Int Surg 2019;**104:**283–290
DOI: 10.9738/INTSURG-D-18-00014.1

Burn Injury Induces Intestinal Inflammatory Response Mediated by Th17 in Burn-Primed Endotoxemic Mice

Kazuhiko Sekine[1,2], Takayuki Shibusawa[1], Seitaro Fujishima[1,3], Naoki Aikawa[1,4], Junichi Sasaki[1]

[1]*Department of Emergency and Critical Care Medicine, Keio University School of Medicine, Tokyo, Japan*

[2]*Current position: Department of Emergency and Critical Care Medicine, Tokyo Saiseikai Central Hospital, Tokyo, Japan*

[3]*Department of General Medicine, Keio University School of Medicine, Tokyo, Japan*

[4]*Keio University School of Medicine, Tokyo, Japan*

Objective: This study aimed to elucidate the mechanism underlying the susceptibility to infection-related acute lung injury by focusing on the role of gut mucosal T-helper (Th) 17 cells that preferentially produce IL-17 with probiotics in a burn-primed endotoxemic mice model.

Methods: Mice were subjected to a 15% total body surface area third-degree burn. Survival from lethal lipopolysaccharide (LPS) administration (3 mg/kg) on 11th day post-burn was assessed in mice fed by chow with or without 1.2% *Lactobacillus* powder after burn injury. Lamina propria mononuclear cells were enzymatically isolated from the ileum removed on 11th day post-burn and incubated along with 1 μg/mL LPS or 10 μg/mL anti-CD3 antibody for 24 hours; subsequently, the following 7 cytokines were analyzed in the supernatant: IFN-γ, TNF-α, IL-2, IL-4, IL-6, IL-10, and IL-17.

Results: *Lactobacillus* treatment post-burn injury markedly improved survival after lethal endotoxemia in burn-primed mice (64.3% versus 21.4%, $P = 0.03$). The production of proinflammatory cytokines such as TNF-α, IL-6, and IL-17 by lamina propria mononuclear T-lymphocytes and macrophages including Th17 response was augmented by burn injury but decreased with *Lactobacillus* treatment after burn injury.

Corresponding author: Kazuhiko Sekine, MD, PhD, Department of Emergency and Critical Care Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.
Tel.: +81-3-3225-1323; Fax: +81-3-3353-2232; E-mail: skkazoo@gmail.com

Conclusions: Th17- and Th17-mediated inflammatory responses in the gut mucosa may play a vital role, which could be attenuated by *Lactobacillus* treatment, in survival of lethal endotoxemia in burn-primed mice.

Key words: Th17 cells – Inflammatory response – *Lactobacillus casei* – Lamina propria mononuclear cells – Gut – Burn injury

When faced with an infection, patients with severe injuries (*e.g.*, burn and trauma) are susceptible to multiple organ dysfunction syndrome (MODS) following acute lung injury (ALI).[1] To elucidate the pathologic and immunologic mechanisms underlying organ failure in critically injured patients, we developed a 2-hit animal model that was first subjected to burn, followed by infectious secondary insult with endotoxin injection on 11th day after burn, resulting in lethal ALI and MODS.[2,3] Although several researchers have reported on burn-induced immunosuppression by T-helper (Th) cells and macrophages,[4–11] our analyses of this 2-hit model have shown that administering lipopolysaccharide (LPS) to burn-primed mice causes lethal ALI and MODS due to excessive inflammatory cytokine production in the plasma and lungs.[2] This excessive inflammatory cytokine response by macrophage/monocytes to LPS contributes to ALI after burn injury.[3] These immunological pathways may be involved in the intestinal ischemia and/or gut barrier injury observed after burns or shock.[12–16] The reasons for the development of burn-induced immunosuppression and MODS following ALI need to be clarified.

