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DIFFERENTIAL IMMUNOLOGICAL PHENOTYPES ARE EXHIBITED FOLLOWING SCALD AND FLAME BURNS

Johannes Tschöp^{*,†,‡}, André Martignoni^{*,†,‡}, Maria D. Reid^{*,†}, Samuel G. Adediran[†], Jason Gardner^{*}, Greg J. Noel^{*}, Cora K. Ogle^{*,†}, Alice N. Neely^{*,†}, and Charles C. Caldwell^{*,†}

^{*}Department of Research, Shriners Hospital for Children

[†]The Laboratory of Trauma, Sepsis & Inflammation Research, Department of Surgery, University of Cincinnati College of Medicine, Cincinnati, Ohio, U.S.A.

[‡]Department of Anesthesiology, Klinikum Grosshadern, Munich, Germany.

Abstract

A dysfunctional immune system is known to be part of the pathophysiology after burn trauma. However, reports that support this have used a variety of methods, with numerous variables, to induce thermal injury. We hypothesized that, all other parameters being equal, an injury inflicted by a scald would yield different immunological responses than one inflicted by a flame. Here, we demonstrated that both burn methods produced a full-thickness burn, yet there was more of an increase in subdermal temperature, hematocrit, mortality, and serum IL-6 concentrations associated with the scald burn. On postinjury day 1, the scald-burned mice showed diminished lymphocyte numbers, interferon γ production, and lymphocyte T-bet expression as compared with sham- and flame-burned mice. On postburn day 8, spleens from both sets of thermally injured animals showed an increase in proinflammatory myeloid cells as compared with sham-burned mice. Furthermore, the T-cell numbers, T-bet expression, and phenotype were changed such that interferon γ production was higher in scald-burned mice than in sham- and flame-burned mice. Altogether, the data show that differential immunological phenotypes were observed depending on the thermal injury method used.

Keywords

Thermal injury; spleen; T cell; macrophage; T-bet; IFN- γ

INTRODUCTION

According to the 2005 edition of the American Burn Association's National Burn Repository, which now contains information regarding more than 126,000 cases, 46% of all burns are due to direct flame injuries and 32% are due to scalds. Therefore, it is appropriate that the predominant models used over the years to study burn injury have used either a flame burn (1) or a scald (2). Partial- and full-thickness burn models have been developed by varying the temperature and/or duration of the burn. Furthermore, templates can be used to vary the amount of surface area injured (3). However, the different models of inflicting thermal injury and the variability of the size and thickness of the burn make it difficult to compare reports.

It is well established that thermal trauma induces an inflammatory response. Acute inflammation is terminated by a number of anti-inflammatory processes. Both actions can modulate numbers and functions of leukocytes. In response to thermal injury, it has been reported that splenic T cell 1) numbers are initially decreased (4,5), 2) production of interferon (IFN) γ and IL-2 is reduced (6–8), and 3) proliferation is altered (9,10). Furthermore, it has been shown that macrophages become hyperactive after thermal injury, as determined by increased secretion of inflammatory mediators (11,12).

Here, we hypothesized that postinjury immune dysfunction would vary depending on whether a scald or a flame was used to inflict the thermal injury. To test this, we modified the flame model that we used here (13) by using the same strain and sex of mouse, burn size, burn duration, and anesthesia and resuscitation protocols as are used here in our scald mouse model (14), thereby allowing us to compare directly the effects of a scald versus a flame injury. We then analyzed splenic leukocyte function and numbers 1 and 8 days after the thermal injury.

MATERIALS AND METHODS

Scald- and flame-burn models

Male C57BL/6J mice weighing 22 to 26 g were anesthetized with 3% isoflurane in oxygen. Hair was then clipped from their backs. For the scald burn, the mice were placed in a template that exposed 18% of their backs. The mice were immersed in 9000B0C water for 9 s. For the flame burn, a flame-resistant card was pressed against the shaved area that exposed 18% of their backs, and the exposed back was covered with 0.5 mL ethanol. This was ignited and allowed to burn for 9 s. Immediately after either burn, 1.8 mL of sterile saline was given intraperitoneally, and the mice were allowed to recover for a minimum of 30 min in an oxygen tent consisting of a cloth-covered cage placed on a heating pad with 5 L/min oxygen blown in. Sham-treated mice were given the same treatment except that they were immersed in room-temperature water. Previous experimentation in our laboratory has shown that there are no immunological differences between sham-scalded mice dipped in room-temperature water and sham-flamed mice that had ethanol placed but not ignited on the dorsal region (data not shown).

