Absence of host tumor necrosis factor receptor 1 attenuates manifestations of idiopathic pneumonia syndrome

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Shukla, Mayank, Shuxia Yang, Carlos Milla, Angela Panoskaltsis-Mortari, Bruce R. Blazar, and Imad Y. Haddad. Absence of host tumor necrosis factor receptor 1 attenuates manifestations of idiopathic pneumonia syndrome. Am J Physiol Lung Cell Mol Physiol 288: L942–L949, 2005. First published December 17, 2004; doi:10.1152/ajplung.00260.2004.—The interaction of TNF-α with TNF receptor 1 (TNFR1) activates several signal transduction pathways that lead to apoptosis or NF-κB-dependent inflammation and immunity. We hypothesized that host TNFR1 expression contributes to noninfectious lung injury and inflammation commonly observed after bone marrow transplantation (BMT), termed idiopathic pneumonia syndrome (IPS). C57BL/6 TNFR1-sufficient (TNFR1+/+) and -deficient (TNFR1−/−) mice were total body irradiated with or without cyclophosphamide conditioning and were given bone marrow plus IPS-inducing donor spleen T cells from B10.BR wild-type mice. TNFR1−/− recipient mice exhibited improved early post-BMT survival associated with decreased permeability edema. In addition, the low lung compliance measured in anesthetized, ventilated TNFR1+/+ mice on day 7 after BMT was restored to baseline during TNFR1 deficiency. Importantly, bronchoalveolar lavage fluid (BALF) inflammatory cells from TNFR1−/− vs. TNFR1+/+ mice generated less nitric oxide (NO) and nitrating species and exhibited suppressed programmed cell death as assessed using flow cytometry. However, cellular infiltration and levels of proinflammatory cytokines and chemokines were generally higher in BALF collected on day 7 after BMT from TNFR1−/− compared with TNFR1+/+ recipient mice. Our results support a major role of host TNFR1 in regulation of NO production and lung dysfunction after allogeneic BMT.

cytokines; nitric oxide; peroxynitrite; apoptosis; bone marrow transplantation

IDIOPATHIC PNEUMONIA SYNDROME (IPS), formerly known as interstitial pneumonitis, accounts for up to 40% of non-graft-vs.-host disease (GVHD) deaths in allogeneic bone marrow transplantation (BMT) patients (7, 13). The median time to IPS onset is 35 days after marrow transplant, and case fatality rate exceeds 70% (11). Although the IPS risk has been shown to be related to the intensity of the conditioning regimen and the severity of GVHD, the pathophysiological mechanisms responsible for generating IPS are incompletely understood. Recent evidence from several laboratories indicates that IPS is a net result of conditioning-induced tissue injury, cellular recruitment, and immune activation (4, 8, 9, 25, 29).

Tumor necrosis factor (TNF)-α is a multifunctional cytokine implicated in IPS pathogenesis. TNF-α can contribute to IPS injury via effects on both the afferent T-cell activation phase (1) and the efferent tissue injury phase (27). Neutralization of TNF-α has been shown to be an effective treatment in preclinical models of IPS (10) and, perhaps, in humans (35). Possible sources of early post-BMT TNF-α generation in the lungs include host monocytes/macrophages and epithelial cells and allogeneic donor T cells. The importance of lung-infiltrating donor T cell-derived TNF-α was recently shown by Hildebrandt et al. (17), who demonstrated significantly reduced IPS severity in recipient mice given allogeneic T cells from TNF-α−/− mice, whereas utilization of TNF-α−/− mice as BMT recipients had no effect on IPS assessed by a histopathological injury score.

TNF-α binds two TNF receptors (TNFR). TNFR1 and TNFR2 are structurally related but functionally distinct receptors expressed on the surface of most cell types, with the exception of erythroid and unstimulated T cells (30). The interaction of TNF-α with TNFR1 activates the caspase pathway, leading to programmed cell death, or activates nuclear factor (NF)-κB, leading to gene expression of inflammatory mediators including inducible nitric oxide synthase (iNOS) (3, 6). In the presence of superoxide, iNOS-derived nitric oxide (NO) may lead to the formation of peroxynitrite, a potent oxidant and nitrating agent that has been implicated in IPS injury (16).