Many clinical and experimental studies have been performed to study gut function and disorders such as inflammatory bowel disease (IBD).[17–19] Researchers have shown that a Th17 cell (a new subset of Th cells that produces IL-17A) and other inflammatory cytokines induced by Th17 cells play critical roles in the gut in IBD. Although IL-17 is responsible for promoting inflammation through the recruitment of neutrophils and monocytes, Th17 cells are responsible for the facilitation of intestinal barrier functions and preventing bacterial mucosal invasion.[17,20] As Th17 cells are majorly present in the inflamed intestines of IBD patients, Th17-induced proinflammatory cytokines including IL-17 trigger and amplify inflammation in IBD.[18] In clinical studies and various animal models of IBD,[18,21] the efficacy of probiotics, including *Lactobacillus* and *Bifidobacteria*, has been widely reported via several mechanisms involved in the displacement and suppression of the pathogen growth,[22] functional improvement of the epithelial barrier,[19] and immunomodulation of Th1, Th2, Th17, and regulatory T cell production.[22] The administration of certain *Lactobacillus* species suppressed the Th17-mediated secretion of IL-17 via the down-regulation of TGFβ1 and IL-23 expression and downstream p-STAT3 phosphorylation in experimental colitis;[23] therefore, probiotics directly improve Th17-induced overproduction of downstream inflammatory cytokines and related symptoms through an anti-inflammatory effect in the gut of IBD patients.[18,21,22,23]

As Th17 response is caused by burn injury (directly in the burned skin and indirectly in the cardiac tissue),[20,24,25] we hypothesized that the Th17 response and its modulation with probiotic preparation in the gut contribute to the pathogenesis of ALI and MODS after burn injury. In this study, we analyzed the intestinal cytokine kinetics in the burn-primed endotoxemic 2-hit model to clarify the role of the Th17 response in the gut treated with or without *Lactobacillus* treatment.

Materials and Methods

To assess the effects of probiotic preparation on host survival, a burn-primed murine model with sequential endotoxemia was applied as per a previously used method.[2,3] Seven-week-old Balb/c male mice (23–28 g) from the Charles River Laboratories Japan, Inc, were purchased. All mice were caged and provided *ad libitum* with water and standard powder chow (CE-2, Nippon Kurea, Tokyo, Japan) for at least a week. The mice were then assigned into burn and sham groups. The mice dorsum was shaved using an electrical clipper with appropriate pentobarbital anesthesia, one day pre-burn injury. The shaved dorsum was exposed to a 5-second hot steam using ether anesthesia on day 1, to produce a third-degree burn over 15% of the total body surface area (TBSA). Immediately post-burn injury, 4 mL of sterile saline was intraperitoneally administered to the burned mice and then they were observed. The

same protocols were used for the sham mice except the burn injury.

The mice were bred with the standard powder chow before burn injury. We sought to assess the survival after lethal endotoxin challenge in burn-primed mice fed with a probiotic preparation containing *Lactobacillus casei (L. casei)*. To assess the effects of *Lactobacillus* treatment on the burn-primed endotoxemic model, some of the burned mice were bred with the standard powder chow with 1.2% Biolactis (Yakult Honsha Company, Limited, Tokyo), which contains 500 mg of *L. casei* (1.5×10^9 – 2.1×10^{10} bacteria)/1 g powder, after burn injury. The remaining mice received the standard powder chow containing an amount of cornstarch equivalent to that in the Biolactis as a control.

For survival studies, 3 mg/kg lipopolysaccharide (LPS) (*E. coli* 0111:B4; Sigma-Aldrich) that is not lethal for sham mice was administered through the tail vein on 11th day after burn injury, and cumulative survival was determined for 72 hours (up to 96 hours) after lethal endotoxemia. All of these procedures and care for mice were deliberated and approved by the Keio University Institutional Animal Care and Use Committee, Tokyo, Japan.

Our preliminary experiments showed that cytokine contents were significantly higher in the ileum than those in the jejunum or the colon in burn-primed endotoxemic mice, indicating that the ileum was the primary location of cytokine production (unpublished data). To investigate the immunologic effects of burn injury and *Lactobacillus* treatment on gut mucosal mononuclear cells, we measured cytokine production by the isolated mononuclear cells from lamina propria in the ileum on 11th day after burn injury with or without *Lactobacillus* treatment in the burned mice. We resected the entire ileum from the burned and sham mice on 11th day after burn injury under appropriate pentobarbital anesthesia to isolate the lamina propria mononuclear cells using an enzyme method described in a previous study.[26] Dissected mucosa from the ileum was washed thoroughly using Ca^{2+} and Mg^{2+}-free Hank's buffered saline to remove the mucus, cut into small pieces (2-mm cubes), and incubated at 37°C for 45 minutes in the medium with 1 mmol/L dithiothreitol (Sigma–Aldrich) and 2.5% fetal calf serum (FCS) to promote the release of epithelial and intraepithelial mononuclear cells from the mucosal tissue into supernatant. After the supernatant was removed by pipetting, the mucosal tissue including lamina propria mononuclear cells were incubated and stirred in a medium that contained 0.03% collagenase A from *Clostridium histolyticum* (Sigma–Aldrich) at 37°C for 2 hours. Thereafter, the supernatant in which the lamina propria mononuclear cells were released from the tissue was collected, and the cell suspension was separated using a Percoll gradient. The cell fractions were suspended in a 40% isotonic Percoll solution and then centrifuged using a Ficoll–Hypaque density gradient. After separation, the lamina propria mononuclear cells were resuspended and adjusted to 1.0×10^6 cells/mL in RPMI-1640 with 5% FCS. The lamina propria mononuclear cells rich in T-lymphocytes and macrophages/monocytes (as determined using flow cytometry) were then immediately used for further experiments.

