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Intestine Immune Homeostasis after Alcohol and Burn Injury

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Abstract

Traumatic injury remains one of the most prevalent reasons for patients to be hospitalized. Burn injury accounts for 40,000 hospitalizations in the United States annually, resulting in a large burden on both the health and economic system and costing millions of dollars every year. The complications associated with post-burn care can quickly cause life-threatening conditions including sepsis, multiple organ dysfunction and failure. In addition, alcohol intoxication at the time of burn injury has been shown to exacerbate these problems. One of the biggest reasons for the onset of these complications is the global suppression of the host immune system and increased susceptibility to infection. It has been hypothesized that infections following burn and other traumatic injury may stem from pathogenic bacteria from within the host's gastrointestinal tract. The intestine is the major reservoir of bacteria within the host, and many studies have demonstrated perturbations of the intestinal barrier following burn injury. This article reviews the findings of these studies as they pertain to changes in the intestinal immune system following alcohol and burn injury.

Introduction

Trauma remains a major public health problem in United States and throughout the world. This alone causes 37 million patients to visit emergency departments, and results in 2.6 million hospital admissions and 150,000 deaths each year in the United States (1; 2). Trauma is also a leading of cause of severe disability, and creates a major burden on the

health care system (1–3). There are substantial data suggesting a relationship between the use of alcohol and trauma (4; 5). Nearly half a million burn injuries are reported annually within the United States (6), and approximately 50% of these occur under the influence of alcohol (ethanol) intoxication (7–17). A similar number of other traumatic injuries are also reported to occur under the influence of ethanol intoxication (7; 11; 14; 16). These reports further suggest that ethanol intoxication is not simply a risk factor leading to traumatic injury, but also presents a unique challenge in the treatment of patients who survive the initial insult (7–16). Burn patients who are intoxicated at the time of injury exhibit a higher incidence of infection, and higher morbidity and mortality compared to patients with a similar extent of injury but have not consumed ethanol prior to injury (7–16). Although both chronic and acute ethanol consumption is likely to confound the pathology associated with burn and other traumatic injury, studies have shown that the majority of burn patients are not chronic alcoholics (7; 10–12; 14). Rather, they have consumed ethanol on an acute basis before injury (7; 11; 12; 14). The most common causes of death in patients who survive the initial injury are sepsis, and the development of multiple organ dysfunction and failure. While a number of studies have demonstrated that the size of burn is a critical factor in the overall outcome from the injury (18–20), others have suggested that age and gender can also influence the outcome of burn patients specially in patients with smaller burn injuries (21–29). Likewise, alcohol consumption at the time of burn injury has been shown to further confound post-burn pathogenesis (9; 14; 17; 30–37). Additional findings suggest that a smaller burn which by itself may not have any deleterious effects on host defense, but when combined with intoxication may become detrimental. Regardless of the initial insult a large number of studies have suggested that the gut barrier is compromise after alcohol and burn injury (38–43). The maintenance of gut barrier integrity is complex and is composed of many elements including a mucus layer, epithelial cell lining, and a host of innate and adaptive immune defenses. This article will review studies published in the area of gut immune responses after burn injury, and whether the presence of alcohol exposure at the time of injury influences this complex, highly regulated system.

Intestinal barrier and immunity

With its large surface area, the intestine functions mainly in absorption of nutrients and water. As a result, the gut is regularly exposed to the external environment, and constantly comes in contact with large numbers of microorganisms and dietary antigens. In humans, there are about 10^{9-12} microorganisms per gram of feces, with more than 400 species in the lower part of the intestine. Most bacteria are beneficial and establish a symbiotic relationship with the host, primarily by aiding in nutrient metabolism. Additionally, these commensal bacteria protect the host from infection by both limiting pathogenic bacterial colonization in the intestinal lumen, and by stimulating immune responses against pathogens (44; 45).

In addition to the commensal bacteria that provide host defense against pathogens, the intestine also contains a physical barrier comprised of intestinal epithelial cells (IECs) that serve many functions in preventing pathogen infiltration from the gut lumen (45–47). One of the primary functions of IECs is to form a physical barrier, which is comprised of tight junctions, adherens junctions, and desmosomes. These inter-epithelial spaces permit

selective absorption of nutrients into the circulation, and prevent bacteria and their products from translocating to extra-intestinal sites, such as mesenteric lymph nodes (MLNs), spleen, liver and blood. Specialized epithelial cells, goblet cells, secrete a layer of mucus that covers the luminal surface of the intestine. This mucus layer establishes a barrier that prevents bacteria from directly contacting the epithelial layer. IECs also serve immune functions by secreting antimicrobial proteins (AMP's). Since the intestine contains large amounts of bacteria and has a huge surface area, some bacteria infiltrate this barrier, which results in activation of the intestinal immune system.

