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Review

Th17 cells: critical mediators of host responses to burn injury and sepsis

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RECEIVED FEBRUARY 15, 2012; REVISED JUNE 8, 2012; ACCEPTED JUNE 11, 2012. DOI: 10.1189/jlb.0212083

ABSTRACT

Th cells have long been recognized as vital components of the adaptive immune system. Until recently, $CD3^+CD4^+$ Th cells were divided into cell-mediated Th1 or humoral Th2 responses. However, the Th1-Th2 hypothesis failed to accommodate the more recently described Th17 cells. Today, the major Th cell subsets include Th1, Th2, Th9, Th17, Th22, and Tregs, each of which produce specific effector cytokines under unique transcriptional regulation. Specifically, Th17 cells produce effector cytokines IL-17, IL-21, and IL-22 under the regulation of ROR-γt. Th17 lymphocytes were first described as orchestrators of neutrophil recruitment and activation and as key players in chronic inflammation and autoimmunity. More recent evidence suggest that Th17 lymphocytes and their effector cytokines play a crucial role in maintaining mucosal immunity and barrier integrity, including the skin, lung, and gut. Burn injury induces global changes to the systemic immune response, including suppressed immune function and increased susceptibility to infection. Moreover, burn trauma is associated with remote organ injury. This relationship between burn and remote organ injury supports the hypothesis that immune suppression may facilitate the development of sepsis, systemic inflammatory response syndrome, and multiple organ dysfunction syndrome in critically ill burn patients. Herein, we discuss this emerging adaptive cell subset in critical care settings, including burn injury and clinical sepsis, and highlight the potential therapeutic role of IL-22. *J. Leukoc. Biol.* 92: 529–538; 2012.

Abbreviations: $^{-/-}$=deficient, AhR=aryl hydrocarbon receptor, ALT=alanine aminotransferase, AST=aspartate aminotransferase, Bcl=B cell lymphoma, CLP=cecal ligation and puncture, DSS=dextran sodium sulfate, EAE=experimental autoimmune encephalomyelitis, FICZ=6-formylindolo[3,2-b]carbazole, Foxp3=forkhead box p3, HepG2=hepatocellular carcinoma cell line, HIF=hypoxia-inducible factor, IBD=inflammatory bowel disease, IL-22TG=IL-22 transgenic, ILC22= IL-22 producing innate lymphoid cells, KO=knockout, Mϕ=macrophage(s), Rb2=retinoblastoma 2, ROR=retinoic acid-related orphan receptor, Tc=cytotoxic T cell, TCDD=2,3,7,8-tetrachlorodibenzo-p-dioxin, Treg=regulatory T cell

INTRODUCTION TO Th17 LYMPHOCYTES

Th cells have long been recognized as vital components of the adaptive immune system [1, 2]. Classically, Th cells are generated following antigen presentation by APCs, including DCs and Mϕ, in secondary lymphoid tissues [3]. Until recently, $CD3^+CD4^+$ Th cells were divided into cell-mediated Th1 (IFN-γ-producing) or humoral Th2 (IL-4-producing) responses. The effector cytokines produced by Th1 and Th2 were further predicted to regulate the development and function of the alternate subset [1]. However, the Th1-Th2 hypothesis failed to accommodate the more recently described IL-17-producing $CD3^+CD4^+$ Th17 cells [4]. Today, the major Th cell subsets include Th1, Th2, Th9, Th17, Th22, and Tregs, each of which produces specific effector cytokines under unique transcriptional regulation [5, 6]. Specifically, Th17 cells produce effector cytokines IL-17, IL-21, and IL-22 under the regulation of ROR-γt [7–9].

IL-17 cDNA was originally cloned from murine hybridomas, where it was noted to share 58% homology with ORF 13 from the T lymphotropic herpes virus saimiri [10, 11]. However, IL-17 was not identified as a Th cytokine until 2000, when Infante-Duarte et al. [4] demonstrated IL-17 production from Th cells in response to microbial lipopeptide challenge. IL-22 was first cloned from T cells and found to share 22% aa identity with IL-10 [12]. Further analysis confirmed that IL-22 expression required and induced activation of the STAT proteins [12]. Coexpression of IL-17 and IL-22 eventually led to the recognition of these molecules as effector cytokines of Th17 cells [13, 14].

Th17 effector cytokines IL-17 and IL-22 have been studied immensely since the early 2000s [8, 15, 16]. Although Th17 lymphocytes were first described as orchestrators of neutrophil recruitment and activation and as key players in chronic inflammation and autoimmunity [8, 17], more recent evidence suggests that Th17 lymphocytes and their effector cytokines play a crucial role in maintaining mucosal immunity. Th17