To evaluate the phenotypic changes of T-lymphocytes and macrophages/monocytes in the ileum mucosa after burn injury and *Lactobacillus* treatment, *in vitro* cytokine productions by the lamina propria mononuclear cells were examined in reaction to anti-CD3 antibody and LPS. As previously described,[3] we used LPS for a specific macrophage/monocyte stimulation and anti-CD3 antibody (145-2C11, BD Biosciences) for a T-lymphocyte stimulation. Through preliminary experiments, we determined that 24-hour incubation with LPS (1 μg/mL) and anti-CD3 antibody (10 μg/mL) resulted in optimal cytokine production. To evaluate macrophage/monocyte and T-lymphocyte functions in the ileum mucosa, lamina propria mononuclear cells were adjusted to 1.0×10^6 cells/mL in RPMI-1640 containing 5% FCS and incubated with anti-CD3 antibody (10 μg/mL) or LPS (1 μg/mL) for 24 hours at 37°C. After incubation, the supernatants of the cell suspensions were centrifuged and stored until analysis at –80°C.

For the analysis of cytokines in the supernatant from the gut tissue or a cell suspension of lamina propria mononuclear cells, we used the Cytometric Bead Array Mouse Th1/Th2/Th17 Cytokine Kit (BD biosciences) that allows researchers to determine the values of cytokines such as IFN-γ, TNF-α, IL-2, IL-4, IL-6, IL-10, and IL-17.

We used Fisher's protected least significant difference (PLSD) comparison test for the observed values of cytokines and the Kaplan-Meier test (log-rank) for the 72-hour cumulative survival rates. Data are presented as mean ± SEM, and a P value of < 0.05 was considered significant.

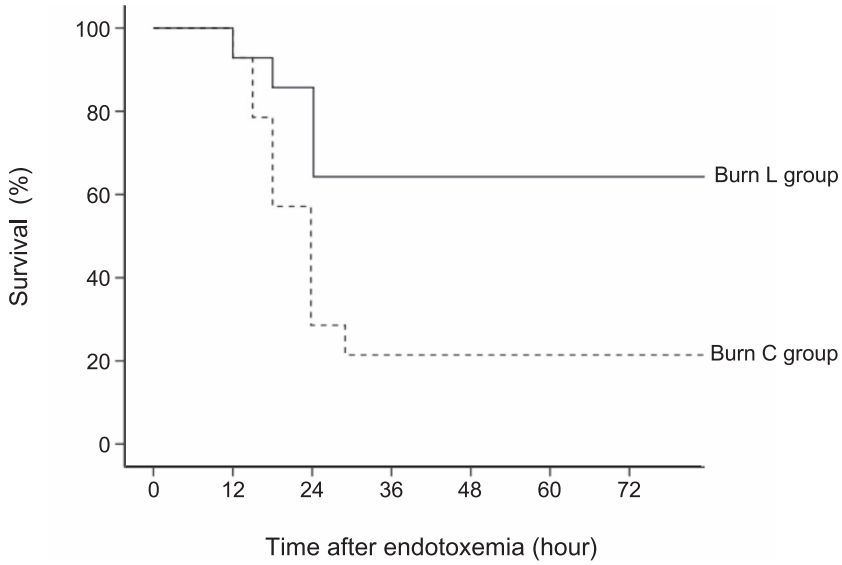

Fig. 1 Cumulative survival after lethal endotoxin administration in the burned mice fed chow with or without 1.2% *Lactobacillus* powder after burn injury. 15% TBSA burned mice fed with or without the *Lactobacillus* treatment were observed to determine the cumulative survival after the administration of 3 mg/kg lipopolysaccharide on the 11th day post-burn. The 72-hour survival rate was 21.4% in the burned mice fed with standard powder chow as controls (Burn C group: dashed line), and 64.3% in the burned mice fed with the special powder chow containing 1.2% *L. casei* powder (Burn L group: solid line) ($P = 0.03$, log-rank test).