Histochemical analysis

On postburn day 8, excised skins were fixed with 4% paraformaldehyde in phosphate-buffered saline and embedded in paraffin. Masson trichrome staining was performed for histological examination.

Subdermal temperature measurements

The thermometer sensor was placed subdermally under the exposed back. At the end of the flame or scald burn, the temperature was recorded.

Hematocrit determination, leukocyte counting, and preparation for flow cytometry

Single-cell suspensions were prepared using standard procedures (15). The hematocrit and cell counts were determined using a Beckman-Coulter AcT 10 cell counter. The leukocytes were stained with fluorescein isothiocyanate, phycoerythrin, peridinin chlorophyll, and allophycocyanin-labeled antibody. Specifically, the anti-F4/80 (clone BM8), anti-CD11b (clone M1/70), and anti-IFN- γ (clone XMG1.2) antibodies were purchased from Invitrogen (Carlsbad, Calif). The anti-CD62L (clone MEL-14), anti-CD4 (clone RM4-5), anti-CD8 (clone 53-6.7), anti-major histocompatibility (MHC) II (clone AF6-120.1), anti-TNF- α (clone MP-XT22), and rat immunoglobulin G1 isotype (clone A85-1) antibodies were purchased from BD Biosciences (San Jose, Calif). Samples were analyzed with an LSR flow cytometer and CELLQUEST Pro software (BD Biosciences).

Cytokine analysis

Intracellular staining of plate-bound anti-CD3 and CD28-activated cells to evaluate IFN- γ cytokine production *in situ* was conducted as previously described (15). Intracellular staining of LPS-activated cells to evaluate cytokine production *in situ* was conducted in permeabilized cells using 2 μ M (final concentration) monensin (Cal-Biochem, La Jolla, Calif) and saponin buffer (phosphate-buffered saline containing 0.1% [wt/vol] saponin, 0.1% bovine serum albumin, 0.01 M HEPES, and 0.1% sodium azide) after 4 h of LPS stimulation. After staining with anti-TNF- α monoclonal antibody (mAb), cells were analyzed by flow cytometry as previously described. Concentrations of IFN- γ in cell-free supernatants from plate-bound anti-CD3 and CD28-stimulated T cells were determined using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (Invitrogen).

T-bet analysis

The intracellular analysis of T-bet expression and intensity was conducted as previously described except that the cells were treated *ex vivo*, not after stimulation. The anti-T-bet (clone 4B10) was purchased from eBioscience (San Diego, Calif).

Statistics

Statistical comparisons were performed using Student *t* test (two groups) or Tukey test and ANOVA (more than two groups). StatView (SAS Institute, Cary, NC) and GraphPad Prism 3.0 were used for statistical analyses. A value of $P \leq 0.05$ was considered statistically significant.

RESULTS

Effects of different modes of thermal injury

In the burn models of thermal injury, the methodology of inflicting a flame and scald burn is fundamentally different. As detailed in the methods, during a flame burn, the mouse is prone, allowing the burn heat to flow away from the torso, whereas during the scald, the burn-generated heat flows into the torso. To verify that both methods inflict a full-thickness burn, burned areas were examined by histology on day 8 after the thermal injury. Skin samples from the leading edge of the burn (Fig. 1, A–C) and the center of the burn (Fig. 1, B–D) were compared between the two types of burn injury. A blinded burn pathologist concluded that both methods generated a full-thickness burn, as indicated by the disruption of the cellular structure in the burned skin. Furthermore, both methods yielded a sharp leading edge between the burn and unburned sections. To quantify the effect of these different burns, we placed a temperature probe subdermal at the burn site. As shown in Figure 2A, the subdermal starting temperature was similar for both methods, whereas at the end of the 9-s scald burn, there was a significantly higher subdermal temperature as compared with the flame burn, 146 versus 11200B0F. We also determined that the mortality associated with each technique was dissimilar. There was a 22% mortality associated with the scald burn as compared with 11% mortality with the flame-burned mice with each group having 45 mice (data not shown). There was no mortality associated with sham-burned mice. Additionally, we determined the hematocrit from mice inflicted with a sham, scald, or flame burn at different time points after the injury (Fig. 2B). We observed an increased hematocrit from both the scald- and flame-burned mice 6 h after injury. The hematocrit was significantly higher from the scalded mice as compared with flame-burned mice. Within 48 h, the hematocrit from both sets of mice return to the level associated with the sham-burned mice. Finally, to determine the systemic immunological response to the differing burn techniques, we quantified serum IL-6 concentrations (Fig. 2C). After 6 h, there was a significantly increased concentration of IL-6 in the scald-burned as compared with the flame-burned, whereas there were no significant