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lymphocytes have been shown to protect against various pathogens, including *Bacteriodes fragilis*, *Klebsiella pneumoniae*, and *Candida albicans*, which if not contained properly, may become sources of local or systemic infection following traumatic injury, such as burn [18–22]. Moreover, Th17 lymphocytes have been proposed to enhance tight junction formation through ERK MAPK signaling and subsequent up-regulation of tight junction proteins [23]. Furthermore, dysregulation of Th17 effector cytokines IL-17 and IL-22 has been implicated in psoriasis [24, 25], rheumatoid arthritis [26, 27], and both forms of IBD [28–30]. IL-22 is especially unique in that it does not act on immune cells but rather on epithelial cells of the skin, pancreas, intestine, liver, lung, and kidney [31, 32], where it is involved in chemotaxis [33], antimicrobial peptide expression [14, 15, 31, 34], tissue repair [16, 35] and epithelial cell survival [36, 37], proliferation [19], and differentiation [25]. Together, these findings suggest that Th17 effector cytokines must be tightly regulated to effectively maintain mucosal immunity and barrier function. As depicted in **Fig. 1**, Th17 effector cytokines exert their functions at mucosal interferes, including the gut epithelial lining, where they help maintain an intact physical barrier and induce expression of antimicrobial peptides to prevent invasion by luminal bacteria. Herein, we discuss this emerging adaptive cell subset in critical care settings, such as burn injury and clinical sepsis, and examine the role of IL-22 as a potential therapeutic agent.

Differentiation of Th17 lymphocytes

The development of Th17 cells has been studied and reviewed extensively in mice and humans [8, 9, 38, 39]. Murine models demonstrate that Th17 lymphocytes begin differentiation under the control of TGF-β and IL-6 [40–42]. Alone, TGF-β induces the expression of Foxp3 and differentiation of Tregs. However, IL-6 induces the expression of IL-21 in a STAT3-dependent manner [43]. Subsequently, IL-21, along with IL-6 and TGF-β, further amplifies STAT3 activation and prompts downstream expression of ROR-γt [7, 44, 45]. In this manner, IL-21 acts in an autocrine loop on differentiating Th17 cells, similar to the actions of IFN-γ and IL-4 on Th1 and Th2 cells, respectively [43, 44, 46]. IL-21 has further been suggested to exert its actions by inducing expression of the IL-23R [7, 44, 45] and suppressing Foxp3 expression [43, 46]. Lastly, IL-23 expands and stabilizes Th17 cells through further activation of STAT3-driven ROR-γt expression and subsequent production of Th17 effector cytokines IL-17 and IL-22 (Fig. 1) [27, 47–49].

IL-23, a member of the IL-12 family, is heterodimeric cytokine composed of the p19 and p40 subunits; the p40 subunit is shared by IL-12, whereas the p19 subunit is unique to IL-23 [50, 51]. IL-23 is synthesized and produced by a variety of cells, including antigen-presenting DCs and Mϕ [50, 51]. In the gut, for example, APCs sample luminal content and travel