Results

In the burn-primed murine model, only 15% TBSA burn was nonlethal after injury for any mice. Figure 1 demonstrates the 72-hour survival rate after lethal endotoxemia on 11th day after burn injury for all groups. The survival rate was significantly lower in burned mice fed with standard powder chow as controls (Burn C group: n = 14, 21.4%) than that in burned mice fed with special powder chow containing 1.2% *L. casei* powder (Burn L group: n = 14, 64.3%) ($P = 0.03$, log-rank test), indicating that *Lactobacillus* treatment directly improved survival after lethal endotoxemia in burn-primed mice.

In the *in vitro* studies, as shown in Fig. 2, the production of proinflammatory cytokines, such as TNF-α, IL-6, and IL-17 in reaction to anti-CD3 antibody significantly increased in the burn control (Burn C) group compared with that in the sham control (Sham C) group, demonstrating that burn injury exacerbated the inflammatory response by T-lymphocytes in the gut mucosa. The values of all the cytokines observed, except IL-4, significantly decreased in the burn with *Lactobacillus* treatment (Burn L) group compared with those in the Burn C group. In contrast, the values of IL-4 and IFN-γ significantly decreased in the sham mice that received *Lactobacillus* treatment (Sham L group) compared with those in the mice of the sham C group.

Regarding the macrophage/monocyte functions in reaction to LPS, the production of all cytokines significantly increased in the Burn C group compared with that in the Sham C group, except TNF-α, and the cytokines were similarly suppressed in the Burn L and Sham L groups (Fig. 3). This indicated that exaggerated inflammatory and compensatory anti-inflammatory responses on the part of macrophages/monocytes were induced by burn injury. With the *Lactobacillus* treatment, the baseline cytokine levels measured in the gut mucosa were remarkably suppressed to the levels observed in the sham C group or even lower levels.

Discussion

This study showed that burn injury increases the intestinal immune response, such as the up-regulation of cytokine productions by T cells and macrophages/monocytes in the ileum of burn-primed mice. It is noteworthy that the ileum cytokine kinetics by the T cells is contrary to past reports that Th1-cytokine production by peripheral monocytes and spleen cells is markedly suppressed, while Th2-cytokine responses became dominant after burn injury.[5,6,27] In contrast, those by macrophages/monocytes are similar to our previous observation of excessive cytokine production in response to LPS by splenic macrophages/monocytes in burn-primed mice.[2,3] The current findings suggest that the Th1-dominant *in vivo* reaction in the ileum causes exaggerated cytokine response and hypercytokinemia after burn injury.

Among CD3-induced productions of Th1 and inflammatory cytokines, the productions of TNF-α, IL-6, and IL-17 by lamina propria T-lymphocytes were significantly increased, whereas CD3-induced

