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 Injury¹

Thomas J. Murphy, Niamh Ni Choileain, Yan Zang, John A. Mannick, and James A. Lederer²

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 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-α, IL-1β, 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.

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3 Abbreviations used in this paper: WT, wild type; CFDA SE, carboxy-fluorescein diacetate, succinimidyl ester; PGN, peptidoglycan; Treg, regulatory T cell.
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-CD4tm1Kssn (CD4−/−), and B6.12952-CD8atm1Mak (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, penicillin/streptomycin/fungizone, 100 mM HEPES buffer, 100 µM 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. 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-Thy1.2), 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 splenocytes on wire mesh. Splenocytes were created for cell culture. Briefly, Mice were isolated using a MACS CD4+ T cell isolation kit according to the manufacturer’s instructions (Miltenyi Biotec). Brieﬂy, non-CD4+ T cells within the splenocyte suspension were indirectly magnetically labeled using a mixture of biotin-conjugated Abs against CD8a (Ly-2), CD45R (B220), DX5, CD11b (Mac-1), and Ter-119 and anti-biotin microbeads. The bead-coated, non-CD4+ T cells were depleted by passing the splenocyte suspension through a MACS LD column within a strong magnetic field. The collected effluent then contained an enriched CD4+ T cell population.

Flow cytometric analysis after staining with FITC-labeled anti-CD4 Ab confirmed that the collected effluent contained >90% CD4+ T cells. Purified CD4+ T cells were transferred to Rag1−/− and CD4−/− mice at a concentration of 5–6 × 105 cells/200 µl PBS via intracardiac injection under anesthesia just before sham or burn injury. Some mice received an intracardiac injection of PBS (200 µl) as a control. In one study designed to assess the influence of burn injury on the proliferation of adoptively transferred CD4+ T cells, the purified CD4+ T cells were transfected with the puromycin resistance gene construct (pMD2G) and with carboxy-fluorescein diacetate, succinimidyl ester (CFDA SE) using the Vybrant CFDA SE cell tracker dye as described by the manufacturer (Molecular Probes) before their transfer into Rag1−/− mice. The percentage of cells that underwent proliferation in vivo within the lymph nodes or spleen of recipient mice was determined by staining cell suspensions with Cy5-labeled anti-CD4 Ab and measuring the level of CFDA SE staining by flow cytometry.

For purification of CD25+ and CD25− T cell subsets, PE-labeled anti-CD25 Ab (contained in the Miltenyi purification kit; 10 µl/107 cells) was added to the splenocyte suspension before application to the MACS LD separation column. The CD4+ T cells in the column effluent were collected by centrifugation at 300 × g and resuspended in 90 µl of MACS buffer. Ten microliters of anti-PE microbeads were added, and after incubation at 4°C, the cells were washed, resuspended in buffer, and applied to a MACS MS column within the field of a MACS separator. The column was washed three times with buffer to elute CD25− cells, then the column was removed from the separator; the retained cells were flushed out with buffer and the plunger supplied with the column and immediately reapplied to a second MS column; and the process was repeated. Samples of the recovered CD25+ and CD25− cell populations were stained with FITC-labeled anti-CD4 Ab, and by two-color flow cytometry, the CD25− and CD25+ CD4+ cell populations were shown to be >90% pure. CD25+ or CD25− CD4+ T cells (2–3 × 106 cells/200 µl PBS) or 200 µl of PBS alone were administered to Rag1−/− mice by intracardiac injection under anesthesia at the time of burn or sham injury. Splenocyte suspensions were performed 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 Interna- tional) 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 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 TLR4 agonist, and using a highly purified preparation of specificity of the TLR responses by repeating those previous studies shown to possess both TLR4 and TLR2 agonist activities (15). We used a preparation of vs spleen cell preparations from Rag1 immune cell populations present in T/B cell-depleted splenocytes depletion by FACS to provide a basis for comparison of the innate cells in WT spleen cell preparations before and after T/B cell de-
mice.