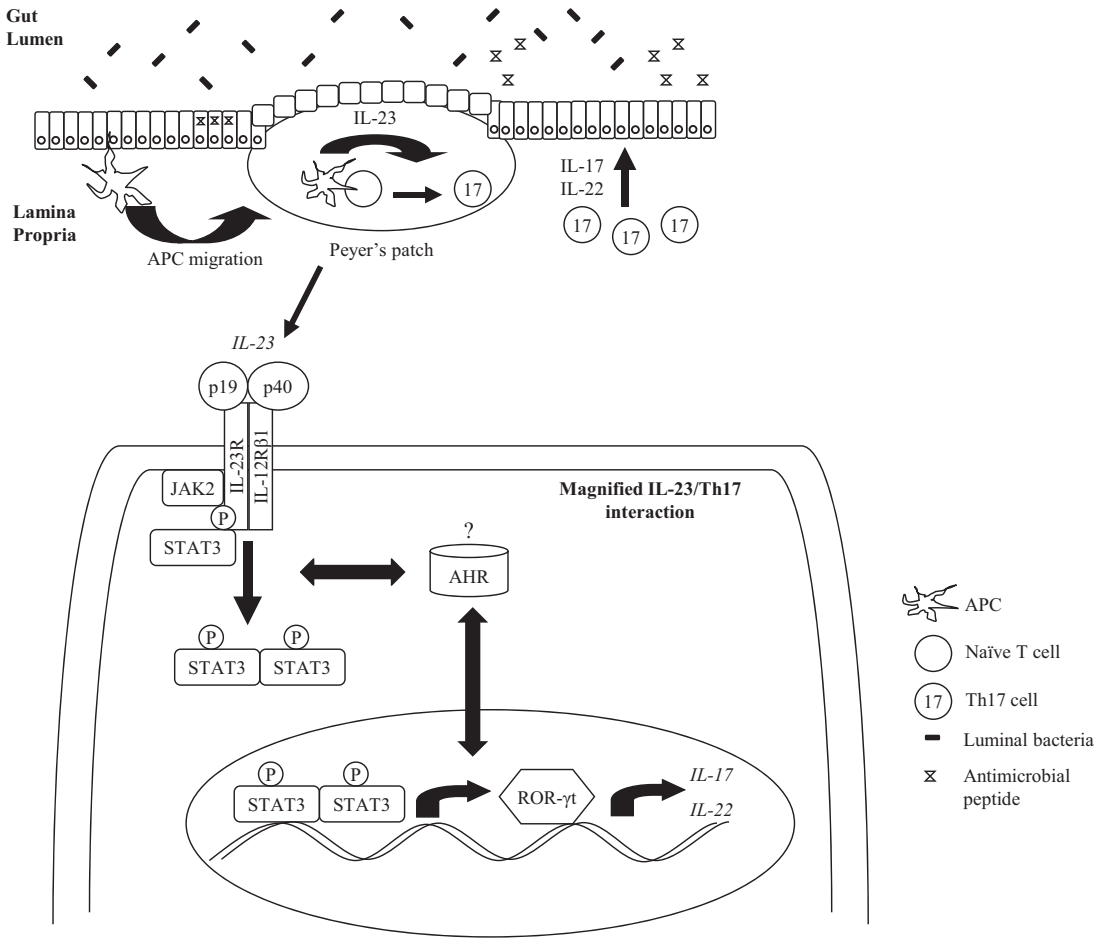

Figure 1. Schematic of Th17 cell/gut mucosal interactions. Under healthy conditions, IL-23 drives expression of Th17 cytokines, IL-17 and IL-22. These Th17 effector cytokines maintain mucosal barrier integrity and immune function, as well as promote expression of antimicrobial peptides. Together, these T cell effector functions prevent evasion of gut microbes.

to local secondary lymphoid organs, such as intestinal Peyer's patches, where they present antigen to differentiating T cells and produce IL-23 to drive Th17 polarization (Fig. 1). Binding of IL-23 to its receptor complex, IL-12Rβ1 and IL-23R, results in Jak2-mediated phosphorylation of tyrosine residues on the cytoplasmic domain of the IL-23R subunit (Fig. 1) [52]. Whereas 12Rβ1 is also part of the IL-12R, IL-23R is unique to the IL-23R complex. Phosphorylated tyrosine residues serve as docking sites for STAT3 proteins, which in turn become phosphorylated [52]. Once phosphorylated, STAT3 proteins homodimerize and translocate to the nucleus to up-regulate downstream signaling pathways, including the expression of Th17 hallmark transcription factor ROR-γt [7, 44, 45, 52, 53]. Activation of ROR-γt results in the production of Th17 cytokines IL-17 and IL-22 [45, 53, 54].

Th17/Treg balance

Similar to Th1 and Th2 cells, Th17 and Tregs regulate the differentiation of one another to maintain equilibrium. Alone, TFG-β induces the expression of Foxp3 in naïve T cells [5], the key transcriptional regulator of Tregs. However, in the presence of IL-6, TGF-β induces naïve T cells to express IL-23R and ROR-γt [40, 55], thereby promoting a Th17 phenotype. Zhou et al. [56] demonstrated that TFG-β-induced Foxp3 expression represses IL-23R and ROR-γt expression, suggesting that the Treg phenotype regulates Th17 cell differentiation. Similarly, Bettelli et al. [40] found that IL-6 inhibits expression of TGF-β-induced Foxp3. More recently, HIF-1 was implicated in the regulation of Th17/Treg balance [57]. HIF-1 regulates the metabolic switch from oxidative phosphorylation to aerobic glycolysis during hypoxic conditions [58] and is a target gene of activated STAT3 [53]. Dang et al. [57] found increased HIF-1 expression in Th17 cells and demonstrated that naïve T cells from $CD4^+$-specific HIF-$1^{-/-}$ mice failed to differentiate into IL-17-expressing cells or up-regulate mRNA expression of IL-17 and IL-23R. Further experimentation revealed that HIF-1 is necessary for STAT3-dependent expression of ROR-γt and that $CD4^+$-specific HIF-$1^{-/-}$ mice are resistant to EAE, a Th17-dependent process. Chromatin immunoprecipitation analysis of the ROR-γt-binding site on the IL-17 promoter region showed localized binding of ROR-γt, HIF-1, and transcription factor p300, which modulates HIF-1-mediated gene expression [57]. In regards to Tregs, Dang et al. [57] further found that HIF binds Foxp3, targeting it for proteasomal degradation. These results convincingly demonstrate that Th17/Tregs interdependently regulate the differentiation of one another.

Role of gut commensal bacteria on Th17 differentiation

Given their role in maintaining mucosal immunity, it is not surprising that recent investigations indicate that Th17 cells may be regulated in the gut through host-commensal bacteria interactions [59–61]. Ivanov et al. [60] took advantage of ROR-$\gamma t^{gfp/+}$ mice to demonstrate that after birth, $TCR\beta^+CD4^+IL\text{-}17^+GFP^+$ cells appear in the small intestine lamina propria at Day 25 and eventually represent the majority of IL-17-producing cells by Day 33. These data suggest that the presence of Th17 cells depends on colonization of the intestine with commensal bacteria. This notion is supported further by results indicating that germ-free mice, which are devoid of bacteria and fungi, lack Th17 cells. Colonization of germ-free mice with fecal homogenates from control mice induced Th17 cells in the gut lamina propria within 2 weeks after colonization [60]. Treatment of control mice with antibiotics, known to affect commensal bacteria, diminished the frequency of Th17 cells in the lamina propria. Through analysis of commensal bacteria from Th17 cell-sufficient mice, the authors concluded that the presence of Th17 cells in small intestine lamina propria correlated with the quantity of Gram-negative cytophaga-flavobacter-bacteroidetes bacteria present in the gut [60]. This group later demonstrated that colonization of germ-free mice with segmented filamentous bacteria, Gram-positive spore-forming bacteria, also induced IL-17- and IL-22-producing Th17 cells in the gut lamina propria [59]. Wu et al. [62] demonstrated that gut colonization of mice with enterotoxigenic *B. fragilis* induced expression of IL-17 in a STAT3-dependent manner. Increased IL-17 was not seen with nontoxigenic *B. fragilis* or in CD4 STAT3-KO mice [62]. Together, these data highlight the vital role of host-commensal bacteria interactions in the regulation of immune homeostasis. Moreover, the duodenum, through up-regulation of Th17 chemokine CCL20, was recently implicated in the control of Th17 immune cells. In a murine model of *Staphylococcus aureus* sepsis, Esplugues et al. [63] reported increased Th17 cells in the duodenum of *S. aureus*-treated mice, 3 days postbacterial injection, which was not noted in the spleen. Further analysis of duodenal Th17 cells revealed that these cells exhibited a suppressive phenotype, as marked by increased expression of IL-10 [63]. Whereas the mechanism by which these Th17 cells acquire a suppressive phenotype remains to be elucidated, these data suggest that the gut is critical in maintaining equilibrium of Th17 cells, which are necessary to clear infection but are immunopathogenic in excess.

