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Burn induces a Th-17 inflammatory response at the injury site

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ABSTRACT

Introduction: The high incidence of morbidity and mortality following major burn can be in part attributed to immune dysfunction and wound healing complications. Inflammation plays a major role in the complex process of wound repair. Recently, a novel class of T-helper cells, termed Th-17 cells, has been found to secrete the pro-inflammatory cytokines IL-17 and IL-22. The Th-17 response also involves other cytokines, such as IL-6 and TGF-β, which have been shown to be associated with burn-induced inflammation. Nonetheless, the relationships between the Th-17 response and post-burn inflammation are unknown.

Methods: C57BL/6 male mice (n = 5–6/group) were subjected to a major burn (25% TBSA) or sham procedure. Three hours thereafter, skin samples were collected (uninjured skin and burn skin) and processed for the determination of Th-17 cytokine (IL-6, IL-17, IL-22, IL-23, IL-27, and TGF-β) levels by ELISA.

Results: At 3 h after burn a significant (~3-fold) increase in tissue levels of IL-17 and IL-22 was observed at the burn site as compared to sham skin. The burn-induced Th-17 response was independent of statistically significant changes in other Th-17 cytokines (i.e., IL-6, IL-23, IL-27 and TGF-β).

Conclusions: These findings indicate the development of a robust Th-17 response at the burn site that may play an important role in subsequent immune and wound healing derangements.

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1. Introduction

The high incidence of morbidity and mortality following major burns can often be attributed to immune dysfunction and wound healing complications. These conditions increase the patient's susceptibility to infection leading to sepsis, systemic inflammatory response syndrome (SIRS), and multiple organ failure. Proper healing of the burn wound limits the risk of infection and is critical to a successful recovery [1,2]. In this regard, inflammation, which involves a variety of immune cells and mediators, plays a major role in the complex process of wound repair [3,4].

The T-cell response to injury can also be classified based upon the cytokine profile that the cells express. The T helper 1 (Th-1) cell and Th-2 cell paradigm was first proposed to explain how immune responses differ to eradicate various pathogens. The induction of a proper T-cell response is critical to the orchestration of sufficient defensive mechanisms to control infection. In this regard, major injury has been shown to decrease the Th-1 response and enhance the Th-2 response leading to immune paralysis and increased susceptibility to sepsis [5,6]. Recently, a novel class of T-helper cells, termed Th-17 cells, has been found to secrete the pro-inflammatory cytokine IL-17, which appears to function in inflammation and

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autoimmunity. Th-17 cells have also been shown to secrete other pro-inflammatory cytokines such as IL-6 and IL-22. Based on these observations, a potentially important and unidentified role for the Th-17 response in the development of immunoinflammatory complications following burns may exist. The relationships between the Th-1, Th-2 and Th-17 responses are shown in Fig. 1. The Th-17 response can be divided into early and late mediators with IL-6, IL-27 and TGF-β regulating the initial differentiation of naïve T-cells and IL-23, IL-17 and IL-22 being associated with the mature Th-17 T-cell phenotype. The role of Th-17 in the post-injury immunoinflammatory response is unknown. The current study was undertaken to determine the relationship between burn-induced inflammation and the Th-17 response.

2. Materials and methods

2.1. Animals

This experiment used C57BL/6 male mice (18–22 g; 8–10 weeks of age, Charles River Laboratories, Wilmington, MA). The mice were given one week to acclimatize in the animal facility before the study began. Animals were randomly assigned to either a sham treatment group or a burn group. The procedures used in this study were approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio, and the experiments were performed in accordance with the National Institutes of Health guidelines for the care and handling of laboratory animals.