In our murine IPS model, lung dysfunction and inflammation and iNOS induction in irradiated mice are dependent on infusion of allogeneic spleen T cells. The addition of cyclophosphamide (Cy) at clinically relevant doses (120 mg·kg−1·day−1 on days −3 and −2) significantly (P < 0.0001) accelerated mortality and enhanced lung injury compared with total body irradiated (TBI) mice given infusion-causing donor T cells without Cy (26, 32). Similarly, in a model of GVHD in TBI mice, Cy enhanced GVHD-induced death, possibly by promoting LPS translocation in the gastrointestinal tract (19). Neutralization of TNF-α with the use of recombinant human TNFR:Fc, a bivalent soluble form of the TNFR bound to the heavy portion of human immunoglobulin molecule, was effective in preventing GVHD death in TBI but not Cy/TBI mice (19). Whether similar differential effects of conditioning regimen occur during interruption of TNF signaling in IPS is currently unknown.

The purpose of this study was to determine the role of TNFR1 on markers of lung dysfunction and inflammation in recipient mice conditioned with TBI ± Cy and infused with wild-type allogeneic spleen T cells. We also were interested in determining whether TNF-α binding to TNFR2 might compen-
sate for TNFR1 deficiency. We hypothesized that TNFR1−/− mice exhibit decreased IPS injury and improved survival after allogeneic BMT. Indeed, our results show attenuation in IPS injury during TNFR1 deficiency that was associated with decreased levels of NO and nitrative stress, consistent with an important role of host TNFR1 in NO generation in vivo. However, lung cellular infiltration and levels of some proinflammatory cytokines and chemokines were higher in TNFR1−/− vs. TNFR1+/+ recipient mice at least in part because of suppressed apoptosis of inflammatory cells during TNFR1 deficiency.

MATERIALS AND METHODS

Mice. B10.BR (H-2k), C57BL/6 (H-2b), and TNFR1−/− mice (backcrossed >10 generations to C57BL/6 mice) were purchased from Jackson Laboratory (Bar Harbor, ME). Mice were housed in microisolator cages in the specific pathogen-free facility of the University of Minnesota (Minneapolis, MN) and cared for according to the Research Animal Resources guidelines of our institution. For BMT, donors were 6–8 wk of age and recipients were used at 8–10 wk of age. Sentinel mice were found to be negative for 15 known murine viruses including cytomegalovirus, K-virus, parvovirus, and pneumonia virus of mice.

BMT protocol. Our BMT protocol has been described previously (34). Briefly, on the day before BMT, C57BL/6 wild-type and TNFR1−/− knockout mice were lethally total body irradiated (TBI; 7.5 Gy) by X-ray at a dose rate of 0.41 Gy/min. Donor B10.BR bone marrow (BM) was T cell depleted (TCD) with anti-Thy 1.2 MAb (clone 30-H-12, rat IgG2b) plus complement (Neiffenegger, Woodland, CA). For each experiment, a total of 5–10 recipient mice per treatment group were transplanted via caudal vein with 20 × 106 B10.BR TCD BM cells, with 15 × 106 spleen T cells (BM plus spleen (BMS)) as a source of IPS-causing T cells. In additional experiments to cause severe IPS, Cy (Cytovax; Bristol Myers Squibb, Seattle, WA) administered by intraperitoneal injection at a dose of 120 mg kg−1 day−1 on days −3 and −2 pre-BMT was added to the conditioning regimen (BMS-Cy). A cohort of BMS+Cy mice was monitored for post-BMT survival.

