CD4⁺CD25⁺ Regulatory T Cells Control Innate Immune Reactivity after Injury

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CD4\(^+\)CD25\(^+\) Regulatory T Cells Control Innate Immune Reactivity after Injury\(^1\)

Thomas J. Murphy, Niambh Ni Choileain, Yan Zang, John A. Mannick, and James A. Lederer\(^2\)

Major injury initiates a systemic inflammatory response that can be detrimental to the host. We have recently reported that burn injury primes innate immune cells for a progressive increase in TLR4 and TLR2 agonist-induced proinflammatory cytokine production and that this inflammatory phenotype is exaggerated in adaptive immune system-deficient (Rag1\(^{-/-}\)) mice. The present study uses a series of adoptive transfer experiments to determine which adaptive immune cell type(s) has the capacity to control innate inflammatory responses after injury. We first compared the relative changes in TLR4- and TLR2-induced TNF-\(\alpha\), IL-1\(\beta\), and IL-6 production by spleen cell populations prepared from wild-type (WT), Rag1\(^{-/-}\), CD4\(^{-/-}\), or CD8\(^{-/-}\) mice 7 days after sham or burn injury. Our findings indicated that splenocytes prepared from burn-injured CD8\(^{-/-}\) mice displayed TLR-induced cytokine production levels similar to those in WT mice. In contrast, spleen cells from burn-injured CD4\(^{-/-}\) mice produced cytokines at significantly higher levels, equivalent to those in Rag1\(^{-/-}\) mice. Moreover, reconstitution of Rag1\(^{-/-}\) or CD4\(^{-/-}\) mice with WT CD4\(^+\) T cells reduced postinjury cytokine production to WT levels. Additional separation of CD4\(^+\) T cells into CD4\(^+\)CD25\(^+\) and CD4\(^+\)CD25\(^{-}\) subpopulations before their adoptive transfer into Rag1\(^{-/-}\) mice showed that CD4\(^+\)CD25\(^+\) T cells were capable of reducing TLR-stimulated cytokine production levels to WT levels, whereas CD4\(^+\)CD25\(^{-}\) T cells had no regulatory effect. These findings suggest a previously unsuspected role for CD4\(^+\)CD25\(^+\) T regulatory cells in controlling host inflammatory responses after injury. The Journal of Immunology, 2005, 174: 2957–2963.

It is generally agreed that injury perturbs immune homeostasis by inducing an initial systemic inflammatory response, which is often followed by a compensatory anti-inflammatory response (1–3). These opposing effects of injury on the immune system are believed to play a major role in the decreased resistance of the injured host to nosocomial infections and the development of multiple organ failure, which is the leading cause of death in seriously injured patients who survive initial resuscitation (3–5). The results of many clinical studies and investigations in relevant animal models of injury have shown that major traumatic or thermal injury alters the behavior of both innate and adaptive immune systems (3–11). The relative importance of the altered reactivity of these two systems in the overall immune dysfunction that occurs after injury has not yet been clearly defined and remains a subject of considerable controversy.

In an attempt to clarify this issue, we have used Rag-deficient (Rag1\(^{-/-}\)) mice to explore the potential influences of a functional adaptive immune system on the innate immune system after injury (12). In that study we made the observation that Rag1\(^{-/-}\) mice could survive a 25% total body surface area burn injury with minimal mortality, similar to that seen in the wild-type (WT),\(^3\) C57BL/6J background mouse strain, and we found that the splenocytes from injured Rag1\(^{-/-}\) mice displayed a more vigorous proinflammatory cytokine response to Escherichia coli LPS stimulation than splenocytes from WT burn mice depleted of T and B cells to yield a cell population resembling Rag1\(^{-/-}\) splenocytes. This increase in LPS reactivity by Rag1\(^{-/-}\) splenocytes was most apparent 7 days after injury. Furthermore, we found that reconstituting Rag1\(^{-/-}\) burn mice with WT splenocytes reduced this augmented LPS response to levels seen in WT burn mice. These observations suggested that cells of the adaptive immune system must play an active role in controlling the proinflammatory nature of the innate immune system after major injury.