The intestinal immune system, referred to as gut-associated lymphoid tissue (GALT), is comprised of Payer's patches (PP), mesenteric lymph nodes (MLN), and the cells present in the lamina propria (LP) and epithelial layers (48–52). Both organized (PP and MLN) and diffuse compartments of GALT contain all conventional immune cells including T and B cells, macrophages (M ϕ) and dendritic cells (DC). PP have specialized epithelial cells called "M cells" through which pathogens normally enter (51–57). Pathogens that cross the gut epithelial barrier through the M cells directly encounter M ϕ and DCs, which are present in intraepithelial pockets under M cells (50–61). These cells phagocytose bacteria and kill the ingested organisms by releasing antimicrobial proteins and reactive oxygen species (44). PPs are generally believed to be the sites responsible for generation of effective immune responses in the intestine. They are the principal sites for T cell priming and B cell differentiation into IgA producing plasma cells (50–57; 61–63). These IgA-producing plasma cells migrate to the lamina propria and secrete dimeric IgA, which is transported across the epithelial layer where it binds to intestinal bacteria to prevent translocation to extra-intestinal host tissues (44). Bacterial translocation is an ongoing physiological phenomenon, but intact PP and MLN immune cell functions kill these bacteria and MLNs remain relatively sterile.

Macrophages

Macrophages are one of the most abundant leucocytes in the intestine, and are the largest population of mononuclear phagocytes in the body (64). Macrophages are found throughout the intestine, both in the mucosa and within the epithelial layer and lamina propria. Findings from clinical and experimental studies have shown that macrophages are an important mediator in the regulation of systemic immune responses after alcohol, burn, and traumatic injury (18; 65–69). During inflammation, large numbers of macrophages rapidly migrate to the intestinal mucosa to capture and kill pathogens, as well as clear apoptotic cells and debris. As a result, macrophages respond by producing pro-inflammatory cytokines and chemokines. TNF α upregulates adhesion molecules on local blood vessels, and IL-8 induces recruitment of mononuclear cells and granulocyte cells to the intestine. These factors result in fibroblast proliferation and collagen deposition in the intestinal epithelium. In addition, macrophages in the mucosa of the small and large intestines produce IL-1 and IL-18 and can cause epithelial apoptosis and barrier dysfunction, as well as vascular damage and necrosis (70). Elevated IL-1, IL-6, TNF α and PGE $_2$ also have been found in serum and tissue samples obtained from burn-injured patients (71–73). Isolated enterocytes and intestinal macrophages from burn animals cultured for 24 hours exhibited an increase in IL-6 (74). In support of these findings, studies from our laboratory have demonstrated that ethanol

intoxication combined with burn injury increased IL-6, IL-18, IL-10 and KC production in the intestine, lung and liver tissue of mice (75–77). While the cellular source of these cytokines in our studies remain to be established, elevated macrophage-derived cytokines, specifically IL-6 has been correlated with severity of the injury and a poor prognosis in burn patients (72; 78; 79). IL-6 and IL-18 were found to delay neutrophil apoptosis and enhance superoxide production in circulation (80). IL-6 also suppresses T cell responses in a murine model of ethanol combined with burn injury. Splenic T cells treated with monoclonal antibodies against IL-6 prevented T cell suppression after ethanol and burn injury (81; 82), and depletion of intestinal resident macrophages prevents mucosal damage following intestinal ischemia reperfusion injury (83). Together these findings suggest that macrophages are important regulators of both systemic and gut mucosal integrity, but their role in response to alcohol and burn injury remains to be investigated.