Pulmonary function analysis. Pulmonary mechanics in pentobarbital sodium-anesthetized, ventilated mice were measured according to the method described by Diamond and O’Donnell (12), with slight modifications. Briefly, after careful dissection of the neck, a short metal cannula was inserted into the trachea and secured with 3.0 silk. A polyethylene catheter was inserted orally into the lower third of the esophagus to estimate pleural pressure. The animal was then placed in a restraint holder and connected to a ventilator (model 1600, Stanford, CA) set at a respiratory rate of 150 breaths/min and a tidal volume of 100 ml. Volume-pressure measurement of lung recoil was performed using a plethysmograph (AJP-Lung Cell Mol Physiol, VoL 288 • May 2005 • www.ajplung.org)
were analyzed using ANOVA or Student’s t-test. B6 TNFR1+/+ (solid bars) and TNFR1−/− (open bars) recipient mice were lethally irradiated (7.5 Gy) on day −1 and infused on day 0 with B10.BR bone marrow with 15 × 10^6 donor spleen inflammation-inducing T cells (bone marrow + spleen; BMS). In additional experiments, irradiated mice were also conditioned with Cy (120 mg·kg⁻¹·day⁻¹ on days −3 and −2; BMS+Cy). Dynamic lung compliance was measured on day 7 after BMT in anesthetized mice placed in a single-chamber plethysmograph and connected to a mouse ventilator set at a respiratory rate of 150 breaths/min. Lung compliance was measured at a transpulmonary pressure of 1. Transpulmonary pressure was calculated as the difference between the respiratory system pressure and the plethysmograph chamber pressure. Transpulmonary pressure was measured on day 3. The pre-BMT weights of mice lost weight after allogeneic BMT, percent weight loss being calculated as the difference between the weight loss after allogeneic BMT and the body weight on the day before BMT. Lung compliance was measured at a tidal volume of 200 µl. Transpulmonary pressure was calculated using airway and intrapleural pressures. Control (C) mice were nonirradiated and nontransplanted. Data are means ± SE representing a total of 5 mice in each experimental group from 2 independent experiments. *P < 0.05 compared with control mice. Lung compliance was improved in TNFR1−/− compared with TNFR1+/+ mice (+P < 0.05) after allogeneic BMT.

RESULTS

Suppressed IPS injury and mortality in TNFR1−/− recipient mice. Our previously obtained data indicate that addition of Cy to conditioning regimen increases lung dysfunction and accelerates mortality after allogeneic BMT (26). Lung dysfunction was assessed on day 7 after BMT in TBI and Cy/TBI TNFR1+/+ and TNFR1−/− mice given allogeneic spleen T cells (BMS and BMS+Cy, respectively) by performing pulmonary function analysis in anesthetized, ventilated mice and measuring BALF total protein levels. This time point was chosen because it represents peak donor T cell-dependent inflammation and activation of host monocytes/macrophages implicated in IPS pathogenesis and outcome (5). Lung compliance values at a tidal volume of 200 µl in control (nonirradiated and nontransplanted) TNFR1+/+ and TNFR1−/− mice were similar. After allogeneic BMT, lung compliance was decreased in BMS and BMS+Cy TNFR1+/+ mice, consistent with development of IPS injury following irradiation, Cy, and allogeneity (Fig. 1). In contrast, lung compliance in BMS and BMS+Cy Cy TNFR1−/− mice was restored toward baseline with values not significantly different from those of non-BMT control mice.

BALF return volumes collected on day 7 after BMT were similar in all groups (>90% of instilled volume). BALF levels of total protein in untreated control TNFR1+/+ and TNFR1−/− were not different (<0.2 mg/ml). After BMT, BALF total protein levels increased in BMS and BMS+Cy TNFR1+/+ mice. Protein in the alveolar compartment may be caused by secretion from inflammatory, and lung parenchymal cells or may reflect protein extravasation across the alveolar-capillary barrier. This transplant-related increase in BALF total protein levels was attenuated in TNFR1−/− mice. †P < 0.05 compared with non-BMT control mice. The pre-BMT weights of mice lost weight after allogeneic BMT, percent weight loss being calculated as the difference between the weight loss after allogeneic BMT and the body weight on the day before BMT. Lung compliance was measured at a tidal volume of 200 µl. Transpulmonary pressure was calculated using airway and intrapleural pressures. Control (C) mice were nonirradiated and nontransplanted. Data are means ± SE representing a total of 5 mice in each experimental group from 2 independent experiments. *P < 0.05 compared with control mice. Lung compliance was improved in TNFR1−/− compared with TNFR1+/+ mice (+P < 0.05) after allogeneic BMT.