Because of recent and past reports showing that both CD4\(^+\) and CD8\(^+\) T cells can modulate immune responses, we wanted to delineate which of these major T cell subsets might mediate regulatory activity after injury (13). We were particularly interested in learning whether the naturally occurring CD4\(^+\)CD25\(^+\) regulatory T cell (Treg) subset might be involved in controlling injury-induced inflammatory responses, because a recent report had suggested that Tregs could suppress the proinflammatory reactivity of innate immune cells (14). To address these questions, we compared the TLR4- (LPS) and TLR2 (peptidoglycan (PGN))-induced proinflammatory cytokine production of innate immune cell populations prepared from the spleens of sham- or burn-injured WT, Rag1\(^{-/-}\), CD4\(^{-/-}\), or CD8\(^{-/-}\) mice. Our findings suggested that CD4\(^+\) T cells might play a significant regulatory role in controlling TLR responses after injury, because the TLR reactivities of spleen cells prepared from Rag1\(^{-/-}\) and CD4\(^{-/-}\) mice were similar, and the responses of cells prepared from WT vs CD8\(^{-/-}\) mice were also indistinguishable. We next performed a series of adoptive transfer experiments first using WT CD4\(^+\) T cells and then purified populations of WT CD4\(^+\)CD25\(^+\) or CD4\(^+\)CD25\(^{-}\) T cells. The results of these adoptive transfer studies revealed that CD4\(^+\)CD25\(^+\) T cells were both necessary and sufficient to control the increased TLR reactivity of the innate immune system after burn injury. We believe that the results presented in this study are the
first to demonstrate that CD4+CD25+ T cells can regulate the inflammatory response to injury.

Materials and Methods

Mice

Male WT C57BL/6J, B6.129-Rag1tm1Mom (Rag1−/−), B6.129S6-CD4tm1Kwe (CD4−/−), and B6.129S2-CD8αtm1Mak (CD8−/−) mice were purchased from The Jackson Laboratory. The mice were maintained in an accredited virus-Ab-free animal facility in accordance with the guidelines of the National Institutes of Health and the Harvard Medical School standing committee on animal research. The mice were acclimated for at least 1 wk before use in experiments at 6–8 wk of age.

Reagents

Highly purified, phenol-extracted LPS from E. coli serotype 0111:B4 was prepared and provided by Dr. E. Kurt-Jones (University of Massachusetts Medical School). The LPS re-extraction was performed as described by Hirschfeld et al. (15). All the purified LPS used in this study was tested and found to be a selective TLR4 agonist without detectable TLR2 stimulatory activity in embryonic kidney cells (HEK293) transfected with TLR2 or TLR4 gene expression constructs (16). PGN was obtained from Sigma-Aldrich. Culture medium for in vitro studies consisted of RPMI 1640 supplemented with 5% heat-inactivated FCS, 1 mM penicillin, pencillin/streptomycin/fungizone, 10 mM HEPES buffer, 100 mM nonessential amino acids, and 2.5 × 10−5 M 2-ME, all purchased from Invitrogen Life Technologies. FITC-labeled anti-CD3, anti-CD4, anti-CD8, and anti-CD19 mAbs were purchased from BD Pharmingen. PE-labeled anti-TLR4-MD-2-specific Ab was purchased from eBioscience.

Mouse injury model

The thermal injury protocol, approved by the National Institutes of Health and the Harvard Medical School standing committee on animal research, was performed as described previously (17, 18). Mice were anesthetized via i.p. injection of ketamine (125 mg/kg) and xylazine (20 mg/kg). The dorsal fur was shaved, and the animal was placed in an insulated plastic mold to expose 25% of the body surface area. The exposed dorsum was then immersed in 90°C water for 9 s to induce a well-demarcated, full-thickness, anesthetic burn injury. Sham mice were treated exactly as burn mice, except that they were exposed to isothermic 24°C water. Immediately after the procedure was performed, sham and burn mice were resuscitated with an i.p. injection of 1 ml of pyrogen-free normal saline. The mortality from burn injury in the present experiments varied from 0 to 5%.