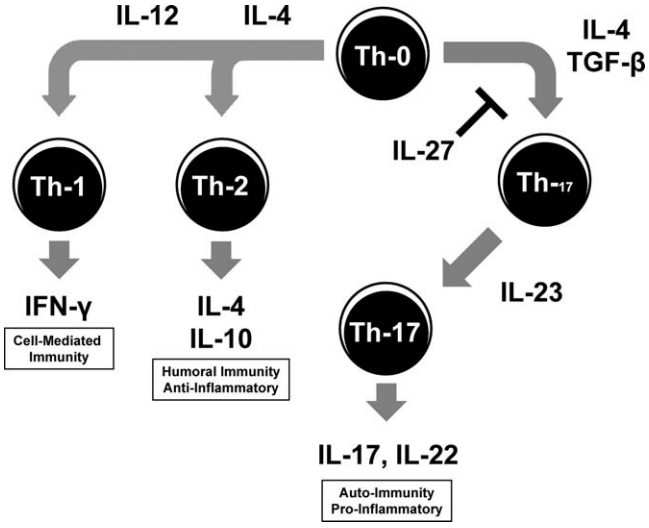

Fig. 1 – T-cell cytokine pathways. Naïve T-cells (Th-0) can differentiate into specific cytokine producing phenotypes. Th-1 T-cells mature under the influence of IL-12 and produce IFN-γ, which is associated with cell-mediated immunity. Th-2 cells mature under the influence of IL-4, produce IL-4 and IL-10, promote humoral immunity and are generally anti-inflammatory in nature. Th-17 T-cells mature under the influence of IL-6, TGF-β, and IL-23. IL-6 and TGF-β are early mediators, whereas IL-23 is a late mediator in the differentiating process. IL-27 acts as an inhibitor of the Th-17 response. Mature Th-17 cells produce IL-17 and IL-22, which are pro-inflammatory in nature.

2.2. Burn injury procedure

Mice received a scald burn as previously described [7]. Prior to the burn procedure the mice were anesthetized with an intraperitoneal injection of ketamine/xylazine. Once fully anesthetized the dorsal surface was shaved, and the mouse was placed in a custom insulated mold exposing 12.5% of total body surface area along the right side. The mold was immersed in 70 °C water for 10 s to produce the burn. The mouse was then repositioned in the mold, and the left side was immersed in 70 °C water for 10 s. The resulting injury covered 25% total body surface area, and previous studies have verified this injury to be a full-thickness burn, as defined by injury to the epidermal, dermal, and subdermal layers [8]. The mice were then given 1 mL of Ringer's lactate solution by intraperitoneal injection and placed on a heating pad. When the mice were fully awake they were put back in their cages and returned to the animal facility. Sham treatment included administering anesthesia, shaving the dorsal surface, and resuscitating with Ringer's lactate solution. Analgesics were not used in this process due to the profound immunomodulatory effects of NSAIDs and opiates [9,10].

2.3. Tissue collection and processing

At 3 h after burn or sham procedure the mice were euthanized and skin samples were collected. Samples from the burn group included injured skin from the wound site as well as uninjured skin from outside of the wound site. The burn wound and uninjured skin was excised down to the level of the musculofascia, including the sub-mucosal layer by sharp dissection with a scalpel blade and/or curved dissecting scissors. The samples were immediately snap frozen in liquid nitrogen and stored at −80 °C for cytokine analysis, fixed in 10% formalin for histological analysis, or weighed for determination of wet/dry weight ratio. Analysis was conducted at 3 h after injury based on early findings showing an elevation in cardiac IL-17 at this time post-burn [11].

2.4. Cytokine determination

The samples for cytokine analysis were homogenized in protease inhibitor cocktail (Calbiochem) as described by Faunce et al. [12] and previously employed in our laboratory [13,14]. The concentrations of interleukin 6 (IL-6), IL-17, IL-22, IL-23, IL-27, and TGF-β were assayed by ELISA according to the manufacturer's recommendations (R&D Systems). The values obtained were normalized to the total protein of the tissue homogenate as determined by BCA assay.

2.5. Histology

Skin samples from sham and burn mice were excised and fixed in 10% formalin. The samples were embedded in paraffin, and slides of 4-μm cross-sections were prepared