The AhR: new regulator of Th17 lymphocytes?

Most recently, the AhR has been implicated in Th17 immunity, particularly in the regulation of IL-22 [64–68]. AhR is a ubiquitous transcription factor found in the cytoplasm of vertebrate cells. Upon ligand binding, AhR translocates to the nucleus, where it regulates gene expression. Ligands for AhR include dioxin and natural chemicals, including bacterial metabolites, derivatives of tryptophan, and phytochemicals. Initially, Veldhoen et al. [64] found selective expression of AhR in mouse and human Th17 cells, as compared with other Th cells, and further demonstrated that addition of FICZ, a high-affinity ligand of AhR, greatly increased expression of IL-17 and IL-22 in vitro. Moreover, $AhR^{-/-}$ T cells failed to respond to FICZ treatment, whereas retroviral transduction of AhR restored IL-22 expression [64]. Quintana et al. [68] provided evidence suggesting that AhR controls Th17 differentiation in a ligand-dependent manner. In these experiments, in vivo activation of AhR by TCDD reduced the frequency of $IL\text{-}17^+CD4^+$ cells and induced the frequency of TGF-β-secreting $CD4^+Foxp3^+$ Tregs, which were capable of attenuating EAE.

In line with Veldhoen et al. [64], in vivo activation of AhR by FICZ boosted Th17 cell differentiation and exacerbated EAE [68].

As AhR drew attention in the field of Th17 immunity, more recent studies revealed that AhR plays a greater role in the modulation of IL-22 than IL-17 [65–67]. Two groups simultaneously described a subset of human Th cells that produce IL-22 but neither IL-17 nor IFN-γ, namely, Th22 cells [65, 66]. Both demonstrated a vital role for AhR; however, these groups presented conflicting data regarding the role of ROR-γt in IL-22 production by Th22 cells. Duhen et al. [65] reported low expression of RORC (ROR-γt in humans) in their Th22 population and concluded that RORC is nonessential for Th22 development. The authors showed further that de novo induction of Th22 cells required IL-6 and TNF-α. Conversely, Trifari et al. [66] used small interfering RNA to demonstrate that silencing of RORC or AhR diminished IL-22 production from memory $CD4^+$ T cells, whereas IL-17 was only affected by silencing of RORC. This group found that IL-1β and IL-23 are required for de novo induction of Th22 cells, whereas the presence of TGF-β induced IL-17 production [66]. The authors confirmed that FICZ treatment increases IL-22 production yet provided protein and mRNA data suggesting that FICZ decreases IL-17 [66], which challenges data by Veldhoen et al. [64]. A third group further confirmed increased IL-22 and decreased IL-17 in response to FICZ treatment but also reported increased IL-22 following TCDD treatment [67], which Quintana et al. [68]had demonstrated inhibits IL-17 but did not assess its effect on IL-22. Collectively, data consistently demonstrating a role for AhR in expression of IL-22 and discrepancies in the requirements for RORC and effect of FICZ/TCDD on IL-17 production support the notion that the requirements for IL-22 and IL-17 production are distinct. Irrespective of whether IL-22 and IL-17 are produced by a single cell type, Th17 cells, or multiple cell types, Th17 and Th22, further studies should explore the molecular mechanism by which AhR regulates IL-22 production. Gaining a comprehensive understanding of the regulation of IL-17 and IL-22 will provide a foundation for the development of targeted treatments for patients with Th17-related pathologies.