Ex vivo studies

Mice were killed by CO2 asphyxiation 7 days after sham or burn injury. Spleens were harvested, and cell suspensions were prepared by mincing the tissues on wire mesh. Splenocytes were treated with Tris-ammonium chloride solution for 3 min to lyse RBC and were washed twice in culture medium before suspension in culture medium or PBS.

Pan-T (anti- Thy-1.2), Pan-B (anti-B220), CD4 (anti-L3T4), and CD8 (anti-Ly-2) Dynabeads (Dynal Biotech) were used according to the manufacturer’s instructions to deplete T and B lymphocytes from WT splenocyte suspensions, so that splenocyte populations comparable to Rag1−/− mice were prepared 7 days after adoptive transfer experiments were assessed for cell surface CD4 expression using FITC-labeled anti-CD4 Ab. All studies were performed with appropriate isotype controls, and in all instances, reconstituted Rag1−/− and CD4−/− mice were compared with WT C57BL/6J mice. The flow cytometry results were analyzed using the accompanying CellQuestPro software (BD Biosciences).

Cytokine ELISAs

TNF-α, IL-1β, IL-6, and IL-10 were measured using ELISA kits purchased from R&D Systems according to the manufacturer’s instructions. Briefly, 96-well microtiter ELISA plates, (Nunc MaxiSorp; Nunc Nalge International) were coated with capture Ab diluted in PBS overnight at 4°C. The plates were then blocked with PBS containing 1% BSA (blocking buffer) for 1 h and washed with wash buffer (PBS and 0.5% Tween 20). Standards of known concentrations and samples were added, then incubated for 1 h at 37°C. The plates were washed, and biotinylated detection Ab was added. After 1 h, plates were washed, avidin-HRP conjugate was added, and plates were incubated for 30 min at 37°C. After additional washing, the developer substrate was added, and the color reaction was stopped with 2 M H2SO4. Absorbance readings were measured using an ELISA plate reader (Molecular Devices) set at 450–570 nm wavelength. Extrapolation of values for sample data from standard curves was calculated using the SoftMax Pro software program (Molecular Devices).

Statistics

The PRISM 3.0 software program (GraphPad) was used for all statistical calculations. Cytokine production differences were compared by ANOVA with the Bonferroni multiple comparisons test. A value of p < 0.05 was considered significant.

Results

Regulatory effects of CD4 and CD8 T cells on TLR4 and TLR2 reactivity after injury

We recently reported that an intact adaptive immune system plays a role in controlling innate immune cell responses after burn injury...
Groups of Rag1

group. Three independent experiments using eight mice per TLR4 agonist, and using a highly purified preparation of specificity of the TLR responses by repeating those previous studies Thus, we wanted to extend the findings with respect to the specificity, shown to possess both TLR4 and TLR2 agonist activities (15). we used a preparation of those from WT mice.

Immune cell populations present in T/B cell-depleted splenocytes depletion by FACS to provide a basis for comparison of the innate I, the T/B cell-depletion protocol effectively eliminated the prepared from sham and burn WT mice resembled those from WT (C57BL/6J) vs spleen cell preparations from Rag1