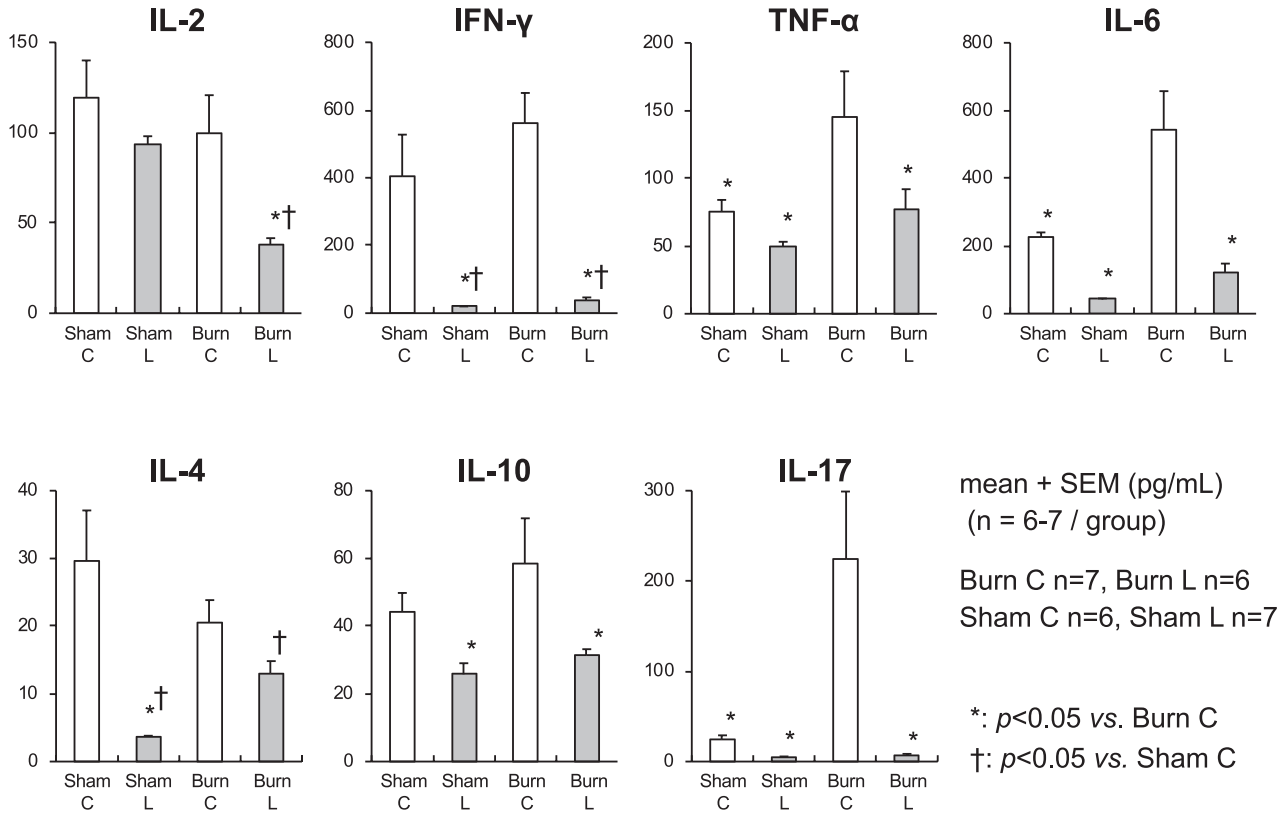

Fig. 2 *In vitro* cytokine production by isolated lamina propria mononuclear cells in reaction to anti-CD3 antibody for a T-lymphocyte stimulation. Anti-CD3-induced production of proinflammatory cytokines including TNF-α, IL-6, and IL-17 was significantly enhanced in the burned mice without *Lactobacillus* treatment (Burn Control: Burn C) group compared with that in the sham without *Lactobacillus* treatment (Sham Control: Sham C) group. The production of all the cytokines, except IL-4, in the burn with *Lactobacillus* treatment (Burn L) group was significantly lower than that in the Burn C group, while the production of specific cytokines, IFN-γ and IL-4, in the sham mice with *Lactobacillus* treatment (Sham L) was significantly lower as compared with that in those in the sham C.

Th2 cytokines such as IL-4 and IL-10 were not altered as a result of burn priming. Additionally, LPS-induced cytokine response mainly from lamina propria macrophages/monocytes was significantly increased, except TNF-α, because of burn priming. We found that post-burn IL-17 production was enhanced from lamina propria lymphocyte and macrophage/monocyte, whereas our result that the other proinflammatory cytokine productions such as TNF-α and IL-6 were almost completely enhanced in the ileum after burn injury is consistent with the past several reports.[13,14,28] As Th17 cells are identified as the primary lymphocyte producer of IL-17, our results suggest that Th17 cells, or possibly other IL-17-producing cells (*e.g.*, CD8 T cells, γδ T cells), should be differentiated and induced in the mucosa of the ileum after burn priming. Furthermore, as Th17 cells directly secrete several proinflammatory cytokines including TNF-α, IL-1β, IL-6, IL-12, IL-23, and TGF-β,[18,21] the post-burn overproduction of these inflammatory cytokines may be responsible for burn-primed induction of Th17 cells in lamina propria. Notably, this study showed that IFN-γ productions from lamina propria mononuclear cells were not only suppressed in T-lymphocytes at all but were also enhanced in macrophages/monocytes; major burns and trauma have been demonstrated to attenuate Th1 response associated with immunosuppression and susceptibility to sepsis.[4–7,11,27] Since some of the IL-17 producing T cells that also produce IFN-γ (termed as Th1/Th17 cells) increased in the gut mucosa from Crohn's disease patients,[29,30] the burn-induced Th17 cells in the gut might resemble those in Crohn's disease. It remains unclear why Th17 cells were rich in the gut after burn injury, as in Crohn's disease. Th17 cells are differentiated from naïve T-helper cells by IL-1β, IL-6, and TGF-β[18,30] and the plasma levels of