levels of IL-6 detected in sham-burned mice (data not shown). We further determined that significant concentrations of systemic IL-6 remain elevated 24 h after thermal injury and return to sham levels at 48 h. Thus, although both burn models produced a full-thickness burn, there was more of an increase in subdermal temperature, mortality, hematocrit, and serum IL-6 concentrations associated with the scald burn.

Effects of thermal injury upon splenic leukocyte number and phenotype 1 day after thermal injury

The spleen is the largest single secondary lymphoid organ in mice and represents approximately 0.2% to 0.4% of total body weight. It is immunologically important because it contains significant numbers of B cells, T cells, and macrophages. In response to thermal injury, the spleen mass and splenocyte numbers were altered differentially by burn type. Specifically, in sham-burned mice, it was observed that the spleen represented approximately 0.35% of the total body weight. The scalded mice spleen percentage dropped to 0.23% 1 day after thermal injury, whereas the flame-burned mice spleens showed no significant difference as compared with the sham-burned mice spleens (Fig. 3A). To explore the impact of burn injury on leukocyte numbers and function, we inflicted a sham, scald, and flame burn upon mice. We assessed changes in lymphocyte numbers in differing tissues 1 day after injury. In peripheral blood, the scald burn diminished circulating levels of naive CD4 and CD8, as well as nonnaive CD4 T cells as compared with the sham burn (Fig. 3B). After the flame burn, only the significant decrease in nonnaive CD4 T cells was observed. In the thymus, significant reductions in CD4, CD8 double-positive thymocytes were observed after both types of burn injury (Fig. 3C). In addition, significant reductions in CD4 single-positive and CD4 CD8 double-negative cells were observed after scald injury.

The number of macrophages and neutrophils showed no significant change in any of the treated mice as compared with untreated mice 1 day after burn injury (data not shown). We examined T-lymphocyte absolute numbers from the spleens of mice after either a flame or scald burn was inflicted. As shown in Figure 3D, we observed that naive CD4 and CD8 T cells were decreased after scald injury as compared with the numbers of these cells from sham- and flame-burned animals 1 day after thermal injury.

In a temporal study of splenic T-cell depletion after a sham or scald burn, we determined that T cells were depleted from the spleen from scalded mice starting after 6 h (Fig. 4A). T-Cell depletion continued for 24 h when the 50% depletion was observed (Fig. 3D). To gain insight into the mechanism of depletion, we isolated splenic T cells 8 h after either a sham or a scald burn. Immunoblot analysis showed a 3-fold increase in active caspase 3 in the T cells taken from scald-burned mice (Fig. 4, B and C). We also examined the functionality of splenic T cells extracted from mice 1 day after thermal injury. Using a T-cell receptor (TCR)-specific mitogen to stimulate splenic T cells, we found that the percentage of IFN- γ producing nonnaive CD4 T cells taken from both the scald- or flame-burned mice was decreased as compared with sham-burned mice (Fig. 5A). Of the cells that produced IFN- γ , the intensity of expression was significantly decreased in both naive and nonnaive CD8 cells isolated from scald-burned mice (Fig. 5B). Finally, splenocytes activated with a TCR-specific mitogen produced 20-fold less IFN- γ in contrast to cells taken from flame-burned mice (Fig. 5C).

T-Bet is a member of the T-box family of transcription factors. It is a master switch in the development of proinflammatory T-cell responses and promotes the production of IFN- γ (16). As we had observed differences in IFN- γ expression and accumulation, we next investigated the T-bet expression in splenic T cells. On postburn day 1, we found that T-bet expression was significantly decreased on naive (CD62L^{high}) T cells taken from both scald- and flame-burned mice and nonnaive (CD62L^{low}) CD8 T cells from scald-burned mice (Table

1). Of the cells that expressed T-bet, we did not observe significant differences in expression intensity.