Moreover, we found that the T/B cell-depleted spleen cells pre-

in macrophages in spleen cells prepared from burn vs sham WT

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 responsiveness (19–21). As shown in Fig. 2A, LPS or PGN stimulated splenocytes from burn-injured CD4+ mice produced higher levels of TNF-α, IL-1β, and IL-6 than splenocytes from WT burn mice. Moreover, the phenotype of CD4+ mice appeared similar to that of Rag1−/− mice with respect to their LPS and PGN reactivity after burn injury (Figs. 1 and 2A). In contrast, splenocytes from burn-injured CD8−/− mice produced approximately the same levels of responsivi
ty to LPS 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.

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.

### 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 19.1 (0.9)</td>
<td>Burn 12.8 (0.1)</td>
<td>12.8 (0.1)</td>
</tr>
<tr>
<td>CD8 T cells</td>
<td>Sham 13.6 (0.06)</td>
<td>Burn 8.9 (0.08)</td>
<td>8.9 (0.08)</td>
</tr>
<tr>
<td>B cells</td>
<td>Sham 25.1 (0.2)</td>
<td>Burn 21.1 (0.3)</td>
<td>21.1 (0.3)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Sham 3.2 (0.12)</td>
<td>Burn 5.4 (0.15)</td>
<td>5.4 (0.15)</td>
</tr>
<tr>
<td>Macrophages</td>
<td>Sham 4.7 (0.2)</td>
<td>Burn 4.7 (0.2)</td>
<td>4.7 (0.2)</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>Sham 14.0 (0.8)</td>
<td>Burn 14.0 (0.8)</td>
<td>14.0 (0.8)</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.
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<sup>−/−</sup> mice did not demonstrate significant differences in LPS- or PGN-induced cytokine production (Fig. 2A). These results suggest that CD4<sup>+</sup> 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<sup>−/−</sup>, CD4<sup>−/−</sup>, and CD8<sup>−/−</sup> 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<sup>+</sup> and CD8<sup>+</sup> 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<sup>+</sup> T cells into Rag1<sup>−/−</sup> or CD4<sup>−/−</sup> 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<sup>+</sup> T cells might regulate the development of the proinflammatory phenotype after injury. To address this possibility, groups of Rag1<sup>−/−</sup> or CD4<sup>−/−</sup> mice received highly purified WT CD4<sup>+</sup> 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<sup>+</sup> T cells in the spleens of Rag1<sup>−/−</sup> and CD4<sup>−/−</sup> sham and burn mice by flow cytometry. As illustrated in Fig. 4A, CD4<sup>+</sup> T cells were easily detected in spleen cells prepared from sham or burn Rag1<sup>−/−</sup> or CD4<sup>−/−</sup> mice, although not to the same levels as those found in WT mice. Additionally, we wanted to confirm that the adoptively transferred CD4<sup>+</sup> T cells were able to expand and survive equally well in sham and burn Rag1<sup>−/−</sup> mice to assure that the injury did not markedly influence the adoptive transfer process. This was accomplished by transferring CFDA SE-labeled WT CD4<sup>+</sup> T cells into sham vs burn mice and measuring the halving of the dilution of fluorescent CD4<sup>+</sup> T cells 7 days later. As illustrated in Fig. 2B, burn injury did not significantly alter the adoptive transfer of CD4<sup>+</sup> T cells into Rag1<sup>−/−</sup> 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<sup>+</sup> T cell reconstitution in CD4<sup>−/−</sup> 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 × 10^6 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 seen 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<sup>+</sup> 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> mice could 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 CD25 on their cell surface and are thus referred to as CD4<sup>+</sup> Tregs. Recent work from our laboratory has indicated that burn injury markedly augments Treg activity, as evidenced by increased expression of cell surface TGF-β1, increased IL-10 production, and enhanced ability of these cells to inhibit the proliferation of CD3-stimulated T cells (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> CD4<sup>+</sup> T cell population and transferred them separately into Rag1<sup>−/−</sup> mice to compare their relative abilities to control TLR4 or TLR2 reactivity 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 burn Rag1<sup>−/−</sup> mice and WT burn mice with respect to TNF-α and IL-1β production (p > 0.05). *p < 0.05, sham vs burn groups; #p < 0.05, burn CD4<sup>+</sup>CD25<sup>−</sup> vs CD4<sup>+</sup>CD25<sup>+</sup> T cell-recipient mice groups.
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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