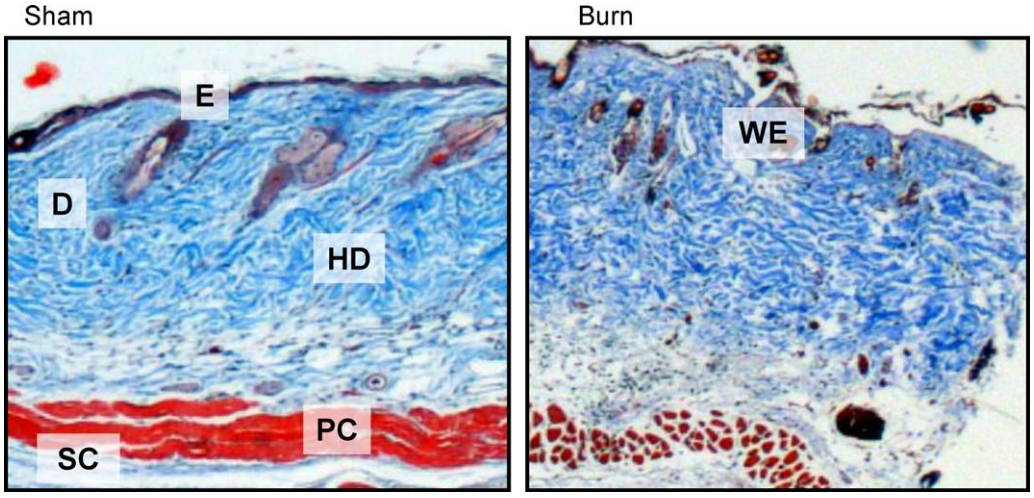

Fig. 2 – Skin histology. Skin samples were collected from sham and burn mice at 3 h after injury, formalin-fixed and processed for Masson-Trichrome staining. Representative samples are shown. Epithelium (E); dermis (D); hypodermis (HD); *Panniculus carnosus* (PC); sub-cutis (SC); wound edge (WE). Magnification = 100×.

and stained with Masson-Trichrome. Representative pictures are presented at a magnification of 100×.

2.6. *Determination of skin wet-to-dry weight ratios*

Skin samples were collected and weighed (wet weight) and then subjected to desiccation for 3 days until a stable dry weight was achieved and wet-to-dry weight ratios were determined.

2.7. *Statistical analysis*

Data are expressed as mean ± SE. Comparisons were analyzed using ANOVA, and a P value of <0.05 was considered to be statistically significant for all analyses.

3. Results

3.1. *Burn injury*

The burn procedure induced a full-thickness injury as shown in Fig. 2. At 3 h after injury marked tissue disruption is evident with destruction and separation of the epidermis and edema in the hypodermis and *Panniculus carnosus*. In addition, infiltration of viable cells is evident in the hypodermis and disseminated intravascular coagulation was observed throughout the skin layers. In parallel with these qualitative changes after burn there was also a significant increase in tissue wet/dry weight ratio after burn as compared with sham skin and uninjured skin from burn mice obtained from the ventral surface (Table 1).

3.2. *Skin cytokine levels*

Analysis of the early Th-17 mediators (IL-6, IL-27, TGF-β) in the skin from burn mice revealed that while significant levels of the cytokines were present in the tissue, they were not significantly different from that of skin samples obtained from sham mice (Fig. 3). In contrast, the late Th-17 mediators (IL-17, IL-22, IL-23) were significantly altered at 3 h after burn. Burn tissue showed a significant 3 to 4-fold increase in the levels of IL-17 and IL-22 as compared with sham skin (Fig. 4). IL-23 levels in the skin were not significantly altered by burn.

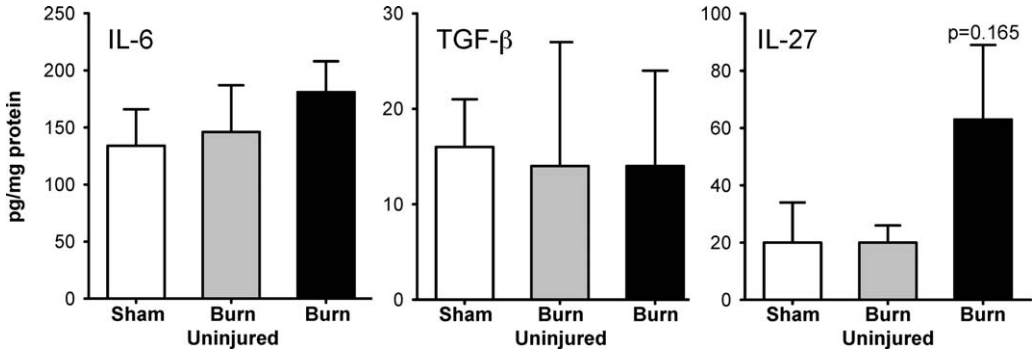

Fig. 3 – Early Th-17 cyokines. Samples of skin were collected from sham mice and from 2 sites on burn mice (normal [i.e., uninjured] and the burn site) at 3 h after injury. Tissue levels of IL-6, TGF-β and IL-27 were determined by ELISA as described in Section 2. Data are mean ± SEM; n = 5–6 mice/group.

Table 1 – Skin wet/dry weight ratio[a].