Effects of thermal injury upon splenic leukocyte number and phenotype 8 days after thermal injury

On postburn day 8, we observed a trend toward increased splenocytes in both the flame- and scald-burned as compared with sham-burned mice (data not shown). Flow cytometric analysis of the size and granularity of the cells suggested that this increase was due, in part, to myeloid cells. To verify this observation and to further elucidate the type of myeloid cells, flow cytometric analysis was conducted using the surface markers CD11b and F4/80. In the scald- and flame-burned spleen, there was no significant change in the absolute numbers $F4/80^{-}/CD11b^{+}$ cells. However, there was an increase of 228% and 250% in $F4/80^{+}/CD11b^{+}$ cells in the scald- and flame-burned spleens, respectively (Fig. 6A). Furthermore, both sets of myeloid cells expressed the MHC II, and after both the scald and flame burn, the expression of this molecule was down-regulated 40% to 60% (Fig. 6B). To distinguish the inflammatory potential of the $F4/80^{-}/CD11b^{+}$ and $F4/80^{+}/CD11b^{+}$ cell types, cells were extracted from both sham-, scald-, and flame-burned mouse spleens; stimulated with LPS; and intracellular production of TNF- α was determined by flow cytometry (Fig. 6C). The data show that TNF- α production by the $F4/80/CD11b$ doubly positive cells is greater as compared with the $F4/80^{-}/CD11b^{+}$ cells. Importantly, this was true for the cells isolated from all three groups. In addition, the intensity of TNF- α expression was not significantly different between the three groups. Finally, because the spleen contains dendritic cells, we excluded CD11c-expressing cells in our analysis (data not shown). Thus, 8 days after thermal injury, there is a significant increase in proinflammatory $F4/80^{+}/CD11b^{+}$ myeloid cells in the spleens of both sets of thermally injured mice.

On postburn day 8, we also assessed changes in lymphocyte numbers in blood, thymus, and spleen. In peripheral blood, the scald-burned mice exhibited diminished circulating levels of naive CD4 and CD8 T cells as compared with the sham burn (Fig. 7A). In the blood from the flame- and scald-burned mice, there was a significant increase in nonnaive CD8 T cells. In the thymus, we observed significant reductions in both CD4 and CD8 single-positive and a significant increase in CD4 CD8 double-negative cells after both types of burn injury (Fig. 7B). The absolute numbers of splenic naive CD4 T cells from both the scald- and flame-burned mice were significantly decreased as compared with these cells in sham-burned mice (Fig. 7C). Furthermore, the absolute numbers of nonnaive CD4 and CD8 T cells from the scald-burned mice were significantly higher as compared with these cells taken from the flame- and sham-burned mice. Thus, 8 days after thermal injury, the two types of burns altered T-cell populations in the blood, thymus, and spleen.

On postinjury day 8, we examined splenic T cells extracted from scald-, flame-, and sham-burned mice. Using a TCR-specific mitogen to stimulate splenic T cells, we found that the percentage of IFN- γ -producing naive CD4 T cells taken from both scald- or flame-burned mice was increased as compared with sham-burned mice (Fig. 8A). Furthermore, the percentage of nonnaive CD4 T cells producing IFN- γ was increased in cells taken from scald-burned mice. Of the cells that produced IFN- γ , the intensity of expression was significantly decreased in both naive and nonnaive CD4 and CD8 cells isolated from scald-burned mice (Fig. 8B). However, scalded mice splenocytes activated with a TCR-specific mitogen produced 3-fold more IFN- γ in contrast to cells taken from flame-burned mice (Fig. 8C).

Similar to postburn day 1, we investigated the percentage and intensity of T-bet expression in T cells (Table 2). We found that the percentage of cells expressing T-bet was significantly decreased on T cells taken from scald-burned mice as compared with sham-burned mice. The percentage of naive T cells isolated from flame-burned mice also had significantly decreased T-bet expression. Of the cells that expressed T-bet, we observed significant decreases in the

expression intensity of nonnaive CD4 and CD8 as well as naive CD8 T cells isolated from the scald-burned mice.