Dendritic cells

Dendritic cells are present in all GALT sites in the small and large intestines. In healthy individuals, DCs aid in maintaining intestinal homeostasis by activating regulatory T cells (T_{reg} Cells) to reduce the immune response to normal commensal bacteria. DCs can act as a bridge between the innate and adaptive immune systems due to their ability to interact directly with both arms of the immune system through differential receptor expression. DCs take up antigens from both commensal and pathogenic bacteria, and migrate via the lymphatic vessels to MLN, where they activate naive CD4 positive T cells (84). The differentiation of naive CD4⁺ T cells into effector T helper cells requires activation of their T cell receptor and co-stimulatory molecules in the presence of specific cytokines produced by innate immune cells. Intestinal DCs produce IL-12 to drive the differentiation of Th1 cells, and IL-4 to initiate the differentiation of Th2 cells against extracellular pathogens. Furthermore, DCs-producing IL-10 and TGF- β trigger regulatory T cell (T_{reg} cell) differentiation to produce anti-inflammatory cytokines for intestinal tolerance (84; 85). DCs are not well studied in response to burn injury with or without ethanol exposure. One study carried out by Fujimi et al. suggest that burn injury does cause some changes in splenic DCs but these changes do not appear to alter their antigen-presenting activity significantly (86). However more studies are needed to fully understand their role in the maintenance of gut mucosal immunity after burn alone or combined with prior alcohol exposure.

T cell subsets

Several studies have shown that major burn injury results in global suppression of host immunity. This suppression in host immune defenses is attributed to a host of differential T cell immune effector function deficits (see Table 1). Additional studies suggest that alcohol further exacerbates these T cell function deficits. For example, alcohol combined with burn injury results in decreased delayed type hypersensitivity, greater suppression of mitogen-induced splenic-lymphocyte proliferation, and increases susceptibility to bacterial infection (7; 11; 14; 68; 81; 82; 87–91). Further findings suggest suppression of intestinal immunity and increases in intestinal permeability in rodents receiving a combined insult of alcohol and burn injury (30; 92–94). Such changes in intestinal barrier integrity can lead to increased translocation of gut bacteria or their products. Gut bacterial translocation has been

demonstrated in critically injured patients as well as in patients with terminal illnesses (39; 43; 95; 96). Similar increases in gut bacterial translocation are also reported in animal models of acute injuries (38; 40; 42; 97–104). These gut-derived bacteria can cause infection and perpetuate/exacerbate post-injury pathogenesis leading to sepsis and multiple organ dysfunction in patients with acute injuries (38–43; 59; 95–107).

Bacteria or their products can stimulate intestinal immune cells to release inflammatory cytokines such as IL-1, IL-6, IL-10, IL-12, IL-18, IL-23 and TGF β . The release of these cytokines induces the recruitment of neutrophils to the site of inflammation (108), and plays a decisive role in the differentiation of naive T cells into Th1, Th2, Th17 and regulatory T cells (Treg), which are a crucial components of intestinal homeostatic regulation (Figure 1). Additionally, intestinal innate lymphoid cells (ILCs), such as NK-like cells, lymphoid tissue inducer (LTi) cells, and $\gamma\delta$ intestinal lymphocytes ($\gamma\delta$ IEL) respond to pro-inflammatory cytokines by producing IL-22, IL-17A and IL-17F. IL-22 acts on the intestinal epithelial cells to enhance antimicrobial defense and epithelial barrier integrity, while IL-17A and IL-17F are involved in the recruitment of neutrophils (109). Collectively, the cells of the intestinal immune system play an important role in minimizing harmful effects from resident bacteria. Findings from our laboratory and others have clearly demonstrated that traumatic conditions such as burn injury combined with alcohol exposure cause a disruption in intestinal T cell functions in many regions within the gut (30; 110–114).

Th1 effector functions

Under physiological conditions, ligation of the T cell receptor (TCR) provides the primary stimulus required for T cell activation (Figure 2). Additionally, co-stimulation of T cells through CD28 with CD80 (B7-1) or CD86 (B7-2) molecules on antigen presenting cells (M ϕ and DC) further augments and sustains T cell activation (137–140). Conversely, an interaction of T cell cytotoxic lymphocyte-associated molecule-4 (CTLA4; CD152) with the same B7-1 or B7-2 molecules on M ϕ and DC results in attenuation of T cell responses. Thus, CD28 and CTLA4 have crucial, yet opposing functions in T cell activation (Figure 2). In addition, the cytokine milieu at the time of T cell activation as shown in Figure 1 dictates subsequent differentiation of T cells to IL-2/IFN- γ producing Th-1, or IL-4/IL-10 producing Th-2 cells (18; 136; 141–143). In this regard, IL-12 and IL-18 released from M ϕ and DC are shown to promote the IL-2/IFN- γ producing Th-1 type cells.