There are multiple mechanisms by which AhR may control IL-22 (Fig. 1). One such possible mechanism is the interaction between STAT molecules and AhR; for example, STAT1 and STAT5 have been coimmunoprecipitated with AhR [69]. Thus, it is possible that AhR and STAT3 interact to differentially regulate ROR-γt and expression of IL-17 and IL-22. Most recently, AhR-induced notch signaling was implicated in the development of gut IL-22-producing ILC22, including NKp46 and lymphoid tissue inducer cells, as well as in the formation of postnatal lymphoid tissues [70]. Whereas these data focus on innate producers of IL-22, the mechanisms involved may affect the development of adaptive IL-22-producing Th cells, as well as the secondary lymphoid tissues necessary for the differentiation of Th17 cells. In line with previous literature, Lee et al. [70] found reduced production of IL-22 in gut-related lymphoid organs and impaired response to *Citrobacter rodentium* infection in $AhR^{-/-}$ mice. The authors also noted decreased ILC22 and the lack of cryptopatches and isolated lymphoid follicles in the small intestines of $AhR^{-/-}$ mice, which they attributed to the absence of AhR signaling. Administration of TCDD induced expression of notch1 and notch2, which was absent in $AhR^{-/-}$ mice [70]. Moreover, mice lacking expression of RBP-J_κ, the DNA-binding protein that associates with the intracellular regions of all notch molecules to mediate transcriptional activity of notch, demonstrated less ILC22 as compared with WT mice [70]. Together, these data suggest that the development of ILC22 is regulated by AhR-mediated notch activity, a mechanism that may also be involved in the adaptive immune response. Further studies should test the role of AhR and notch in Th17 cell development, as well as establish whether AhR signaling produces differential immune responses when activated by endogenous versus exogenous ligands.

Th17 CELLS AND BURN INJURY

Whereas the role of Th17 lymphocytes has been studied extensively in the context of regulating infection, little work has focused on these cells in the context of injury. Following traumatic injury, such as burn, there are global changes to the systemic immune response, including suppressed immune function and increased susceptibility to infection [71–75]. Moreover, burn trauma is associated with remote organ injury, affecting the lung [76, 77], kidney [78–80], gut [81–85], and bone marrow compartment [86–88] in human and animal studies. This inter-relationship between burn and remote organ injury supports the hypothesis that immune suppression may facilitate the translocation of gut-derived bacteria and/or their products and contribute to the development of sepsis, systemic inflammatory response syndrome, and multiple organ dysfunction syndrome in critically ill burn patients [83, 85, 89]. Given their central role in mediating mucosal immunity, including the gut and lungs, Th17 lymphocytes may regulate immune perturbations following burn injury.

Murine models of burn injury demonstrate that Th17 responses are elicited by burn injury. At the site of burn injury, Th17 cytokines IL-17 and IL-22 have been shown to be elevated approximately threefold as compared with sham injury, within 3 h of burn in the absence of significant changes in IL-6, IL-23, or TGF-β [90]. Whereas these changes are transient, an early perturbation of IL-17 and IL-22 postburn injury may disrupt the wound-healing process and promote burn wound sepsis [91]. Moreover, elevated levels of IL-17 have been observed at distant sites and in the systemic circulation. IL-17 is elevated in cardiac tissue, 3 h postburn injury [92] and in the circulation, 1 and 7 days after injury [93]. Similarly, Oppeltz et al. [94] used cells isolated from BAL fluid, 7 days postburn injury to show heightened IL-17 production following stimulation with TLR2 agonist zymosan. Whereas this study did not suggest a specific source for IL-17 nor identify a cell type responsive to zymosan, it further highlights the importance of IL-17 at mucosal barriers, such as the lung, and provides reason to investigate the role of Th17 cells and their immunomodulatory products, in acute lung injury following burn. Together, these data indicate that local and systemic Th17 immune responses are elicited early on postburn injury

and that sustained elevations in levels of IL-17 may contribute to the burn-induced inflammatory response and subsequent immune aberrancies.

Other experimental models of burn injury demonstrate bimodal skewing of Th17 lymphocytes. Neely et al. [95] reported increased Th17 cells ($IL\text{-}17^+CD4^+$), 3 and 14 days postburn injury in draining peripheral LNs but not spleens of injured mice. More importantly, this group demonstrated a decreased Th17/Th1 ratio Day 3 after injury, which normalized at Day 7 and increased at Day 14 [95]. These findings suggest that early postburn injury, a decreased Th17/Th1 ratio may contribute to increased susceptibility to extracellular pathogens, including *K. pneumoniae* and *C. albicans*. Conversely, late postburn injury, an increased Th17/Th1 ratio may perturb Th1-dependent immune responses.

Patient data specific to the Th17 axis following burn injury and its possible role in remote organ damage are quite limited. However, data from the Inflammation and Host Response Glue Grant indicate that serum IL-17 is elevated in adult and pediatric burn patients within 1 week after injury [96, 97]. IL-17 levels in children were increased to a greater extent than those in adult patients [96], suggesting an age-dependent difference to burn injury, which may exacerbate the development of a hypermetabolic state. Recently, a single center study examined Th17 cell development in 26 burn patients with third-degree thermal injuries. The results revealed that peripheral blood mononuclear cells isolated from these patients had a decreased ability to express ROR-γt and produce IL-17 in response to TCR activation and *C. albicans* challenge [98]. *C. albicans* has been shown to increase mortality in burn patients [72]. Inatsu et al. [98] further reported measureable levels of IL-10 in sera from burn patients and demonstrated an inhibitory role for IL-10 in the development of $CD4^+ROR\text{-}\gamma t^+$ IL-17-producing T cells. Although the molecular mechanism by which burn injury-induced IL-10 inhibits IL-17 remains to be elucidated, this study highlights the perturbation of Th17 cells following burn injury and warrants further investigation in the context of traumatic injury, including burn.

