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Burn-injury affects gut-associated lymphoid tissues derived CD4+ T cells

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ABSTRACT

After scald burn-injury, the intestinal immune system responds to maintain immune balance. In this regard CD4+T cells in Gut-Associated Lymphoid Tissues (GALT), like mesenteric lymph nodes (MLN) and Peyer's patches (PP) respond to avoid immune suppression following major injury such as burn. Therefore, we hypothesized that the gut CD4+T cells become dysfunctional and turn the immune homeostasis towards depression of CD4+ T cell-mediated adaptive immune responses. In the current study we show down regulation of mucosal CD4+ T cell proliferation, IL-2 production and cell surface marker expression of mucosal CD4+ T cells moving towards suppressive-type. Acute burn-injury lead to up-regulation of regulatory marker (CD25+), down regulation of adhesion (CD62L, CD11a) and homing receptor (CD49d) expression, and up-regulation of negative co-stimulatory (CTLA-4) molecule. Moreover, CD4+CD25+ T cells of intestinal origin showed resistance to spontaneous as well as induced apoptosis that may contribute to suppression of effector CD4+ T cells. Furthermore, gut CD4+CD25+ T cells obtained from burn-injured animals were able to down-regulate naïve CD4+ T cell proliferation following adoptive transfer of burn-injured CD4+CD25+ T cells into sham control animals, without any significant effect on cell surface activation markers. Together, these data demonstrate that the intestinal CD4+ T cells evolve a strategy to promote suppressive CD4+ T cell effector responses, as evidenced by enhanced CD4+CD25+ T cells, up-regulated CTLA-4 expression, reduced IL-2 production, tendency towards diminished apoptosis of suppressive CD4+ T cells, and thus lose their natural ability to regulate immune homeostasis following acute burn-injury and prevent immune paralysis.

1. Introduction

Burn-injury leads to immunosuppression [1]. This deficit in host immune defenses is attributed to T cell immune suppression, including depletion of T cells [2], decrease in T cell proliferation [3], Th17 cells produced cytokines [4], deficiencies in pro-inflammatory cytokine production [5], induction of T cell anergy [6], dysfunctional dendritic cells [7], nitric oxide production by macrophages [8], TLR4 co-stimulation [9], nuclear factor-kB (NF-kB) and activated protein 1 (AP-1) alterations [10], and Fas-mediated apoptosis [11]. Despite all this published information little is known about the precise role of gut-associated lymphoid tissue CD4 + T cells. We have previously shown that T cells and neutrophils both contribute to immune suppression observed following day-3 post-burn [7,12–15]. We have also observed that immunosuppression following burn injury leads to loss of intestinal mucosal barrier thus allowing bacteria to translocate and subsequently appear in intestinal lymph nodes such as mesenteric lymph nodes (MLN) and even circulation, culminating in septicemia and death [15]. Apart from neutrophil associated factors causing intestinal tissue injury and loss of gut barrier function, we also reported a role of CD4 + T cells and antigen presenting cells (APC). The current study examining the role of CD4 + T cells of intestinal origin (GALT) is a progression of our previous published studies [7,12]. In an established scald burn injury model we documented that intestinal CD4 + T cells obtained from mesenteric lymph nodes (MLN) have suppressed IL-2 production and proliferation, accompanied by substantial deletion via apoptosis [12]. The data has started to appear delineating the role of CD4 + T cells in burn-injury as well as other injury conditions [6]. However, all these published studies in burn model so far have looked at CD4 + T cells of splenic and/or draining lymph nodes. Nobody to our knowledge has specifically examined the gut-associated lymphoid tissue (GALT)-CD4 + T cells, specifically originating from Peyer's patches and mesenteric lymph nodes. In the current study we elaborated the functional and phenotypic characterization of GALT-CD4 + T cells, the adoptive transfer of burn-injured-associated CD4 + T cells and their ability to modulate immune response of naïve CD4 +

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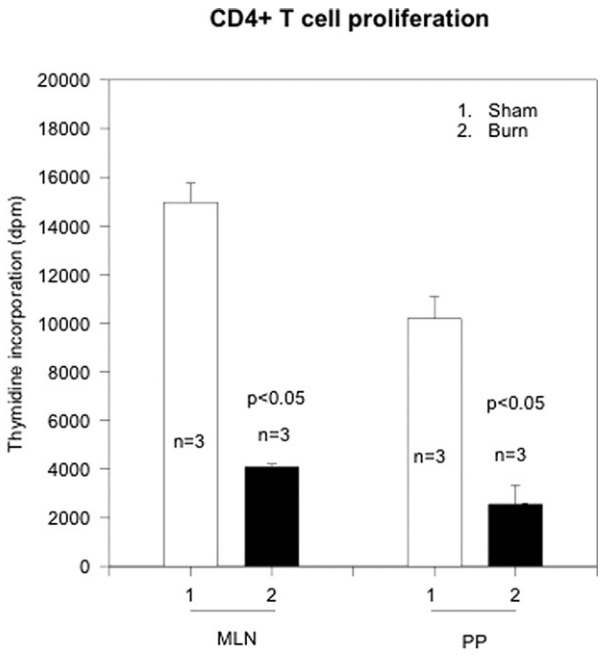

Fig. 1. The figure shows CD4 + T cell proliferation as assessed by Thymidine incorporation (dpm). CD4 + T cells were obtained from gut-associated lymphoid tissue (GALT), i.e., mesenteric lymph nodes (MLN) and Peyer's patches (PP) from sham (open bars) and burn (closed bars). The data represents Mean ± SD Thymidine incorporation (dpm) values obtained from sham and day-3 burn rats (n, number of animals = 3). $p < 0.05$ values show statistical significance.