To define which T cell subset might be involved in regulating TLR4 and TLR2 reactivity after injury, we performed a series of experiments to directly compare the LPS and PGN responsiveness of WT splenocytes with that of splenocytes from CD4 or CD8 T cell-deficient mice. These T cell-deficient mouse strains were chosen for these studies because both CD4+ and CD8+ T cells have been described to mediate regulatory activity and thus might be expected to play a role in suppressing TLR4 and TLR2 agonists than did T/B cell-depleted splenocytes prepared from burn-injured WT mice. Importantly, we found that WT and Rag1−/− splenocytes from sham mice did not significantly differ in the levels of these cytokines produced in response to either LPS or PGN stimulation. A significant increase in cell surface TLR4 or TLR2 expression after burn injury does not appear to be responsible for the observed increase in LPS- or PGN-induced cytokine production at 7 days (18). Furthermore, macrophages prepared from burn-injured Rag1−/− mice do not display differences in cell surface TLR4-MD-2 expression, suggesting that the increased LPS responsiveness by Rag1−/− splenocytes is not due to up-regulated TLR4 expression levels (data not shown). In total, these findings support the idea that injury primes the innate immune system for increased TLR4 and TLR2 activity in adaptive immune-deficient, Rag1−/−, mice.

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In these studies, TNF-α, IL-1β, and IL-6 produced in response to LPS or PGN were used to indicate the level of TLR4 or TLR2 responsiveness, respectively. As shown in Fig. 1, splenocytes from Rag1−/− burn mice produced significantly higher levels of TNF-α, IL-1β, and IL-6 in response to either TLR4 (LPS) or TLR2 (PGN) agonists than did T/B cell-depleted splenocytes prepared from burn-injured WT mice. Importantly, we found that WT and Rag1−/− splenocytes from sham mice did not significantly differ in the levels of these cytokines produced in response to either LPS or PGN stimulation. A significant increase in cell surface TLR4 or TLR2 expression after burn injury does not appear to be responsible for the observed increase in LPS- or PGN-induced cytokine production at 7 days (18). Furthermore, macrophages prepared from burn-injured Rag1−/− mice do not display differences in cell surface TLR4-MD-2 expression, suggesting that the increased LPS responsiveness by Rag1−/− splenocytes is not due to up-regulated TLR4 expression levels (data not shown). In total, these findings support the idea that injury primes the innate immune system for increased TLR4 and TLR2 activity in adaptive immune-deficient, Rag1−/−, mice.

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Table I. Immune cell populations in WT, adaptive immune cell-depleted WT, and Rag1−/− spleen cell preparations*

<table>
<thead>
<tr>
<th>Cell Types</th>
<th>C57BL/6J (WT)</th>
<th>T/B Cell-Depleted WT</th>
<th>Rag1−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 T cells</td>
<td>Sham Burn</td>
<td>Sham Burn</td>
<td>Sham Burn</td>
</tr>
<tr>
<td></td>
<td>19.1 (0.9)</td>
<td>11.6 (0.8)</td>
<td>0.95* (0.09)</td>
</tr>
<tr>
<td></td>
<td>13.6 (0.06)</td>
<td>11.3 (0.44)</td>
<td>1.24* (0.07)</td>
</tr>
<tr>
<td>B cells</td>
<td>25.1 (0.2)</td>
<td>19.1 (3.4)</td>
<td>2.23* (0.09)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>3.2 (0.12)</td>
<td>6.1 (0.6)</td>
<td>9.0 (0.8)</td>
</tr>
<tr>
<td>Macrophages</td>
<td>4.7 (0.2)</td>
<td>11.0 (0.4)</td>
<td>15.7 (0.5)</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>14.0 (0.8)</td>
<td>9.1 (0.8)</td>
<td>7.9 (0.5)</td>
</tr>
</tbody>
</table>

* Rag1−/− splenocytes and WT splenocytes were harvested at 7 days after sham or burn injury. As indicated, one group of WT spleen cells were depleted of T cells and B cells (T/B cell-depleted) using a magnetic bead-mediated depletion procedure as described in Materials and Methods. Cell preparations were then stained with FITC-labeled Abs specific for CD4, CD8, CD19 (B cells), F4/80 (macrophages), GR-1 (neutrophils), or CD11c (dendritic cells). The stained cell preparations were analyzed by flow cytometry. The results shown are the mean (SEM) percent cells expressing the indicated cell surface marker for three mice per group. *, p < 0.05 WT splenocytes versus WT T/B cell-depleted splenocytes.