Table 1. Day 7 after BMT BALF total protein and post-BMT weight loss

<table>
<thead>
<tr>
<th>Group</th>
<th>BALF Total Protein, mg/ml</th>
<th>Weight Loss, % baseline weight</th>
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<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>TNFR1+/+</td>
<td>0.16±0.03</td>
<td>0</td>
</tr>
<tr>
<td>TNFR1−/−</td>
<td>0.17±0.04</td>
<td>0</td>
</tr>
<tr>
<td>BMS</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>TNFR1+/+</td>
<td>0.34±0.03*</td>
<td>−19.0±1.6*</td>
</tr>
<tr>
<td>TNFR1−/−</td>
<td>0.23±0.02*†</td>
<td>−11.4±0.9*†</td>
</tr>
<tr>
<td>BMS + Cy</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>TNFR1+/+</td>
<td>0.73±0.10*</td>
<td>−22.4±3.8*</td>
</tr>
<tr>
<td>TNFR1−/−</td>
<td>0.58±0.08*†</td>
<td>−17.7±1.5*†</td>
</tr>
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*B6 TNF receptor 1-sufficient (TNFR1+/+) and -deficient (TNFR1−/−) mice were lethally irradiated without (BMS) or with cyclophosphamide (Cy; 120 mg/kg on days −3 and −2) (BMS + Cy) and infused with B10.BR bone marrow with inflammation-inducing donor spleen T cells (i.e., BMS). Control mice were nonirradiated and nontransplanted. Values for body weight and bronchoalveolar lavage fluid (BALF) total protein are means ± SE for n = 10–22 mice/group obtained from 2 or 3 independent bone marrow transplantation (BMT) experiments. *P < 0.05 compared with non-BMT control TNFR1+/+ mice. †P < 0.05 compared with BMS or BMS + Cy TNFR1+/+ mice.
Suppressed production of ·NO and nitrotyrosine in TNFR1−/− mice. TNFR1 has been shown to induce iNOS-derived ·NO generation in alveolar macrophages (21). Because reactive oxygen and nitrogen species are implicated in IPS pathogenesis (16), we expected to observe suppressed levels of reactive nitrogen species after allogeneic BMT in mice lacking TNFR1. Indeed, on day 7 after BMT, BALF from BMS and BMS+Cy TNFR1−/− mice contained less nitrite plus nitrate, the stable by-products of ·NO, compared with similarly treated TNFR1+/+ mice (Fig. 3A). In addition, macrophages from BMS+Cy TNFR1−/− mice generated less nitrite compared with cells from BMS+Cy TNFR+/+ mice (Fig. 3B). Intracellular nitrosative stress by macrophages/monocytes from control and BMS+Cy mice was assessed by detection of antigenic sites related to nitrotyrosine. Nitration of monocytes/macrophages obtained from TNFR1−/− vs. TNFR1+/+ BMT mice was decreased (Fig. 4). Nitration was specific, because staining was completely blocked in the presence of excess antigen, 10 mM nitrotyrosine. Cells from non-BMT TNFR1−/− mice exhibited baseline levels of staining (data not shown).

Persistent generation of proinflammatory cytokines and chemokines in TNFR1−/− mice after allogeneic BMT. Despite suppressed ·NO generation in TBI and TBI/Cy mice infused with donor spleen T cells from wild-type mice, the BALF levels of the proinflammatory cytokines TNF-α and IFN-γ and the monocyte and T-cell chemoattractants MCP-1 and MIP-1α were generally higher in TNFR1−/− compared with TNFR1+/+ mice on day 7 after allogeneic BMT (Fig. 5). Furthermore, BALF cellular infiltration was increased in TNFR1−/− vs. TNFR1+/+ BMS mice (5.41 ± 0.32 × 10^4 vs. 3.12 ± 0.25 total cells/ml, respectively, n = 7; P < 0.05) and in TNFR1−/− vs. TNFR1+/+ BMS+Cy mice (7.38 ± 0.94 × 10^4 vs. 4.02 ± 0.34 total cells/ml, respectively, n = 7; P < 0.05). However,
on day 7 after allogeneic BMT, BALF cell differential was not significantly modified during the absence of TNFR1 (30–40% T cells, 50–60% alveolar macrophages and monocytes, and 1–10% neutrophils and other cell types in both TNFR1+/− or TNFR1−/− BMS and BMS+Cy mice). Increased cellularity in BALF from TNFR1−/− recipient mice was not due to an increased number of inflammatory cells in peripheral blood (0.9 ± 0.4 × 10⁶ cells/ml in TNFR1−/− and 1.1 ± 0.2 × 10⁶ cells/ml in TNFR1+/+ BMS+Cy mice, n = 5; P > 0.05). In addition, on day 7 after allogeneic BMT, the numbers of Mac-1- and CD3-positive cells in lung sections from TNFR1−/− were the same as or higher compared with those in TNFR1+/+ mice (Fig. 6), consistent with persistent monocytic and lymphocytic lung infiltration during TNFR1 deficiency. Similarly, the activation states of lung monocytes and dendritic cells assessed by the expression of B7.1 and B7.2 costimulatory molecules in lung sections collected from TNFR1−/− and TNFR1+/+ BMS mice were not different (data not shown).