Taken together, murine and human studies demonstrate that burn injury elicits disruption of Th17 immune responses at multiple time-points after injury. Murine models indicate that burn injury induces increased tissue levels of IL-17 and IL-22 in a nonantigen-specific manner, as suggested by elevated cytokine levels as early as 3 h postburn [90, 92]. Studies from burn patients further demonstrate impaired Th17 differentiation in response to Th17-specific pathogenic challenge [98]. Thus, it is possible that nonantigenic-induced and prolonged elevations of IL-17 weaken pathogen-specific Th17 responses. Subsequently, suppression of pathogen-specific Th17 responses, following burn or other traumatic injury, may lead to increased bacterial and fungal infection, particularly at mucosal interfaces. Studies examining the Th17 axis following burn and other traumatic injuries are necessary to define the normal, time-dependent Th17 responses to injury and to identify whether these changes contribute to remote organ damage, susceptibility to infection, and mortality.

Th17 CELLS AND CLINICAL SEPSIS

There have been limited investigations into the importance of Th17 cell effector functions in clinical sepsis. To date, no direct perturbations of $CD3^+CD4^+$ lymphocyte-derived IL-17 have been reported in clinical data or experimental models of sepsis. However, Flierl et al. [99] did show increased IL-17 in a murine model of CLP, although this was attributed to γδ T cells, not $CD3^+$ αβ T cells. Moreover, the authors demonstrated that neutralization of IL-17 correlated with decreased bacteremia, increased survival, and decreased plasma levels of proinflammatory cytokines TNF-α, IL-1β, and IL-6 [99]. Together, these data suggest that γδ T cell-derived IL-17 negatively impacts outcomes from sepsis, through modulation of proinflammatory cytokines [99]. This observation was confirmed when γδ T cell KO mice showed improved survival and decreased plasma IL-17 following CLP [99]. More recently, these findings were substantiated when the same laboratory took advantage of Rag-1 KO mice, which lack T and B cells, to demonstrate that lack of $CD3^+$ T cells does not affect outcome of severe sepsis following CLP [100], again eliminated the role of $CD3^+$ Th17 cells.

IL-22, the other major immunomodulatory secretory product of Th17 cells, has also been shown to negatively affect outcomes during septic injury. Whereas IL-22 is generally considered immunoprotective, models of peritonitis, as well as clinical data from patients with abdominal sepsis, suggest that higher levels of IL-22 may facilitate bacterial burden and septic complications. Specifically, Weber et al. [101] demonstrated increased levels of IL-22 in kidney and spleen following CLP peritonitis. Inhibition of IL-22 following CLP enhanced bacterial clearance, promoted phagocyte recruitment, attenuated organ dysfunction, and decreased expression of IL-10 [101]. Similarly, a rat model of CLP revealed measureable levels of IL-22 in serum from septic animals [102]. Clinically, a single center hospital study of 16 patients who developed abdominal sepsis following surgery, demonstrated significant elevations in serum IL-22 as compared with healthy volunteers and control surgery patients without septic complications [103]. As compared with control groups, IL-10 was also increased significantly in patients with abdominal sepsis [103], again highlighting that IL-22 may modulate IL-10 levels resulting in heightened bacterial growth [101]. Together, these findings indicate an interdependent relationship between IL-22 and IL-10, which modulates a critical balance between bacterial burden and host immune/barrier homeostasis. As such, adequate levels of IL-22 must be maintained to regulate host immune/barrier integrity, whereas higher levels may induce increased expression of IL-10, subsequently enhancing bacterial growth, and increasing the risk of sepsis. Further studies evaluating this interdependency among IL-22, IL-10, bacterial burden, and host immune/barrier defense may provide a mechanism by which these interactions are regulated and lead to the identification of novel therapeutic targets for patients who develop septic complications.

PROTECTIVE ROLE OF IL-22: UNDERLYING MECHANISMS AND THERAPEUTIC POTENTIAL

Despite being recognized initially for their role in chronic inflammation and autoimmunity [8, 17], Th17 lymphocytes and their effector cytokines have gained interest as therapeutic targets. Specifically, the protective and regenerative effects of IL-22 have led to the investigation of IL-22 as a treatment agent, which has produced promising results, particularly in the field of gastrointestinal-related disease. However, it is important to note that Th17 cells are only one source of IL-22. Major sources of IL-22 include cells of the innate and adaptive immune systems. In mice and humans, innate sources of IL-22 include NK cells and ILC22 [16]. In humans, T cell subsets that produce IL-22 include Th1, Th17, Th22, Tc22, Tc17, as well as γδ T cells [16]. In mice, IL-22 is produced by Th17, Tc17, innate-like NKT, and γδ T cells [16]. Nonetheless, the following section will focus on the potential therapeutic implications of IL-22.