FIGURE 1. Enhanced TLR2 and TLR4-induced proinflammatory cytokine production by Rag1−/− splenocytes after burn injury. Rag1−/− splenocytes and WT splenocytes harvested on day 7 after burn or sham injury and depleted of B and T cells as described in Materials and Methods were stimulated for 48 h with LPS (1 μg/ml) or PGN (10 μg/ml). TNF-α, IL-1β, and IL-6 production was measured by ELISA. Data are expressed as the mean ± SEM and are representative of three independent experiments using eight mice per group. *, p < 0.001, Rag1−/− burn mice vs WT burn mice.
TNF-α, IL-1β, and IL-6 as splenocytes from WT burn mice (Fig. 2B). Interestingly, the effect of CD4 deficiency on TLR4 and TLR2 responses was specific to burn injury, because sham splenocytes prepared from WT vs CD4−/− mice did not demonstrate significant differences in LPS- or PGN-induced cytokine production (Fig. 2A). These results suggest that CD4+ T cells might be responsible for controlling TLR4 and TLR2 responses after major injury.

We also examined the influence of burn injury on LPS- and PGN-stimulated production of IL-10 by T/B-depleted splenocytes prepared from WT, Rag1−/−, CD4−/−, and CD8−/− mice. As shown in Fig. 3, we observed a marked increase in PGN-induced IL-10 production by splenocytes from burn compared with sham mice, whereas injury did not enhance LPS-induced IL-10 production. Therefore, injury does not enhance TLR4-induced IL-10 production, but does have a marked effect on TLR2-induced IL-10. Because IL-10 is a potent counterinflammatory cytokine, this difference between TLR2 and TLR4 responsiveness might have a significant influence on the endogenous control of inflammation after exposure to different TLR ligands (22, 23). Moreover, CD4+ and CD8+ T cells appear to modulate the injury-induced increase in TLR2-induced IL-10 production in a fashion similar to TLR4-induced cytokine production (Fig. 3). These findings demonstrate that TLR4 and TLR2 stimulations result in both overlapping and distinct cytokine production profiles after injury.

Adoptive transfer of WT CD4+ T cells into Rag1−/− or CD4−/− mice suppresses the injury-induced enhancement of TLR4 and TLR2 reactivity

The observed effect of CD4 deficiency on LPS and PGN responses after burn injury suggested that CD4+ T cells might regulate the development of the proinflammatory phenotype after injury. To address this possibility, groups of Rag1−/− or CD4−/− mice received highly purified WT CD4+ T cells at the time of sham or burn injury, and 7 days later, T cell-depleted splenocytes were tested for LPS or PGN reactivity ex vivo. First, we confirmed the successful transfer of cells into mice by measuring the relative levels of WT CD4+ T cells in the spleens of Rag1−/− and CD4−/− sham and burn mice by flow cytometry. As illustrated in Fig. 4A, CD4+ T cells were easily detected in spleen cells prepared from sham or burn Rag1−/− or CD4−/− mice, although not to the same levels as those found in WT mice. Additionally, we wanted to confirm that the adoptively transferred CD4+ T cells were able to expand and survive equally well in sham and burn Rag1−/− mice to assure that the injury did not markedly influence the adoptive transfer process. This was accomplished by transferring CFDA SE-labeled WT CD4+ T cells into sham vs burn mice and measuring the halving of the dilution of fluorescent CD4+ T cells 7 days later. As illustrated in Fig. 2B, burn injury did not significantly alter the adoptive transfer of CD4+ T cells into Rag1−/− and did not significantly change their capacity to proliferate in vivo. We did, however, observe that burn injury resulted in a lower end-point level of CD4+ T cell reconstitution in CD4−/− mice (Fig. 4C), suggesting that injury did influence the expansion of...
transferred CD4⁺ T cells in CD4⁻/- mice. Despite this lower end-point percentage of CD4⁺ T cells found in burn CD4⁻/- mice, we observed that reconstituting Rag1⁻/- or CD4⁻/- mice with CD4⁺ T cells at the time of injury resulted in nearly complete down-modulation of TLR4 and TLR2 responsiveness to levels observed in burn WT mice (Fig. 5). With the exception of IL-6 production in burn-injured Rag1⁻/- mice, there was no significant difference in cytokine production levels by splenocytes from WT vs CD4⁺ T cell-recipient Rag1⁻/- or CD4⁻/- mice. Nonetheless, there remained a significant level of priming for augmented LPS- or PGN-induced TNF-α, IL-1β, and IL-6 release by the adaptive immune cell-depleted splenocytes prepared from CD4⁺ T cell-reconstituted burn compared with sham-injured mice. Thus, the in vivo presence of CD4⁺ T cells appears to be sufficient to reduce the enhanced TLR4 and TLR2 responses observed in burn-injured Rag1⁻/- or CD4⁻/- mice to levels comparable to those seen in WT burn mice.

