2 experiments. A: groups receiving allogeneic BM alone. B: groups receiving allogeneic BM + splenocytes. *P < 0.05 vs. pre-BMT level.

A

![](https://example.com/image1)

B

![](https://example.com/image2)
elevated post-BMT levels of IL-10 in the KO mice were T cell dependent, consistent with our previous findings in WT mice (30).

Graft-vs.-host disease-mediated mortality is accelerated in the absence of host CCL2 or its receptor CCR2. To determine whether host expression of CCL2 or CCR2 would have an effect on the generation of graft-vs.-host disease (GVHD; that is associated with IPS), lethally irradiated C57BL/6, CCL2−/−, or CCR2−/− mice were transplanted with B10.BR bone marrow with or without 5 or 25 × 10^6 splenocytes. Figure 6A shows that CCL2−/− recipients of allogeneic splenocytes had an accelerated lethality compared with WT recipients at both spleen cell doses (P < 0.05). Similar findings were found with CCR2−/− mice (Fig. 6B), and these recipients appeared to be five times more sensitive to the GVHD induced by allogeneic T cells since CCR2−/− recipients of 5 × 10^6 spleen cells had a mortality rate equivalent to WT B6 mice receiving 25 × 10^6 splenocytes. GVHD-induced weight loss paralleled the survival data (not shown). Therefore, preclusion of the CCL2/CCR2 pathway does not hinder GVHD. On the contrary, GVHD mortality is accelerated.

**DISCUSSION**

We demonstrate in this study that the presence of the CCL2/CCR2 pathway is not needed for the induction of early post-BMT-related lung injury. Previous data from our laboratory indicated an induction of CCL2 by resident pulmonary cells post-BMT that preceded the influx of host macrophages. These cells comprise the initial infiltrate into the lung early after BMT and may serve to costimulate alloantigen-reactive donor T cells that enter the lung subsequently. The preferred receptor for CCL2 is CCR2 present on monocytes, and this ligand–receptor interaction has been shown to be critical for monocyte recruitment in various rodent models of inflammation. We had, therefore, reasoned that the use of CCL2 or CCR2 knockout mice as recipients would hinder the influx of these cells. Contrary to what we expected, manifestations of
IPS were not ameliorated, nor was the cellular influx hindered, most likely due to a compensatory increase of other chemotactic agents for host APC (e.g., CCL20).

The paucity of systemic and pulmonary levels of CCL2 in the CCL2−/− recipients post-BMT confirms our earlier hypothesis that CCL2 is host derived post-BMT (29). Because CCL2 production in the lung preceded the influx of host monocytes, we anticipated that mice deficient in CCL2 would exhibit less lung injury post-BMT. This was not the case. Others have also found a minimal role for CCL2 in immune complex-mediated lung injury in rats (9). We did not find a compensatory increase in other monocyte attractants such as CCL7 (MCP-3) or MCP-5, so it remains unknown what the potential recruitment pathway may be. Furthermore, we found

Fig. 5. IFN-γ, IL-10, and CCL2 levels in the bronchoalveolar lavage fluid (BALF; A) and serum (B) on day 7 postallogeneic BMT of WT, CCL2−/−, and CCR2−/− recipient mice. Levels of the indicated cytokines/chemokines were determined by ELISA. Mean values ± SD are indicated for 6 mice/group pooled from 2 experiments. *P < 0.05 vs. corresponding WT group.
ROLE OF HOST CCL2 AND CCR2 IN IPS

Fig. 6. Acceleration of graft-vs-host disease-mediated mortality in the absence of host CCL2 or CCR2. TBI-conditioned WT, CCL2−/− (A), or CCR2−/− (B) mice were transplanted with B10.BR TCD bone marrow with or without 5 or 25 × 10^6 splenocytes. CCL2−/− recipients had an accelerated lethality compared with WT recipients of allogeneic bone marrow and spleen (BMS groups). P < 0.04 for comparisons of both cell doses shown. CCR2−/− recipients had an accelerated lethality compared with WT recipients of allogeneic bone marrow and spleen (BMS groups). P < 0.04 for comparison of 5 × 10^6 cell dose; P = NS for CCR2−/− recipients of 5 × 10^6 cells vs. WT recipients of 25 × 10^6 cells. N = 8/group. NS, not significant.