IL-22 and liver disease

In a model of Con A-induced, T cell-mediated hepatitis, in vivo treatment with IL-22 prevented liver injury, as marked by increased levels of hepatic enzymes ALT and AST [37]. Conversely, treatment of animals with anti-IL-22 antibody exacerbated Con A-induced ALT and AST levels [37]. This observation was confirmed later when IL-22$^{-/-}$ mice were shown to be more susceptible to Con A-induced hepatitis than WT counterparts [36]. Radaeva et al. [37] further demonstrated a role for STAT3 activation in IL-22-dependent modulation of liver injury. With the use of the human HepG2 heptoma cell line, IL-22 was shown to inhibit apoptosis through induction of antiapoptotic proteins Bcl-xL, Bcl-2, and myeloid cell leukemia sequence 1 and promote proliferation through activation of c-myc, cyclin D1, and Rb2. In vivo, Radaeva et al. [37] injected IL-22-transfected or null HepG2 cells into mice and found that mice injected with IL-22-transfected HepG2 cells developed larger tumors with heightened levels of activated STAT3, Bcl-xL, and Rb2 compared with null HepG2-injected mice. Together, these results confirm a protective role for IL-22 in T cell-mediated liver injury, which is dependent on activation of STAT3, and subsequent induction of proliferation and inhibition of apoptosis in hepatocytes, both in vivo and in vitro. To further elucidate the protective role of IL-22 in Con A-induced hepatitis, Zenewicz et al. [36] injected differentiated Th17 cells from IL-22$^{-/-}$ or WT mice into IL-22$^{-/-}$ mice and found that recipients of IL-22$^{-/-}$ Th17 cells exhibited higher ALT and AST levels, confirming a protective role for IL-22 against T cell-mediated liver injury.

More recently, the same group demonstrated that treatment with a single dose of IL-22 attenuates chronic-binge alcohol-induced liver injury. Ki et al. [104] used a modified Lieber-DeCarli diet and single high dose of ethanol by oral gavage to produce fatty liver and liver injury, as marked by increased levels of hepatic enzymes ALT and AST, increased hepatic triglyceride levels, and steatosis. Although the authors did not establish decreased IL-22 in the context of alcohol-induced liver injury, their data did demonstrate increased expression of IL-22R1 in mice and patients with alcoholic hepatitis. Thus, IL-22 may be required for normal liver homeostasis, and in the presence of alcohol, the IL-22 pool may not be sufficient for normal hepatic functions. Ki et al. [104] found that IL-22 treatment prevented alcohol-induced elevations in serum AST/ALT and hepatic triglyceride levels and steatosis. The authors [104] attributed the protective effect of IL-22 to induction of liver antimicrobial lipocalin 2 and the antioxidant gene metalothione, as well as decreased expression of a fatty acid transport protein, although modulation by STAT3 was variable. These results were later confirmed using liver-specific IL-22TG mice, which proved resistant to Con A-induced T cell hepatitis [105]. IL-22TG mice recapitulated up-regulation of antioxidant and mitogenic genes noted with exogenous IL-22 treatment [105]. Collectively, these data highlight the antioxidant, antisteatotic, and antimicrobial effects of IL-22 and elucidate a possible role for IL-22 in the treatment of alcoholic liver disease.

IL-22: regulator of autophagy?

IL-22 was recently shown to modulate pancreatitis through regulation of autophagy [106]. In a mouse model of cerulein-induced pancreatitis, IL-22KO and IL-22TG mice were used to elucidate the role of IL-22 in mediating pancreatic injury and function. IL-22KO and WT mice demonstrated similar phenotypes in response to acute and chronic cerulein injections; both strains had increased pancreatic weight to body weight ratios, amylase and lipase levels, MPO^{+} cell infiltration, and apoptosis [106]. Knocking out of IL-22 did not exacerbate injury. However, IL-22TG mice, which overexpress IL-22, showed no pathologic response to cerulein treatment [106], suggesting a protective role for IL-22 against pancreatic injury. The therapeutic potential for IL-22 in pancreatitis was highlighted further when treatment of WT animals with IL-22, prior to acute cerulein injection, attenuated changes in amylase, lipase, and inflammatory cell infiltration. Similarly, IL-22 treatment via adenoviral delivery ameliorated chronic cerulein-induced pancreatitis [106]. As mentioned previously, IL-22 inhibits apoptosis via activation of antiapoptotic genes, including Bcl-2 and Bcl-xL. Additionally, Bcl-2 proteins inhibit Beclin-1-dependent autophagy [107], which has been implicated in the progression of pancreatitis [108]. To dissect the mechanism by which IL-22 modulates the development of pancreatitis, Feng et al. [106] tested the role of Bcl-2 and Bcl-xL in the regulation of autophagy and found that cerulein-treated IL-22TG mice had decreased formation of autophagosomes as compared with cerulein-treated WT counterparts. Feng et al. [106] showed further that IL-22TG mice displayed increased Bcl-2/Bcl-xL and Beclin-1 interactions as compared with WT mice [106]. Thus, IL-22 protects against pancreatic and inhibits autophagy through modulation of Bcl-2/Bcl-xL and Beclin-1 interactions.

IL-22 and IBD

Studies of IBD consistently emphasize the role of Th17 cells in the pathogenesis of ulcerative colitis and Chron's disease [9,