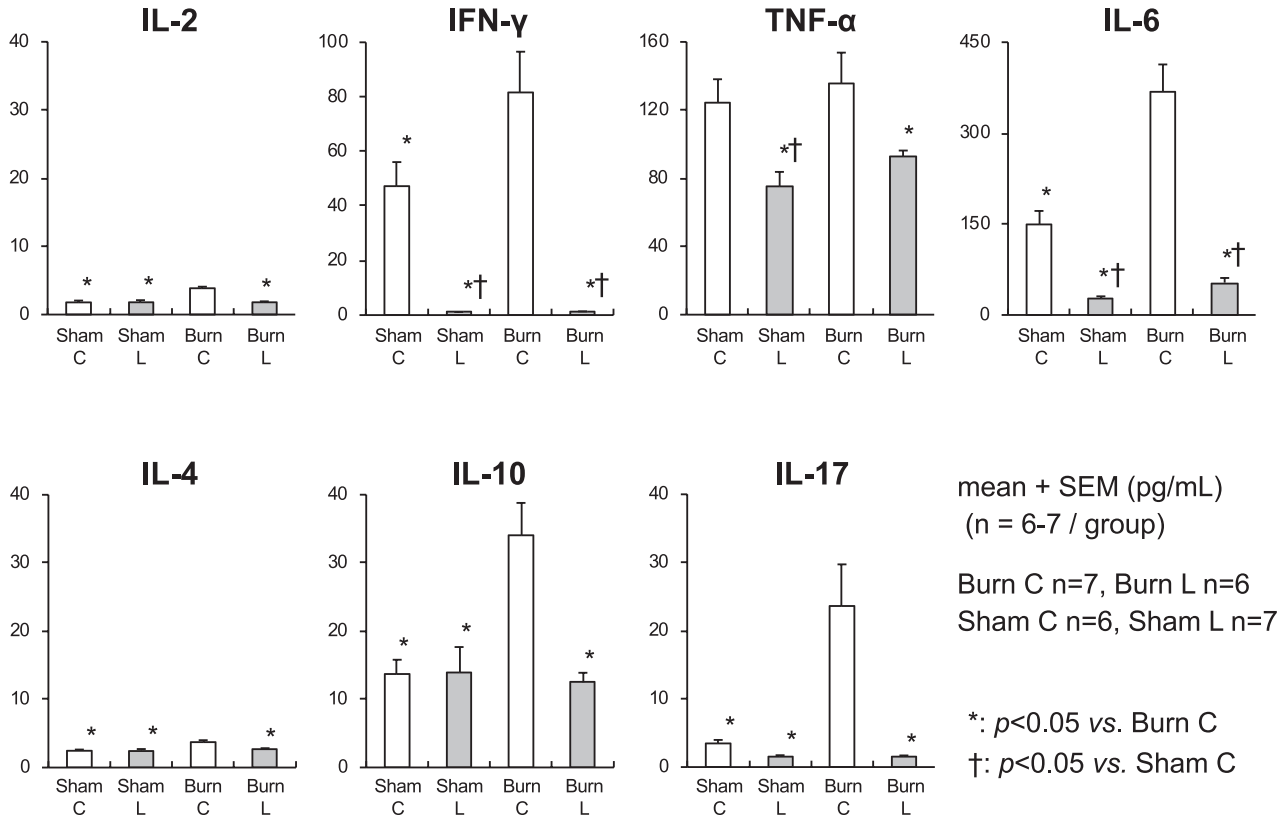

Fig. 3 *In vitro* cytokine productions by isolated lamina propria mononuclear cells in reaction to LPS for a macrophage/monocyte stimulation. All LPS-induced cytokine production that was examined was significantly enhanced in the burned mice without *Lactobacillus* treatment (Burn Control: Burn C) group compared with that in the sham mice without *Lactobacillus* treatment (Sham Control: Sham C) group, except TNF-α; the production was totally suppressed in the burned mice with *Lactobacillus* treatment (Burn L) group and in the sham mice with *Lactobacillus* treatment (Sham L) group.