DISCUSSION

Our data show that both types of thermal injury inflicted a clearly demarcated full-thickness burn. We also observed that the scald burn method as compared with the flame method resulted in increased mortality, hematocrit, systemic IL-6, subdermal temperatures (Fig. 1 and Fig. 2), and lymphocyte depletion (Fig. 3). These data strongly suggest that the scald model produced a greater severity of injury. We suggest that the scald method transfers more thermal energy, and this results in an increased amount of tissue damage. This difference in the severity of injury alters the immune response as previously shown (17). Altogether, we believe this is the first study to directly compare the injury severity and immunological response to two distinct methods of inflicting thermal injury.

On postburn day 1, the spleens from scalded mice had decreased mass as compared with spleens from sham- and flame-burned mice (Fig. 3A). Additionally, splenic cell numbers were significantly decreased in the scalded mouse spleen as compared with sham and flamed mouse spleens. Further examination of purified T cells taken from scald-burn spleens showed that these T cells expressed increased activated caspase 3 as compared with T cells purified from sham-burned mice, demonstrating that these cells were undergoing apoptosis. Our data showed that lymphocytes were depleted after the scald burn as compared with the flame burn. We suggest that this depletion contributed to the immunosuppression after trauma in two distinct ways. First, the elimination of naive lymphocytes limits the adaptive immune system's ability to respond to antigens. Second, tens of millions of apoptotic cells appearing within 24 h likely results in immunosuppression as previously reported (18). In this report, the presence of apoptotic cells was linked with a decrease in IFN- γ production after *ex vivo* stimulation of splenic T cells. On postburn day 1, a similar reduction in IFN- γ production was observed in scald-burned splenic T cells, but not in the flame- or sham-burned splenic T cells. Thus, we suggest that the suppressed IFN- γ production was at least partly associated with apoptosis-driven lymphocyte depletion.

We observed that splenic T cells from scalded mice produced significantly less IFN- γ as compared with sham- and flame-burned mice 1 day after thermal injury (Fig. 5D). Intracellular staining showed little differences in the percentage of cells producing IFN- γ from all three groups of mice. However, the intensity of IFN- γ expression (Fig. 5C) and T-bet expression (Table 1) was decreased in the CD8 T cells from scalded mice. These decreases, coupled with the significant depletion of naive T cells (Fig. 3C), is likely partly responsible for the decreased IFN- γ production. Interestingly, this is not the case for T cells isolated from flame-burned mice. After a scald burn, it has been reported that mitogen-activated protein kinases and nuclear factor- κ B and activator protein 1 activity are altered in T cells (7,19). Inhibition of these proteins is known to result in decreased IFN- γ production. Thus, the combination of decreased numbers, inhibition of key transduction molecules, and decreased T-bet expression are all likely playing a key role in the reduced IFN- γ accumulation from scalded mice. This is significant in that it has been shown that IFN- γ plays a pivotal role in combating bacterial infections (18,20).

Previous studies have shown that after burn injury, tissue edema becomes evident (21,22). The amount of tissue edema is a result of a combination of events to include the size and location of the burn, the type burn, and the amount of fluid resuscitation (23). At 6, 24, and 48 h after the sham or burn injury, we isolated the lungs, kidneys, intestine, and heart. We then conducted wet and dry weight measurements on these tissues to determine edema. Although we observed increases in tissue edema in the tissues tested, we observed no significant differences between

the two models or between the flame and scald model. We speculate that the lack of differences seen are due to differences in burn size, rodent used, anesthesia, and resuscitation amounts.

We and others have demonstrated that burn injury can cause an enhanced Toll-like receptor-mediated proinflammatory response by cells of the innate immune system (24,25). Further studies have shown that treating C57BL/6 mice with 50 µg of staphylococcal enterotoxin B demonstrated lethality in burn-injured, but not sham-injured, C57BL/6 mice (26). Here, we showed that there was a significant increase in splenic CD11b⁺/F4/80⁺ macrophages 8 days after both the flame and scald burn, whereas there were no significant differences found in the absolute number of CD11b⁺/F4/80⁺ macrophages (Fig. 6A). Thus, after both types of burns, there was an increase in hyperinflammatory macrophages. This is significant in that upon treatment with LPS, the CD11b⁺/F4/80⁺ macrophages were large producers of the proinflammatory cytokine TNF-α (Fig. 6C). It is likely that both flame- or scald-burned mice would have a robust proinflammatory response similar to LPS. Interestingly, it has been recently shown (27) that when scalded mice were challenged with *Escherichia coli* 7 days after a scald burn, the burned mice were less susceptible to the infection. Furthermore, the report demonstrated more F4/80-expressing cells at the site of infection. We think that the presence of these hyperinflammatory F4/80-expressing macrophages is beneficial when clearing an infection.