no compensatory increase in chemokine receptors CCR1-5 as assessed by RNase protection assay (data not shown). In another murine system in which a minimal role for CCL2 in recruiting monocytes was found, it was shown that another chemokine, MIG (CXCL9), was responsible for monocyte recruitment (22). Although we did not measure MIG levels in our BMT recipient mice in the current study, in other studies we have used CXCR3−/− mice and anti-CXCR3 MAb

(CXCR3 is the receptor for MIG and IP-10) and found that IPS and GVHD occurred independently of donor CXCR3 expression (B. R. Blazar, P. A. Taylor, and A. Panoskaltsis-Mortari, unpublished data).

We found that mice deficient in CCR2 have elevated levels of the myeloid dendritic cell chemokine CCL20 (MIP-3α) in their lungs both pre-BMT and post-BMT. This correlated with elevated levels of CD11b+IA^- cells coexpressing CD11c consistent with the myeloid dendritic cell lineage that expresses CCR6 (23). CCR6 is the only known receptor for CCL20 that is produced predominantly in epithelial-rich tissues including the lungs (19). Therefore, it would be interesting to determine whether the CCR6/CCL20 pathway plays a critical role in the recruitment of host APC into the lungs post-BMT.

The elevation in IFN-γ levels in the lungs of CCL2−/− recipients is consistent with in vitro data of Hogaboam et al. (18) showing enhanced production of IFN-γ by, and proliferation of, CD4^+ T cells in coculture with stimulated lung fibroblasts and neutralizing CCL2 antibody. This is in contrast to other recent findings demonstrating decreased IFN-γ production in the lungs of CCL2-neutralized mice with pulmonary Cryptococcus neoformans infection (36). Furthermore, these authors found that CCL2-neutralized mice did have Th1 inflammatory cells in lung-associated lymph nodes, but that these T cells failed to traffic to the lungs with resultant Th2 T cell predominance in the lungs. In our study, we found no decrease in T cell influx into the lungs post-BMT and no significant Th1/Th2 skewing. In addition, we found no differences in the percentage of cells expressing mRNA for cytolytic granymes by in situ hybridization (data not shown). Contradictory findings in the literature reasonably stem from the different forms of inflammatory insult (e.g., exogenous infectious agents or allergens vs. internally derived BMT-related injury), so the roles of chemokine/chemokine receptor pathways will differ.

Mice deficient in CCL2 or CCR2 exhibited elevated levels of IL-10 systemically early post-BMT. Induction of IL-10 by blockade of CCL2 was also found in a mouse model of acute septic peritonitis in vivo (26). Indeed, we found higher systemic levels of IL-10 in otherwise non-manipulated CCL2−/− mice compared with WT B6 mice (22 ± 13 pg/ml serum vs. 0 ± 0 pg/ml, P = 0.0002). Therefore, CCL2−/− mice have a significantly elevated level of IL-10 even before BMT. IL-10 is normally considered an anti-inflammatory cytokine being produced by Th2-type cells. However, we have previously demonstrated that high levels of exogenously administered IL-10 can exacerbate both CD4 and CD8 T cell-mediated GVHD in a dose-dependent fashion (6, 7). In fact, we observed that deficiency of CCL2 or CCR2 did not ameliorate GVHD but accelerated mortality and body weight loss. Therefore, preclusion of the CCL2/CCR2 pathway does not hinder GVHD. However, cells other than monocytes can also express CCR2, and it has been recently described that bronchiolar and alveolar epithelial cells express CCR2 receptors that functionally respond to CCL2, resulting in proliferation (13, 14). Therefore, we suggest that the induction and presence of CCL2 in the lung post-BMT may be an attempt by the host to stimulate epithelial cells to reepithelialize the alveolar basement membrane and not to recruit monocytes per se. Furthermore, the production of CCL2 by these same cell types post-BMT (29) raises the possibility of...
an autocrine loop for epithelial cell repair via binding to CCR2 or other chemokine receptor/ligands as well.

In conclusion, we report that IPS and GVHD can occur independently of host expression of CCL2 or CCR2. The data suggest redundancies in the chemokine recruitment pathways in the BMT setting and that other chemokine/chemokine receptor pathways suffice to execute post-BMT complications.

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