proinflammatory cytokines, such as IL-1β and IL-6, are elevated after burn injury;[31,32] thus, these predisposing high systemic inflammatory cytokine levels might induce Th17 cells in the gut and other sites, such as the skin and cardiac tissue, after burn injury, as reported in previous studies.[8,24,25]

This study also showed that probiotic treatment exerted a beneficial effect that burn-induced cytokine overproductions were suppressed through anti-inflammatory response, and resulted in an improved survival rate from lethal ALI in burn-primed endotoxemic mice. These anti-inflammatory effects exerted by probiotics against Th17-mediated hypercytokinemia may contribute to the attenuation of local and systemic inflammation, preventing distant lung injury and improving survival after lethal endotoxemia in our burn-primed model. Although the mechanism of the beneficial effects of *Lactobacillus* treatment is not fully elucidated in this study, probiotics prophylaxis may be a cheap and effective treatment for preventing septic ALI and MODS after burn injury.

This study has certain limitations; we did not perform investigation regarding the cross-reaction of cytokine production between T-lymphocytes and monocyte/macrophages and the direct flowcytometric analysis of Th17 cells using specific cell surface markers. Further studies are awaited regarding the beneficial mechanisms of probiotics in burn-primed mice and the clinical application of *Lactobacillus* treatment for burned patients.

Conclusions

Burn injury induced intestinal Th17 and Th17-mediated inflammatory response, which was associated with the survival of lethal endotoxemia after burn injury. *Lactobacillus* treatment after burn injury decreased intestinal Th17 and their inflammatory

response and the mortality of lethal ALI in burn-primed endotoxemic mice.

Acknowledgments

This work was supported by a grant-in-aid from the Kato Memorial Trust for Nambyo Research, Tokyo in 2009 (grant number: 2009 #2) and the Yakult Bio-science Organization, Tokyo in 2010 (grant number: 313). This work was financially supported in part by the Yakult Bio-science Organization (¥1500000: approximately $12,500).