On postburn day 8, T-cell numbers in blood, thymus, and spleen were returning to those seen in the sham-burned mice (Fig. 7). In addition, on postburn day 8, IFN-γ production and T-bet expression in T cells from flame-burned mice were similar to that found in sham-burned mice. However, IFN-γ accumulation was approximately 3-fold higher in the T-cell samples from scalded mice. The percentage of T cells producing IFN-γ was generally higher in these cells (Fig. 8A). In contrast, the amount of IFN-γ was somewhat lower (Fig. 8B). In general, our data suggest that compared with the other conventional T cells tested, a higher percentage of nonnaive CD8 cells produced IFN-γ. Furthermore, T-bet expression was higher in these cells (Table 1 and Table 2). We found that in the T cells isolated from scald mice, there was a 6-fold higher number of nonnaive CD8 T cells as compared with sham- and flame-burned mice. We speculate that it is this increased number in nonnaive T cells that is responsible for the observed increase in IFN-γ accumulation.

In summary, our findings indicate that the scald burn induces a greater severity of injury as compared with the flame burn. Associated with this increased severity is an increase in systemic IL-6, mortality, hematocrit, lymphocyte depletion, and functionality. However, another finding is that 8 days after trauma in scald-burned mice, both the innate and adaptive arms of immunity are primed more toward a proinflammatory phenotype. It is likely that this will allow for a better bacterial clearance if infected. Altogether, these findings show that depending on the severity of injury and the timing, the response to an infection can be different. Furthermore, the characterization of both models here will allow for better interpretation of previously reported results and the design of future experiments.

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Fig. 1. Thermal injury induced by flame or scald method results in a full-thickness burn. A–D, Representative histological sections of dorsal skin taken from a scald- (A/B) and flame-burned mouse (C/D), respectively. The magnification is $\times 12.5$.

Fig. 2.

Changes in subdermal temperature, hematocrit, and systemic IL-6 vary in response to different thermal injury. A, Subdermal temperature at the beginning and end of a 9-s burn is measured as described in “Materials and methods.” B, The hematocrit associated with each model of thermal injury. C, IL-6 concentrations at the indicated time after thermal injury from scald- and flame-burned mice as measured by ELISA. The sample size is four to six for each experiment. * $P < 0.05$ as compared with wild type; † $P < 0.05$ as compared with flame. ND indicates not detected.

Fig. 3.

Differential spleen mass and absolute lymphocyte numbers 1 day after thermal injury 24 h after the indicated thermal injury. A, The ratio of the body and spleen mass was determined.

Furthermore, lymphocytes were counted and analyzed by flow cytometry. The numbers and type of T cells from peripheral blood (B), thymus (C), and spleen (D) were determined. * $P < 0.05$ as compared with wild type; † $P < 0.05$ as compared with flame. The sample size is 7 to 11 from two combined experiments. DP indicates double positive for CD4 and CD8; DN, double negative for CD4 and CD8; SP, single positive.

Fig. 4. Thermal injuries result in differential T-cell function and caspase-3-mediated T-cell depletion. After sham burn or thermal injury, splenic T cells were isolated, purified, counted, and prepared for immunoblot. A, The percentage of conventional T cells as compared with sham-burned mice taken from scalded mice at the indicated time. B, An immunoblot demonstrating active caspase 3 in T-cell samples isolated from sham- or scald-burned mice. C, Denosometric analysis of immunoblot. * $P < 0.05$ as compared with sham burn.

Fig. 5.

Varied functional differences in T cells 1 day after scald and flame thermal injury. *Ex vivo* splenic T cells were stimulated with plate-bound anti-CD3 and CD28 mAb, and 18 h later, the cells were analyzed for the percentage (A), and intensity (B) of IFN- γ production. In a parallel set of experiments, the supernatants (C) were analyzed for IFN- γ accumulation by ELISA. The sample size for the number determination is seven to nine and is combined from two independent experiments. * $P < 0.05$ as compared with wild type; † $P < 0.05$ as compared with flame. MFI indicates mean fluorescence intensity.