Conversely, IL-10 release helps T cell differentiation towards a Th-2 phenotype (18; 136; 141–143). Studies from our laboratory carried out in a rodent model of ethanol intoxication combined with burn injury demonstrated a significant decrease in PP and MLN T cell IFN- γ release (30; 92; 93). This was accompanied with a decrease in IL-12 and increase in IL-18 levels (30; 75; 110; 113; 114). Although an increase in IL-18 may compensate for the decrease in IL-12, as both IL-12 and IL-18 are known to have synergistic effects on T cell IFN- γ production, our findings suggest that IL-18 alone does not induce IFN- γ in the absence of IL-12.

Th17 effector functions

Similarly, the presence of IL-6, IL-23 and TGF- β promote T cell differentiation into Th17 cells (Figure 1). Recent studies have indicated that IL-17-producing Th17 cells function in the clearance of specific pathogens that are not adequately killed by Th1 and Th2 immunity. However, only a few studies have examined Th17 effector functions in response to burn injury. One clinical study reported that peripheral blood mononuclear cells isolated from burn patients with third-degree burn injury exhibited decreased Th17 differentiation and IL-17 production in response to TCR activation and *C. albicans* challenge (121). Consistent with these findings, we have shown that acute ethanol intoxication combined with burn injury significantly decreases IL-17 and IL-22 production in PP cells stimulated with CD3 and CD28. Furthermore, there are significant decreases in intestinal IL-22, RegIII β and RegIII γ expression, which are accompanied with increases in intestinal permeability and bacterial overgrowth in animals receiving ethanol intoxication combined with burn injury. Treatment of animals with rIL-22 normalizes the expression of RegIII β and RegIII γ in the small intestine, and prevents the increase in intestinal permeability, as well as reduces intestinal bacterial growth following ethanol and burn injury (123; 144). Together, these findings suggest that Th17 cells play a critical role in the maintenance of the intestinal mucosal barrier and immune responses after ethanol and burn injury.

In contrast to these findings, several studies have reported that severe burn injury alone (25% TBST) results in a 3-fold increase in IL-17 and IL-22, but not IL-6, TGF- β and IL-23 in the injury site 3 hours after burn injury (145). The elevated levels of IL-17 have also been observed in cardiac tissue 3 hours after burn injury (146). One clinical study reported that elevated levels of IL-17 in the circulation are observed in adult and pediatric burn patients within 1 week after injury (147). These studies suggest that burn injury disrupts Th17 responses at multiple time points and in multiple organs after injury. It is possible that the early elevated IL-17 may contribute to the burn induced inflammatory response and subsequent immune aberrancies. Later suppression of Th17 responses may result in decreased resistance to bacterial infection and disruption of the intestinal mucosal barrier following burn and other traumatic injuries (122). However, the mechanism(s) by which changes in Th17 cells and their effector cytokines modulate intestinal barrier function after burn and traumatic injury remains largely unexplored and needs further studies.

Regulatory T cells

Tregs have a highly important role in modulating immune responses by balancing the activation of the effector T cells in the gut. Tregs in the intestines are derived from naïve CD4⁺ T cells in the gut, which are driven to a Treg phenotype in presence of TGF- β , IL-10, and Foxp3 expression (Figure 1). Additionally, Tregs can migrate to the intestine from thymic CD4⁺CD25⁺Foxp3⁺ precursors. One study in a mouse model of burn injury demonstrated that Tregs are also able to regulate innate immune responses by inhibiting TLR2 and TLR4 cytokine signaling in spleen (148). Others have reported Treg TCR signaling is preferentially activated following traumatic injury. These studies show Tregs suppress Th1 cytokine responses, and are the largest producers of IL-4, IL-5, and IL-10 following burn injury (126; 127; 149). Currently, there is no information about Tregs in the

intestines following burn or combined alcohol and burn injury. Future studies will be needed to examine if the balance between effector T cells and Tregs is disrupted and how this may potentiate further complications associated with traumatic injury.