Sham	Burn uninjured	Burn
2.62 ± 0.12	2.86 ± 0.31	4.43 ± 0.60[b]

[a] Skin samples were collected at 3 h after burn or sham procedure and wet/dry weight ratios were determined as described in Section 2. Data are mean ± SEM; $n = 5$ mice/group.
[b] $P < 0.05$ as compared with burn injured and sham.

4. Discussion

These findings indicate the development of a robust Th-17 response at the burn site early after injury, which may play an important role in subsequent inflammatory, immunological and wound healing derangements. Specifically, our findings show an elevation in the late Th-17 mediators IL-17, IL-22, early after injury. The elevation in injured skin levels of IL-17 at 3 h after burn is consistent with our previous observations showing a similar increase in cardiac IL-17 [11].

For over 20 years the so-called Th1/Th2 paradigm has been employed to explain the adaptive immune response. A third member of the T helper set, IL-17-producing $CD4^+$ T-cells, now called Th-17 cells, has recently been described as a distinct lineage that does not share developmental pathways with either Th-1 or Th-2 cells [15]. The relationship of the Th-17 response to the Th-1 and Th-2 responses is shown in Fig. 1. Th-17 cells are preferential producers of IL-17A, IL-17F, IL-21 and IL-22. Th-17 cells mediate host defensive responses against extracellular pathogens and are also involved in many autoimmune diseases. In general, the Th-17 response is pro-inflammatory in nature. While the role of Th-17 cells in the response to injury is unclear, a number of major cytokines involved in the inflammatory response after injury, such as IL-6 and tissue growth factor-β (TGF-β), are critical in Th-17 induction. The Th-17 response is uniquely positioned to influence both aspects of the innate and adaptive immune responses. Since both the innate and adaptive arms of the immune system have been implicated in immunoinflammatory complications following major injury, a potential role for the Th-17 response in such derangements can be speculated.

Interleukin-17A is elevated in a range of inflammatory conditions that include rheumatoid arthritis, pneumonia, systemic lupus erythematosus and allograft rejection [16,17]. Based on the varied disease states associated with IL-17 it is not surprising that IL-17 acts on a similar range of cell types such as neutrophils, fibroblasts, epithelial cells, and endothelial cells [16,17]. Several sources of IL-17 have been identified including Th-17 cells, CD8 T-cells, natural killer (NK) cells, γδ T-cells, and neutrophils [18]. Based on these observations, a potentially important and unidentified role for the Th-17 response in the development of immunoinflammatory complications following injury may exist. Moreover, a T-cell subset, γδ T-cells, which appear to be critical in the inflammatory and healing response after burn have been shown to be an important source of IL-17 [19–21]. Recent experimental findings have shown a causative relationship between IL-17, γδ T-cells and survival following sepsis [17]. Thus, a role for γδ T-cells in the generation of a Th-17 response after injury, independent of infection, can be speculated based upon those observations.

The early elevation in skin IL-17 levels is consistent with previous findings showing elevated IL-17 in lung injury models at 4 h after injury [22,23] and in sepsis models at 6 h after infection [17,24]. While the precise role of IL-17 in the early post-burn response remains to be elucidated, previous studies in other experimental models suggest a role for IL-17 in recruiting immune cells, such as neutrophils and monocytes and combating infection [22,24–27]. We have shown in the current study an association between IL-17 levels and infiltration of the wound site, qualitatively (Fig. 2). Whether this relationship is causative remains to be determined. Importantly, we have previously shown that infiltration of the burn wound site in this model system is dependent upon the presence of γδ T-cells [13,28] and others have shown that γδ T-cells are an important source of IL-17 [21]. Therefore, we speculate that the early elevation in skin IL-17 levels after burn is due to activation of the resident γδ T-cell population. Interestingly, our findings showed only an elevation in the late mediators of the Th-17 response (IL-17, IL-22), suggesting that the cellular source is a terminally differentiated cells. The γδ T-cells is ideally suited to serve is this role as in produces IL-17 and can respond directly to danger associated molecular