Fig. 6.

Differential absolute myeloid cell numbers and phenotypes 8 days after thermal injury. Splenic myeloid cells were counted and analyzed by flow cytometry for the absolute numbers of macrophage subsets (A) 8 days after the indicated thermal injury. B, Mean fluorescence intensity (MFI) of MHC II expression. Cells were stimulated with LPS and then analyzed by CD11b, F4/80, and intracellular TNF- α expression by flow cytometry (C). The sample size for the number determination is 8 to 12 and is combined from two independent experiments. The M1 gate is set such that the isotype control yields less than 5% expression. * $P < 0.05$ vs. sham.

Fig. 7.

Differential absolute lymphocyte numbers 8 days after thermal injury. Splenic lymphocytes were counted and analyzed by flow cytometry 8 days after the indicated thermal injury. The number and phenotypes of peripheral blood (A), thymus (B), and splenic T lymphocytes (C) were determined. * $P < 0.05$ as compared with wild type; † $P < 0.05$ as compared with flame. The sample size is 8 to 12 from two combined experiments. DN indicates double negative for CD4 and CD8; DP, double positive for CD4 and CD8; SP, single positive.

Fig. 8.

Varied functional differences in T cells 8 days after scald and flame thermal injury. *Ex vivo* splenic T cells were stimulated with plate-bound anti-CD3 and CD28 mAb 8 days after the indicated thermal injury, and 18 h later, the percentage (A) and intensity (B) of IFN- γ production were determined. In a parallel set of experiments the supernatants were analyzed for IFN- γ accumulation by ELISA (C). The sample size for the number determination is seven to nine and is combined from two independent experiments. * $P < 0.05$ as compared with wild type; † $P < 0.05$ as compared with flame.

Table 1
The percent T-bet expression and intensity in splenic T cells 1 day after sham or burn injury

PBD1	T-Bet expression, %			T-Bet MFI		
	Sham	Scald	Flame	Sham	Scald	Flame
CD4, 62L ^{high}	8.3 ± 0.4	12.0 ± 1.1 [*]	11.4 ± 0.4 [*]	429 ± 36	376 ± 43	417 ± 50
CD4, 62L ^{low}	26.4 ± 2.4	22.0 ± 3.5	25.2 ± 2.2	725 ± 64	602 ± 68	669 ± 50
CD8, 62L ^{high}	25.9 ± 1.2	20.9 ± 1.3 [*]	21.3 ± 0.7 [*]	468 ± 36	435 ± 37	488 ± 28
CD8, 62L ^{low}	50.9 ± 3.6	35.5 ± 5.6 [*]	48.9 ± 5.0	657 ± 44	760 ± 139	670 ± 40

The sample size is seven to eight from two combined experiments.

^{*} *P* < 0.05 as compared with wild type.

MFI indicates mean fluorescence intensity.

Table 2
The percent T-bet expression and intensity in splenic T cells 8 days after sham or burn injury

PBD8	T-Bet expression, %			T-Bet MFI		
	Sham	Scald	Flame	Sham	Scald	Flame
CD4, 62L ^{high}	8.3 ± 0.4	5.7 ± 1.0 ^{*,†}	15.6 ± 1.8 [*]	429 ± 36	290 ± 35	408 ± 62
CD4, 62L ^{low}	26.4 ± 2.4	7.4 ± 1.3 ^{*,†}	21.7 ± 2.4	725 ± 64	228 ± 25 ^{*,†}	505 ± 34 [†]
CD8, 62L ^{high}	25.9 ± 1.2	13.2 ± 0.1 ^{*,†}	34.4 ± 2.3 [†]	468 ± 36	252 ± 32 ^{*,†}	500 ± 18
CD8, 62L ^{low}	50.9 ± 3.6	27.1 ± 9.5 [*]	43.7 ± 6.0	657 ± 44	444 ± 124 [†]	703 ± 45

The sample size is six to eight from two combined experiments.

^{*} $P < 0.05$ as compared with wild type.
[†] $P < 0.05$ as compared with flame.

MFI indicates mean fluorescence intensity.