Collectively, these data indicate persistent lung cellular infiltration and activation in mice lacking TNFR1 after allogeneic BMT.

Suppressed apoptosis of BALF cells from TNFR1−/− mice after BMT. Upon ligand binding, TNFR1 trimerizes and recruits death domain proteins, leading to activation of a caspase cascade and apoptosis. Therefore, we hypothesized that suppressed apoptosis of lung infiltrating inflammatory cells may represent one mechanism for persistent cellular infiltration and increased generation of inflammatory mediators in TNFR1−/− mice after BMT. Apoptosis/necrosis of BALF cells obtained on day 7 after allogeneic BMT was determined by performing flow cytometry after double staining the cells with annexin V antibody and PI. Apoptosis levels in BALF cells from unmanipulated control TNFR1−/− and TNFR1+/+ mice were not different (Fig. 7). Exposure to irradiation, conditioning drugs, and allogeneity increased apoptosis and necrosis in BALF cells from BMS and BMS+Cy B6 mice. In BMS mice, the percentage of annexin V-positive cells decreased from 85 ± 6% in TNFR1+/+ to 64 ± 8% in TNFR1−/− mice (n = 2; P < 0.05) (Fig. 7A). In BMS+Cy mice, annexin V mean fluorescence intensity decreased from 421 ± 36 in cells from TNFR1+/+ to 231 ± 45 in cells from TNFR1−/− mice (n = 2; P < 0.05) (Fig. 7B). These data are consistent with an important role of TNFR1 in the apoptosis of inflammatory cells after allogeneic BMT.

DISCUSSION

In these studies, we investigated the role of host TNFR1 in TBI or Cy/TBI B6 mice infused with spleen T cells from TNFR1+/+ major histocompatibility complex (MHC)-mismatched B10.BR donor mice. The main findings are that TNFR1−/− recipient mice exhibit attenuated manifestations of IPS injury and enhanced early post-BMT survival compared with wild-type mice despite persistent lung cellular infiltration. Suppressed lung dysfunction during host TNFR1 deficiency was associated with decreased generation of reactive nitrogen species and occurred in the presence of high levels of the proinflammatory cytokines TNF-α and IFN-γ and the chemokine MCP-1 and MIP-1α. These results support an important role of host TNFR1 in the development of IPS injury.

There are several potential reasons for persistent cellular infiltration and lung inflammation in TNFR1−/− mice after
allogeneic BMT. TNFR1 activation is a potent signal for programmed cell death or apoptosis of inflammatory cells (14). Exposure to irradiation, conditioning drugs, and allogeneity during the course of BMT enhances cellular apoptosis in the lungs. Impaired transplant-related apoptosis during TNFR1 deficiency will decrease clearance of inflammatory cells, thus increasing their number in the lung. Indeed, our results show that BALF from TNFR1−/− mice collected on day 7 after BMT contained significantly higher numbers of cells that exhibited decreased annexin V staining compared with TNFR1+/+ mice, indicative of impaired apoptosis. Another and perhaps more important reason for persistent inflammation in TNFR1−/− mice is the presence of TNFR1 on the donor spleen T cells that were infused during BMT. Stimulated T cells express TNFR1, which acts as an autocrine growth factor for T cells, leading to clonal expansion and cytokine production. The importance of donor T cell TNFR1 was demonstrated by Hill et al. (20), who reported that T cells lacking TNFR1 have impaired responses to alloantigen in vitro, including reduced proliferation, type 1 cytokine production, and cytolytic function. In vivo, T cells lacking TNFR1 have reduced capacity to induce GVHD and IPS (17, 20). In our BMT experiments, donor T cells from TNFR1+/+ B10.BR mice were infused into TNFR1−/− B6 mice. The full MHC incompatibility between donor T cells and host tissues causes severe T cell alloactivation and the generation of large amounts of proinflammatory cytokines and chemokines, including TNF-α, IFN-γ, MCP-1, and MIP-1α.