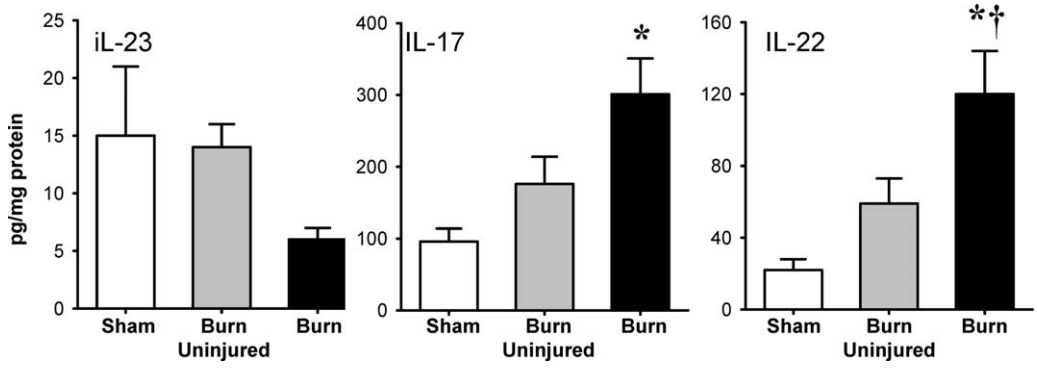

Fig. 4 – Late Th-17 cyokines. Samples of skin were collected from sham mice and from 2 sites on burn mice (normal [i.e., uninjured] and the burn site) at 3 h after injury. Tissue levels of IL-23, IL-17 and IL-22 were determined by ELISA as described in Section 2. Data are mean ± SEM; $n = 5–6$ mice/group. *$P < 0.05$ as compared with sham; †$P < 0.05$ as compared with burn uninjured.

patterns (DAMPS) via toll-like receptors [29]. Conversely, other cell types (NKT cells, NK cells, neutrophils, eosinophils, mast cells) have been reported to produce IL-17 [30,31]. Therefore, the net IL-17 expression in a given tissue bed may arise multiple cell types. Further experiments will be needed to verify this concept that γδ T-cell derived IL-17 orchestrates immune cell infiltration of the burn site.

It is unclear whether the early robust Th-17 response is beneficial or detrimental to the healing response, or whether it persists to times later than 3 h post-injury. Recent findings from our group have shown that IL-17 levels are elevated in the heart early after burn (i.e, 3 h) and were associated with cardiac injury (i.e., elevated plasma troponin levels) suggesting that an overly robust early Th-17 response may be detrimental [11]. In conclusion, the specific elevations in this group of cytokines suggest that they might provide unique targets for therapeutic interventions or serve as biomarkers for evaluation of proper healing. Nonetheless, appropriate pre-clinical data on the expression and function of these Th-17 cytokines in human burn wound healing will need to become available before intervention can be properly considered.

Conflict of interest

The author has no financial or personal relationship(s) with other individuals or organizations that could inappropriately influence or bias the work presented in this manuscript.