**CD4⁺CD25⁺ T cell subset is responsible for controlling TLR4 and TLR2 responses after injury**

Because CD4⁺ T cells appeared to regulate LPS- and PGN-induced cytokine production after injury, we wanted to learn whether CD4⁺CD25⁺ Treg might be the cell type responsible for this finding. To address this question, WT CD4⁺ T cells were purified and then separated into CD4⁺CD25⁺ and CD4⁺CD25⁻ populations. These fractionated CD4⁺ T cell subsets were subsequently transferred into Rag1⁻/- mice (2 × 10⁶ cells of either subset/mouse) at the time of sham or burn injury. Seven days after sham or burn injury, T cell-depleted splenocytes prepared from these mice were tested for LPS- or PGN-stimulated TNF-α, IL-1β, or IL-6 production. The results presented in Fig. 6 demonstrate that the adoptive transfer of CD4⁺CD25⁺ T cells, but not CD4⁺CD25⁻ T cells, into Rag1⁻/- mice reduced LPS- or PGN-induced TNF-α, IL-1β, or IL-6 production levels to those observed in WT burn mice. Although transferring CD4⁺CD25⁺ T cells into Rag1⁻/- mice did not reduce TLR-induced cytokine production levels to those in sham mice, they had a comparatively higher ability to suppress the burn-enhanced TLR4 and TLR2 reactivities than CD4⁺CD25⁻ T cells. Thus, the regulatory effect of CD4⁺ T cells on TLR4 and TLR2 responses in burn-injured mice Rag1⁻/- mice appears to be mediated principally by the CD4⁺CD25⁺ T cell subset.

**Discussion**

A heightened reactivity of the innate immune system to microbes would presumably be of benefit to the injured host. In contrast, an increased systemic inflammatory response against pathogens and their toxic products can contribute to the development of severe and potentially fatal inflammatory responses. Our previous studies had established that mice develop an increase in TLR4 or TLR2 responsiveness by 1 wk after injury, and that this increase in TLR reactivity was even more pronounced in burn-injured, Rag1-deficient mice (12, 18). This suggested that the adaptive immune system must play a significant role in controlling innate immune responses after injury. Indeed, we showed that the adoptive transfer of WT splenocytes into Rag1⁻/- could suppress the amplified TLR4 and TLR2 reactivity that was evident in burn-injured Rag1⁻/- mice (12). We undertook the present study to determine which adaptive immune cell type(s) might be responsible for controlling the development of the augmented proinflammatory phenotype after injury.