Our results indicate that donor T cells are efficiently recruited and activated in the lungs of TNFR1−/− mice, yet they are less effective in causing IPS injury and mortality. A main pathway by which TNFR1 stimulates effector and cytotoxic functions is induction of iNOS and generation of high levels of iNOS-derived NO (28). NO can rapidly react with superoxide to form potent oxidants and nitrating species (15, 33). Therefore, we reasoned and have shown that TNFR1−/− recipient mice exhibit attenuated manifestations of IPS on day 7 after allogeneic BMT in TNFR1−/− recipient mice infused with spleen T cells from fully disparate allogeneic wild-type mice. The lack of TNFR1 on host inflammatory cells results in suppressed generation of high-output NO and decreased formation of the potent oxidant and tissue-injurious agent peroxynitrite (ONOO−). However, TNFR1−/− recipient mice continue to exhibit donor T cell-dependent inflammation because of suppressed apoptosis of inflammatory cells, and the presence of TNFR1 on donor T cells is known to facilitate T-cell proliferation and activation. iNOS, inducible NO synthase.
mice exhibit suppressed -NO and nitrotyrosine associated with attenuated manifestations of IPS injury. Consistent with our data are reports showing dependence of macrophage-derived -NO generation on TNFR1 (21) and decreased -NO levels associated with prevention of endotoxemia-related injuries in rats treated with TNF-α-neutralizing antibodies (31) or in mice lacking TNFR1 receptors (22). Because we have previously shown that BALF collected on day 7 after BMT from iNOS−/− mice given iNOS +/+ donor BM plus donor spleen T cells contained undetectable levels of -NO (33), we reasoned that the source of -NO generated after BMT in the current study was of host cell and not donor cell origin. Although our studies implicate -NO as an important mediator responsible for end(product toxicity after allogeneic transplantation, there are likely other TNFR1-dependent inflammatory mediators that contribute to IPS injury (24).

The addition of Cy to TBI exaggerated T cell-dependent inflammatory responses and IPS injury assessed on day 7 after BMT. Both TBI- and Cy/TBI-conditioned TNFR1−/− mice exhibited attenuated manifestations of IPS injury compared with similarly treated TNFR1 +/+ mice. In contrast, Hill and colleagues reported that injection of recombinant human TNFR:Fc to suppress TNF-α levels prevents GVHD mortality in TBI- (10, 18) but not in Cy/TBI-conditioned mice (19). One reason offered for the differential effects of TNF-α neutralization in TBI vs. Cy/TBI mice was that the type of conditioning regimen may influence the dominant type of cytokine response. For example, exposure to TBI alone induced TNF-α, whereas Cy/TBI mainly upregulated IL-1 production, the main culprit in gastrointestinal (GI) tract injury during GVHD (2). Because GI tract injury is a major determinant of GVHD mortality, neutralization of IL-1 but not TNF-α enhanced survival in Cy/TBI-conditioned mice (19). In the lung, however, we reported that Cy facilitated IPS mortality via depletion of glutathione stores and generation of strong oxidants and nitrating species (16, 36). The absence of TNFR1 would be expected to suppress reactive nitrogen species in both TBI and Cy/TBI mice, which may clarify the reason why interruption of TNF-α signaling was independent of conditioning regimen in our study. It is also possible that differences in our acute MHC disparate IPS model and the subacute minor histocompatibility GVHD model of Hill and colleagues may underlie the real reason for the critical role of the type of conditioning regimen on responses following interruption of TNF-α signaling.

Targeting TNF-α or TNFR1 may represent effective strategies to limit IPS injury and mortality. Preliminary encouraging results obtained with the use of soluble TNFR1 for the treatment of IPS are emerging (35). Additional advantages of these approaches is suppression of donor T-cell proliferation and direct cytotoxicity as recently demonstrated in a BMT model of decreased lung injury in irradiated mice given donor T cells from TNF-α−/− mice (17). However, one potential consequence of suppressed T-cell activation is impaired graft-vs.-leukemia responses that may increase the risk of cancer relapse (19). In addition, targeting TNF-α may increase the risk of opportunistic infections in an already immunocompromised host (23). Further studies focused on evaluating the efficacy and safety of TNF-α inhibition during IPS injury are needed.

In summary, we observed decreased -NO generation and attenuated IPS injury despite persistent cellular infiltration in TNFR1−/− mice given donor T cells from wild-type mice. These results are consistent with the main physiological functions of TNFR1 on host and donor cells in regulation of cellular apoptosis and immune responses (Fig. 8). Improved understanding of the biological significance of TNF-α and TNFR1 following transplantation will optimize therapeutic strategies to prevent or limit IPS injury.

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SUPPRESSED LUNG INJURY IN TNFR1−/− MICE AFTER BMT