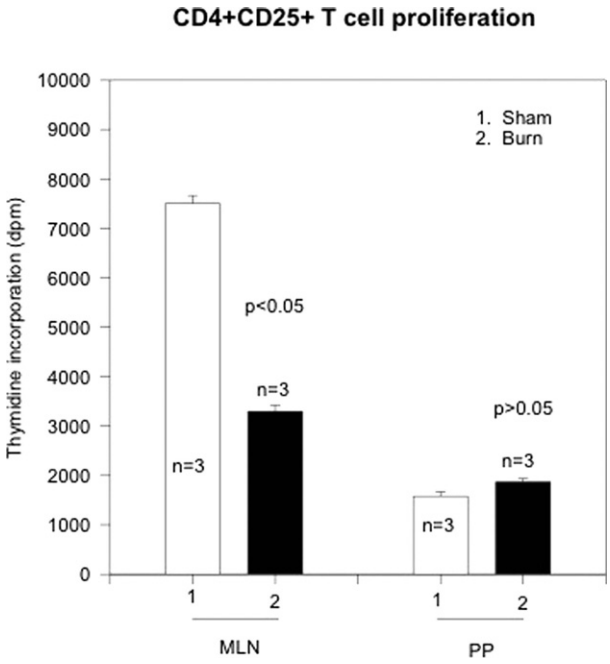

Fig. 2. The figure shows CD4 + CD25 + T cell proliferation as assessed by Thymidine incorporation (dpm). CD4 + CD25 + T cells were obtained from mesenteric lymph nodes (MLN) and Peyer's patches (PP) from sham (open bars) and burn (closed bars). The data represents Mean ± SD values of sham and day-3 burn rats (n, number of animals = 3). Statistical analysis of $p < 0.05$ shows significance and $p > 0.05$ shows no significance.

T cells of the recipient animals. Our data showed that acute burn-injury altered the function of GALT-CD4 + T cells, including cell proliferative responses, cell surface marker expression and IL-2 sensitivities. Furthermore, burn-injury also decreased the sensitivity of GALT-CD4 + CD25 + T cells to camptothecin-associated, as well as spontaneous apoptosis. Finally, the ability of burn-induced GALT-CD4 + CD25 + T cells to transfer their depressive effects to sham rats following adoptive transfer over 1-3 days postburn. We conclude, GALT-derived CD4 + T cells, theoretically known to be of tolerant-type being at the fore front of antigenic challenge, both from commensals and gut antigens; following burn-injury, tend to modulate towards losing their normal regulatory function, and play a role in breach of the normal immune-homeostasis towards an unwanted immune-paralysis.

2. Materials and methods

2.1. Scald burn-injury

Male Sprague Dawley rats, ~2 months old, weighing 200–250 g (Harlan, Indianapolis, IN) were used in this investigation. All animal housing, experimentation and treatment were based on previously published protocols approved by Chicago State University Animal Care and Use Committee (IACUC) committee and in accordance with NIH guidelines. For adoptive transfer experiments male Lewis rats (250–300 g) were used. All rats were acclimatized in the animal facility for at least one week before being subjected to any treatment and were given free access to water and standard lab rat chow ad libitum. The criteria stated here are based on previous published protocols of the acute burn injury model (see references Fazal et al. [12–15]). As pain therapy, buprenorphine (2 mg/kg) intra-peritoneal were given to rats 30 min prior to burn injury for preemptive analgesia, and every 8 h till ready for sacrifice and/or terminally euthanized. Major burn injury protocols have shown to be associated with full thickness 3rd degree skin burns over ~30% of the total body surface associated with fairly consistent inflammation-linked immune as well as gut barrier abnormalities. Moreover, almost all rats survive this injury/trauma and mortality rate is <5% over 3-days. Scald burn destroys pain receptors and most rats handle this scald well and are able to move around, eat and drink normally during the three-day post-burn period. Accordingly, upon verification of deep anesthesia with sodium pentobarbital (40–50 mg/kg intra-peritoneal, i.p.), as determined by absence of any signs of awareness including response to hind limb pinch, rats were subjected to the following steps in quick succession: (i) the back fur was shaved off ~30% Total Body Surface Area (TBSA), (ii) rats were securely placed in an appropriately sized bottomless plastic mold that allows the shaved area of the skin on the back to be immersed in water while the rest of the body is protected, (iii) the back was immersed in a hot water bath (95–97 °C) for 10 s, (iv) excess hot water was immediately wiped off to avoid an additional injury, and (v) 10 ml of normal saline was injected intra-peritoneal for resuscitation. The resuscitation procedure was sufficient to restore control level of urine output in the experimental group of rats over a period of ~24 h. Therefore, we did not find any need to compensate for the loss of fluids. Moreover, animals start feeding and drinking normally; hence do not require artificial feeding. The experimental animals were divided into two groups and each group had at least six rats; [1] Burn, [2] Sham control, which go through clipping of back hair, anesthesia, immersion in water bath at room temperature, resuscitation with 10 ml normal saline. All the data reported and analyzed in this study was obtained from first two groups. Each groups had minimum of six animals, three separate samples were collected from each animal for analysis. In the subsequent experiments "n" represents the number of either samples or animals in the study as detailed in the legends of the respective figures. This injury protocol is well established, repeatable and extensively used by the peers in the field of acute burn-injury studies.