Although B cells are absent in Rag1⁻/- mice and could potentially control inflammatory responses through cellular or Ig-mediated mechanisms, we chose to focus on T cells in this study because they have been shown to act more often as regulatory or suppressor cells (19, 20, 24, 25). Taking advantage of the availability of CD4 or CD8 T cell-deficient mice bred into the same background strain as the Rag1⁻/- mice, we first questioned whether either of these deficiencies could lead to higher TLR responses after burn injury. We found that CD4⁻/- mice, in contrast to CD8⁻/- mice, exhibited an injury response phenotype similar to what we observed in Rag1⁻/- mice, in that the response to LPS or
PGN stimulation was markedly higher in splenocytes prepared from burn-injured CD4<sup>+</sup> mice than in those from WT mice. This indicated that CD4 cells (most likely T cells) must play an active role in suppressing the development of heightened TLR responses at 1 wk after injury. This finding also ruled out a contributing role for CD8<sup>+</sup> T cells in regulating TLR4 or TLR2 reactivity after injury. Because it seemed likely that CD4<sup>+</sup> T cells might be the principal cell type responsible for controlling TLR responses after injury, we next tested whether the adoptive transfer of WT CD4<sup>+</sup> T cells into Rag1<sup>−/−</sup> or CD4<sup>−/−</sup> mice might restore the injury-induced increase in TLR4 and TLR2 reactivity to WT levels. The results of those experiments showed that this was, in fact, the case. These findings confirm that CD4<sup>+</sup> T cells play a dynamic role in regulating the innate host response to injury.

Over the past several years, a number of investigators have described the existence of a naturally occurring subset of CD4<sup>+</sup> T cells with the capacity to control a variety of inflammatory and autoimmune diseases (13, 26, 27). These cells express the IL-2 receptor (CD25) on their cell surface and are thus referred to as CD4<sup>+</sup>CD25<sup>+</sup> Treg (28). Based on these findings, we wondered whether Treg cells might be the subset of CD4<sup>+</sup> T cells responsible for controlling the injury-induced increase in TLR reactivity. Thus, we separated purified CD4<sup>+</sup> T cells into a CD25<sup>+</sup> or CD25<sup>−</sup> cell population and transferred them separately into Rag1<sup>−/−</sup> mice to compare their relative abilities to control TLR4 or TLR2 activity after burn injury. We found that the transfer of CD4<sup>+</sup>CD25<sup>+</sup> T cells into Rag1<sup>−/−</sup> burn mice reduced the TLR4 or TLR2 reactivity of splenocytes to levels observed in burn-injured WT mice. In comparison, the transfer of CD4<sup>+</sup>CD25<sup>−</sup> T cells into Rag1<sup>−/−</sup> mice did not significantly alter LPS or PGN responses in burn-injured recipient mice. Moreover, the observed modulation of LPS or PGN responses of burn Rag1<sup>−/−</sup> mice that received CD4<sup>+</sup>CD25<sup>−</sup> T cells resembled that in CD4<sup>+</sup> T cell reconstituted Rag1<sup>−/−</sup> or CD4<sup>−/−</sup> mice. Thus, it appeared that Treg cells or, more specifically, CD25-expressing CD4<sup>+</sup> T cells, were responsible for controlling the heightened TLR reactivity observed 7 days after injury in adaptive cell-deficient mice. We are aware of only one other publication that demonstrates that the CD4<sup>+</sup>CD25<sup>+</sup> T cells can control the inflammatory potential of the innate immune system. In that study, Powrie and coworkers (14) showed that CD4<sup>+</sup>CD25<sup>+</sup> T cells could suppress the development of bacterium-induced colitis in Rag-deficient mice.

In summary, the present studies indicate that CD4<sup>+</sup> T cells and, in particular, the naturally occurring CD4<sup>+</sup>CD25<sup>+</sup> T cell subset play important roles in controlling the inflammatory response for which the innate immune system is primed after serious injury.
These findings uncover another example of the regulatory interplay between cells of the innate and adaptive immune systems and suggest a protective role for Treg cells in the mammalian injury response. The mechanistic basis for how injury might modulate Treg activity or how Treg cells suppress TLR responses after injury is the subject of ongoing investigations in our laboratory. We believe that an increased understanding of how injury modulates Treg activities will reveal new insights into ways in which normal immune function could be restored after critical injury.

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Disclosures
The authors have no financial conflict of interest.

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