109]. Yet, most of these studies highlight the role of IL-17 and pay less regard to IL-22. Zenewicz et al. [110] eloquently explored the role of IL-22 in IBD by taking advantage of B and T cell null Rag1$^{-/-}$ and IL-22$^{-/-}$ mice. Rag1$^{-/-}$ mice lack T cells, including Tregs; thus, when naïve CD4^{+} T cells from WT mice are adoptively transferred to Rag1$^{-/-}$ mice, they rapidly expand and acquire effector functions in an uncontrolled manner, causing the development of massive inflammation in the gut, similar to that found with IBD. These adoptive transfer studies revealed increased colitis and colonic levels of IFN-γ, IL-17, and IL-22, as well as weight loss, as compared with Rag1$^{-/-}$ mice that did not receive T cells [110]. To clarify the role of IL-22 in intestinal colitis, IL-22$^{-/-}$Rag1$^{-/-}$ mice were injected with naïve CD4^{+} T cells from IL-22$^{-/-}$ or WT mice. Both treatments resulted in the development of colitis, yet IL-22$^{-/-}$Rag1$^{-/-}$ mice injected with naïve T cells from IL-22$^{-/-}$ donor mice demonstrated more severe colitis and weight loss than mice that received WT T cells [110], suggesting a protective role for IL-22-producing cells in the development of gut inflammation. Interestingly, Rag1$^{-/-}$ mice injected with T cells from IL-22$^{-/-}$ donors demonstrated the same expression of IL-22 as IL-22$^{-/-}$Rag1$^{-/-}$ mice that received T cells from WT mice [110], indicating a host-derived source of IL-22. Further analysis revealed that innate NK cells are a major contributor of IL-22 in the gut. This graded response, in which abolishment of IL-22 in recipient mice and donor T cells as compared with either alone produced increasingly worse colitis and weight loss, convincingly indicates that IL-22 plays a role in the development of gut inflammation. To further underscore the protective role of IL-22, Zenewicz et al. [110] used a model of innate colitis—DSS-induced colitis—which causes disruption of colonic epithelial integrity and subsequent inflammation and colitis. IL-22$^{-/-}$ mice, treated with DSS, lost significantly more weight and exhibited greater mortality than DSS-treated WT mice [110], confirming the protective role of IL-22. In yet another model of intestinal colitis, Sugimoto et al. [111] used IL-22 gene delivery in a murine model of Th2-mediated colitis. Their results indicate that IL-22 treatment increased goblet cell expression and reduced colonic diameter and thickness in a STAT3-dependent manner [111]. These results were extended in a DSS model of colitis, where peak levels of colonic IL-22 correlated with increased production of membrane-bound mucins (Muc1, -3, -10, and -13) during the recovery phase of DSS colitis [111]. IL-22 failed to modulate colitis in mice treated with a mucolytic agent, which removes the colonic mucus layer, blocking the actions of IL-22 on mucin production. Similarly, mice concurrently treated with IL-22-neutralizing antibody and IL-22 exhibited greater weight loss, decreased STAT3 activation, and decreased goblet cell accumulation as compared with mice treated with control antibody and IL-22 [111], confirming that IL-22 restores goblet cells in a STAT3-dependent manner.

Therapeutic potential: future directions

Studies demonstrating the protective role of IL-22 in liver, pancreas, and intestinal tract provide promising evidence for the use of IL-22 in the treatment of gastrointestinal-related disease. These early animal studies provide a strong foundation and offer a unique challenge to translation research teams. At the bench, studies exploring whether IL-22, IL-22R, and/or its signaling pathway are perturbed in patients with gut-related pathologies may reveal epigenetic changes or genetic polymorphisms specific to patients who develop disease. These findings would help advance the treatment of disease through the development of new therapeutic agents, as well as through modulation of gene expression with gene therapy. Clinically, research exploring the possibility of treating patients with rIL-22 should be considered in patients with severe gastrointestinal disease who have failed standard therapy. Results from these studies would give a better understanding of the short- and long-term effects of IL-22 and help redirect related basic research. Lastly, investigation of IL-22 as a therapeutic agent may prove beneficial in the treatment of critically ill patients who sustain secondary organ damage, such as burn and trauma patients.

CONCLUDING REMARKS

Th17 cells are now well-established modulators of immune and host epithelial homeostasis, particularly at host/environment interfaces. Whereas ROR-γt remains the hallmark Th17 transcription factor, new data scrutinizing the regulation of Th17 effector cytokines IL-17 and IL-22 propose complex networks involving metabolic changes, host/commensal bacteria interactions, and environmentally or endogenously derived chemicals in the maintenance of proper Th17 balance. Where impaired Th17 responses diminish bacterial clearance and increase epithelial vulnerability, uncontrolled Th17 effector functions promote autoimmune disease. Thus, the multiple pathways regulating Th17 immunity are interdependent and must be regulated tightly. Injuries, such as burn, trauma, and clinical sepsis, have global systemic consequences, many of which affect the delicate mechanisms that govern Th17 immunity. Suppression of antigen-specific Th17 responses, as noted with burn injury, may contribute to infection, remote organ damage, and mortality. Given the importance of this emerging subset, the role of Th17 cells following clinically relevant host injury remains relatively unexplored and warrants deeper investigation.

AUTHORSHIP

J.L.R. prepared the manuscript with input and modifications from M.A.C.

ACKNOWLEDGMENTS

This study is supported by U.S. National Institutes of Health grants R01AA015731 (M.A.C.) and R01AA015731-04S1 (M.A.C.). J.L.R. is supported by U.S. National Institutes of Health grants F30AA020167 (J.L.R.) and T32AA013527 (Elizabeth J. Kovacs) and the Loyola University Chicago Stritch School of Medicine Combined M.D./Ph.D. Program.

DISCLOSURES

The authors have no competing financial interests.

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KEY WORDS:
IL-17 · IL-22 · ROR-γt · aryl hydrocarbon receptor