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REFERENCES

[1] Shakespeare P. Burn wound healing and skin substitutes. Burns 2001;27(5):517–22.
[2] DeSanti L. Pathophysiology and current management of burn injury. Adv Skin Wound Care 2005;18(6):323–32.
[3] Singer AJ, Clark RA. Cutaneous wound healing. N Engl J Med 1999;341(10):738–46.
[4] Martin P. Wound healing—aiming for perfect skin regeneration. Science 1997;276(5309):75–81.
[5] Guo Z, Kavanagh E, Zang Y, Dolan SM, Kriynovich SJ, Mannick JA, et al. Burn injury promotes antigen-driven Th2-type responses in vivo. J Immunol 2003;171(8):3983–90.
[6] O'Sullivan ST, Lederer JA, Horgan AF, Chin DHL, Mannick JA, Rodrick ML. Major injury leads to predominance of the T helper-2 lymphocyte phenotype and diminished interleukin-12 production associated with decreased resistance to infection. Ann Surg 1995;222:482–92.
[7] Alexander M, Chaudry IH, Schwacha MG. Relationships between burn size, immunosuppression, and macrophage hyperactivity in a murine model of thermal injury. Cell Immunol 2002;220(1):63–9.
[8] Schwacha MG, Knoferl MW, Chaudry IH. Does burn wound excision after thermal injury attenuate subsequent macrophage hyperactivity and immunosuppression? Shock 2000;14(6):623–8.
[9] McCarthy L, Wetzel M, Sliker JK, Eisenstein TK, Rogers TJ. Opioids, opioid receptors, and the immune response. Drug Alcohol Depend 2001;62(2):111–23.
[10] Alexander M, Daniel T, Chaudry IH, Schwacha MG. Opiate analgesics contribute to the development of post-injury immunosuppression. J Surg Res 2005;129(1): 161–8.
[11] Oppeltz RF, Zhang Q, Rani M, Sasaki JR, Schwacha MG. Increased expression of cardiac IL-17 after burn. J Inflamm (Lond) 2010;7:38.
[12] Faunce DE, Llanas JN, Patel PJ, Gregory MS, Duffner LA, Kovacs EJ. Neutrophil chemokine production in the skin following scald injury. Burns 1999;25(5):403–10.
[13] Alexander M, Daniel T, Chaudry IH, Choudhry M, Schwacha MG. T-cells of the γδ T-cell receptor lineage play an important role in the postburn wound healing process. J Burn Care Res 2006;27(1):18–25.
[14] Toth B, Alexander M, Daniel T, Chaudry IH, Hubbard WG, Schwacha MG. The role of γδ T cells in the regulation of neutrophil-mediated tissue damage after thermal injury. J Leukoc Biol 2004;76:545–52.
[15] Basso AS, Cheroutre H, Mucida D. More stories on Th17 cells. Cell Res 2009;19(4):399–411.
[16] Dong C. Diversification of T-helper-cell lineages: finding the family root of IL-17-producing cells. Nat Rev Immunol 2006;6(4):329–33.
[17] Flierl MA, Rittirsch D, Gao H, Hoesel LM, Nadeau BA, Day DE, et al. Adverse functions of IL-17A in experimental sepsis. FASEB J 2008;22(7):2198–205.
[18] Weaver CT, Hatton RD, Mangan PR, Harrington LE. IL-17 family cytokines and the expanding diversity of effector T cell lineages. Annu Rev Immunol 2007;25: 821–52.
[19] Schwacha MG. Gammadelta T-cells: potential regulators of the post-burn inflammatory response. Burns 2009;35(3):318–26.
[20] Korn T, Oukka M, Kuchroo V, Bettelli E. Th17 cells: effector T cells with inflammatory properties. Semin Immunol 2007;19(6):362–71.
[21] Roark CL, Simonian PL, Fontenot AP, Born WK, O'Brien RL. gammadelta T cells: an important source of IL-17. Curr Opin Immunol 2008;20(3):353–7.
[22] Liu J, Feng Y, Yang K, Li Q, Ye L, Han L, et al. Early production of IL-17 protects against acute pulmonary *Pseudomonas aeruginosa* infection in mice. FEMS Immunol Med Microbiol 2010.
[23] Lo RS, Dumoutier L, Couillin I, Van Vyve C, Yakoub Y, Uwambayinema F, et al. IL-17A-producing gammadelta T and Th17 lymphocytes mediate lung inflammation but not fibrosis in experimental silicosis. J Immunol 2010;184(11):6367–77.
[24] Kasten KR, Prakash PS, Unsinger J, Goetzman HS, England LG, Cave CM, et al. Interleukin-7 (IL-7) treatment accelerates neutrophil recruitment through gamma delta T-cell IL-17 production in a murine model of sepsis. Infect Immun 2010;78(11):4714–22.
[25] Roussel L, Houle F, Chan C, Yao Y, Berube J, Olivenstein R, et al. IL-17 promotes p38 MAPK-dependent endothelial activation enhancing neutrophil recruitment to sites of inflammation. J Immunol 2010;184(8):4531–7.
[26] Shahrara S, Pickens SR, Mandelin AM, Karpus WJ, Huang Q, Kolls JK, et al. IL-17-mediated monocyte migration occurs partially through CC chemokine ligand 2/monocyte chemoattractant protein-1 induction. J Immunol 2010;184(8):4479–87.

[27] Pelletier M, Maggi L, Micheletti A, Lazzeri E, Tamassia N, Costantini C, et al. Evidence for a cross-talk between human neutrophils and Th17 cells. Blood 2010;115(2): 335–43.
[28] Daniel T, Thobe BM, Chaudry IH, Choudhry MA, Hubbard WJ, Schwacha MG. Regulation of the postburn wound inflammatory response by gammadelta T-cells. Shock 2007;28(3):278–83.
[29] Schwacha MG, Daniel T. Up-regulation of cell surface Toll-like receptors on circulating gammadelta T-cells following burn injury. Cytokine 2008;44(3):328–34.
[30] Hueber AJ, Asquith DL, Miller AM, Reilly J, Kerr S, Leipe J, et al. Mast cells express IL-17A in rheumatoid arthritis synovium. J Immunol 2010;184(7):3336–40.
[31] Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. Annu Rev Immunol 2009;27:485–517.