Potential signaling mechanisms of T cell differentiation

As shown in Figures 1 and 2, T cell activation and differentiation into various subsets is complex, and regulated at multiple levels (7; 30; 150–154). T cell stimulation through the T cell receptor results in activation of T cell receptor-associated molecules (e.g. P56^{lck} and P59^{fyn}, Zap-70). These molecules phosphorylate phospholipase C- γ (PLC- γ), which activates IP₃ and DAG. While IP₃ increases intracellular calcium concentration; DAG activates PKC (150; 152). These signals result in the activation of mitogen activated protein kinases (MAPK). MAPK (i.e., p-38, Erk-1/2 and JNK) then relay the information to the nucleus through a series of downstream signaling molecules leading to T cell activation (151). Activated T cells mitotically divide and produce IL-2 and this in turn leads to T cell proliferation and cytokine production.

Previous studies have demonstrated that severe burn (~25% TBSA) alone results in alterations in T cell receptor associated src kinases P59^{fyn} and other downstream signaling molecules PKC activity, and Ca²⁺ signaling (40; 135; 155–161). Additional findings suggest that while a relatively small burn injury (~12.5% TBSA) does not produce alterations in T cells signaling, when combined with prior alcohol exposure it produces changes in T cell signaling similar to those observed after severe burn. These studies have shown a significant decrease in the MAPK signaling pathway (e.g., p38 and ERK phosphorylation) in MLN T cells following ethanol combined with burn injury (30; 110; 114; 162). Since T cell receptor associated molecules (e.g. src kinases and Zap-70 and PKC etc.) are upstream of p38 and ERK, suppression of TCR associated molecules may potentially contribute to p38 and ERK inhibition in T cells following ethanol combined with burn injury. Furthermore, phosphorylation of p38 and ERK is also regulated by protein phosphatases (112; 151; 152; 158; 162–164). T cells express several phosphatases including serine/threonine-specific phosphatases type-1 (PP1), type-2A (PP2A), and protein tyrosine phosphatases (PTP). These phosphatases can inhibit p38 and ERK signaling. Studies from our laboratory have demonstrated that ethanol combined with burn injury (~12.5% TBSA) is accompanied with increases in PP1 phosphatase activity in T cells. Treatment of T cells with phosphatase inhibitors increased the activity of p38 and ERK after alcohol and burn injury (112; 162). These findings suggest that burn or combined ethanol and burn injury disrupts the balance between phosphatases and kinases, resulting in decreased T cell activation and proliferation (165).

In addition to TCR-initiated signals, studies have shown T cell differentiation into Th-1 or Th-2 cells depends on additional signals provided by IL-12 and IL-4, respectively (150–154; 166–174). IL-12 is a heterodimeric protein consisting of p40 and p35 subunits, and interacts with transmembrane glycoproteins of the IL-12 receptor (IL-12R). IL-12R contains two domains, IL-12R β 1 and IL-12R β 2 (170). IL-12 R β 1 binds to IL-12p40 whereas IL-12 R β 2 recognizes either IL-12p40 or IL-12p35 subunits. Signaling of the IL-12R is complex and includes phosphorylation and translocation of several Signal Transducing Activator

Transcription factors (STAT), but is predominately through STAT-4 to promote Th1 differentiation (167; 168; 171; 175). On the other hand, IL-4-mediated Th-2 differentiation involves STAT-6 (154; 166; 168). Furthermore, activation of STAT-4 is also shown to up-regulate IL-18R complex while the activation of STAT-6 in response to IL-4 down-regulates IL-18R expression (176–179). Thus STAT-4 and STAT-6 provide a second signal for expansion of activated T cells into Th-1 (IL-2/IFN- γ) and Th-2 (IL-4/IL-10) subsets, respectively. In addition to STAT signaling, other studies have also implicated a role of p-38 in IL-12-mediated Th-1 differentiation (151). We observed that while IL-12 normalizes both p38 and ERK activation in T cells, results obtained using p38 and ERK inhibitors indicate that the restoration of ERK plays a predominant role in IL-12-mediated restoration of T cell IL-2 and IFN- γ production after ethanol and burn injury (30; 110; 114; 162).

The differentiation of naive T cells to Th17 involves a several step process (Figure 1). First, TGF- β 1 and IL-6 initiate the development of Th17 cells. Second, autocrine production of IL-21 amplifies the Th17 phenotype. Finally, IL-23 expands and stabilizes Th17 cells *in vivo* (180). IL-23 is one member of the IL-12 family (181), and shares the IL-12R β 1 receptor with IL-12. IL-23 binds to its receptor and activates multiple STAT proteins (including STAT1, 3, 4, and 5) (182–184), however, STAT3 is the predominant player in the IL-23 signaling pathway. Once phosphorylated, STAT3 proteins homodimerize and translocate to the nucleus to upregulate downstream signaling pathways, including the expression of the retinoic acid-related orphan receptor (ROR)- γ t transcription factor (185–187). Activation of ROR- γ t results in the production of Th17 effector cytokines IL-17 and IL-22. In addition, other transcription factors including ROR α , interferon regulatory factor (IRF) 4, and the aryl hydrocarbon receptor (AHR) have also been demonstrated to have significant roles in the production of IL-17 and/or IL-22 (185; 188–191). Studies from our laboratory have demonstrated that acute ethanol combined with burn injury suppressed the Th17 cells producing IL-17 and IL-22 release in PP cells after stimulation with CD3 and CD28 compared to shams. Intriguingly, when cultured with IL-23, these cells produce a several-fold increase in IL-22 release in PP cells in both sham and ethanol combined with burn injury. However, IL-17 levels were not significantly influenced by IL-23 restitution, suggesting that IL-23 differentially regulates IL-17 and IL-22 following ethanol and burn injury. Furthermore, our data demonstrate a role for AHR in IL-23-mediated restoration of IL-22, and indicate that AHR may be involved in the well-described IL-23/ROR- γ t pathway (123; 144). Recent studies demonstrate that AHR may interact with multiple signaling molecules, including STAT proteins and members of the ROR family (189; 191). Since the activation of PKC and Ca²⁺ signaling is critical to T cell activation, we cultured PP T cells with PMA and ionomycin to directly activate calcium and PKC pathways. We observed that PP cells cultured with CD3 and CD28 together with PMA and ionomycin prevent the decrease in IL-17 release, but not IL-22. This finding suggests that signaling molecules upstream of calcium and PKC are also likely involved in regulation of Th17 responses following ethanol and burn injury (123). It is important to note that most of the studies performed to examine T cell differentiation signaling have been performed *in vitro*. Thus, future studies should aim to explore these mechanisms *in vivo* to elucidate the potential impact of these molecular pathways.

Conclusion

The effects of trauma on the gut are profound and can lead to life-threatening complications following injury. Due to the complexity of the intestines and the layers of regulation that maintain homeostasis, many questions remain unanswered pertaining to the role of the intestines following burn injury and other forms of trauma. This review has highlighted many aspects of the intestinal immune barrier and immune system, and discussed what is currently known about the roles of various immune cells. Clearly, disruptions in various immune cells, specifically in subsets of T cells, can potentially perturb the host gut barrier integrity after injury. Future studies will certainly need to focus on how these specific immune cell subtypes help or hinder healing, and find translational ways to minimize intestinal damage following injury. The role of Treg cells have been found to be critical in many diseased and inflammatory conditions, and are thus an essential piece of future research for the trauma field. Finally, future work should focus on how alcohol causes additional deterioration of the intestinal barrier after traumatic injury, as a large number of these injuries occur while intoxicated.

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Figure 1. T cell differentiation into various subsets
Flow diagram of T cell differentiation into Th1, Th2, Th17, and Treg subsets under homeostatic (left) and following burn injury (right). Cytokines in red are decreased in intestinal tissues following injury, cytokines in green are increased in intestinal tissues following injury.

Figure 2. Classical T cell activation and inhibition

T cell activation (left) occurs when the T cell receptor is presented with antigen loaded onto MHC molecules expressed on antigen presenting cells. The binding of the T cell co-receptor CD28 with CD80/86 molecules on antigen presenting cells contributes to T cell activation. T cell inhibition (right) occurs when the T cell inhibitory molecules CTLA-4 binds to CD80/86 on antigen presenting cells.

Table 1

List of immune dysfunctions observed after traumatic injury.

Dysfunction	References
T cell Depletion	(115; 116)
Decreased T cell proliferation	(40; 117–120)
Decreased Th17 cytokine production	(114; 121–123)
Deficient inflammatory cytokine production	(124–127)
T cell Anergy	(92; 128; 129)
Dysfunctional Dendritic Cells	(130; 131)
Increased Nitric Oxide Production by macrophages	(132; 133)
TLR co-stimulation	(124; 134)
NFkB and AP-1 alterations	(135; 136)