References

1. Aikawa N, Shinozawa Y, Ishibiki K, Abe O, Yamamoto S, Motegi M *et al*. Clinical analysis of multiple organ failure in burned patients. *Burns* 1987;**13**(2):103–109
2. Sasaki J, Fujishima S, Iwamura H, Wakitani K, Aiso S, Aikawa N. Prior burn insult induces lethal acute lung injury in endotoxemic mice: effects of cytokine inhibition. *Am J Physiol Lung Cell Mol Physiol* 2003;**284**(2):L270–278
3. Sekine K, Fujishima S, Sasaki J, Ishizaka A, Aiso S, Aikawa N. In vivo IL-18 supplementation ameliorates lethal acute lung injury in burn-primed endotoxemic mice: a novel anti-inflammatory role of IL-18. *Shock* 2009;**32**(5):554–562
4. 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):3983–3990
5. Kavanagh EG, Kelly JL, Lyons A, Soberg CC, Mannick JA, Lederer JA. Burn injury primes naive CD4+ T cells for an augmented T-helper 1 response. *Surgery* 1998;**124**(2):269–276; discussion 276–267
6. Lyons A, Kelly JL, Rodrick ML, Mannick JA, Lederer JA. Major injury induces increased production of interleukin-10 by cells of the immune system with a negative impact on resistance to infection. *Ann Surg* 1997;**226**(4):450–458; discussion 458–460
7. O'Sullivan ST, Lederer JA, Horgan AF, Chin DH, 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**(4):482–490; discussion 490–482
8. Rani M, Zhang Q, Schwacha MG. Burn wound gammadelta T-cells support a Th2 and Th17 immune response. *J Burn Care Res* 2014;**35**(1):46–53
9. Schwacha MG. Macrophages and post-burn immune dysfunction. *Burns* 2003;**29**(1):1–14
10. Schwacha MG, Chaudry IH. The cellular basis of post-burn immunosuppression: macrophages and mediators. *Int J Mol Med* 2002;**10**(3):239–243
11. Toliver-Kinsky TE, Varma TK, Lin CY, Herndon DN, Sherwood ER. Interferon-gamma production is suppressed in thermally injured mice: decreased production of regulatory cytokines and corresponding receptors. *Shock* 2002;**18**(4):322–330
12. Clark JA, Coopersmith CM. Intestinal crosstalk: a new paradigm for understanding the gut as the "motor" of critical illness. *Shock* 2007;**28**(4):384–393
13. Costantini TW, Loomis WH, Putnam JG, Drusinsky D, Deree J, Choi S *et al*. Burn-induced gut barrier injury is attenuated by phosphodiesterase inhibition: effects on tight junction structural proteins. *Shock* 2009;**31**(4):416–422
14. Costantini TW, Peterson CY, Kroll L, Loomis WH, Putnam JG, Wolf P *et al*. Burns, inflammation, and intestinal injury: protective effects of an anti-inflammatory resuscitation strategy. *J Trauma* 2009;**67**(6):1162–1168
15. Magnotti LJ, Upperman JS, Xu DZ, Lu Q, Deitch EA. Gut-derived mesenteric lymph but not portal blood increases endothelial cell permeability and promotes lung injury after hemorrhagic shock. *Ann Surg* 1998;**228**(4):518–527
16. Magnotti LJ, Xu DZ, Lu Q, Deitch EA. Gut-derived mesenteric lymph: a link between burn and lung injury. *Arch Surg* 1999; **134**(12):1333–1340; discussion 1340–1331
17. Blaschitz C, Raffatellu M. Th17 cytokines and the gut mucosal barrier. *J Clin Immunol* 2010;**30**(2):196–203
18. Galvez J. Role of Th17 Cells in the Pathogenesis of Human IBD. *ISRN Inflamm* 2014;**2014**:928461
19. Miyauchi E, Ogita T, Miyamoto J, Kawamoto S, Morita H, Ohno H *et al*. Bifidobacterium longum alleviates dextran sulfate sodium-induced colitis by suppressing IL-17A response: involvement of intestinal epithelial costimulatory molecules. *PLoS One* 2013;**8**(11):e79735
20. Kim A, Lang T, Xue M, Wijewardana A, Jackson C, Vandervord J. The Role of Th-17 Cells and gammadelta T-Cells in Modulating the Systemic Inflammatory Response to Severe Burn Injury. *Int J Mol Sci* 2017;**18**(4)
21. Owaga E, Hsieh RH, Mugendi B, Masuku S, Shih CK, Chang JS. Th17 Cells as Potential Probiotic Therapeutic Targets in Inflammatory Bowel Diseases. *Int J Mol Sci* 2015;**16**(9):20841–20858
22. Tanabe S. The effect of probiotics and gut microbiota on Th17 cells. *Int Rev Immunol* 2013;**32**(5-6):511–525
23. Chen L, Zou Y, Peng J, Lu F, Yin Y, Li F *et al*. Lactobacillus acidophilus suppresses colitis-associated activation of the IL-23/Th17 axis. *J Immunol Res* 2015;**2015**:909514
24. Oppeltz RF, Zhang Q, Rani M, Sasaki JR, Schwacha MG. Increased expression of cardiac IL-17 after burn. *J Inflamm (Lond)* 2010;**7**:38
25. Sasaki JR, Zhang Q, Schwacha MG. Burn induces a Th-17 inflammatory response at the injury site. *Burns* 2011;**37**(4):646–651
26. Watanabe M, Ueno Y, Yajima T, Iwao Y, Tsuchiya M, Ishikawa H *et al*. Interleukin 7 is produced by human intestinal epithelial cells and regulates the proliferation of

intestinal mucosal lymphocytes. *J Clin Invest* 1995;**95**(6): 2945–2953

27. Goebel A, Kavanagh E, Lyons A, Saporoschetz IB, Soberg C, Lederer JA *et al*. Injury induces deficient interleukin-12 production, but interleukin-12 therapy after injury restores resistance to infection. *Ann Surg* 2000;**231**(2):253–261
28. Wang Z, Xiao G, Yao Y, Guo S, Lu K, Sheng Z. The role of bifidobacteria in gut barrier function after thermal injury in rats. *J Trauma* 2006;**61**(3):650–657
29. Annunziato F, Cosmi L, Santarlasci V, Maggi L, Liotta F, Mazzinghi B *et al*. Phenotypic and functional features of human Th17 cells. *J Exp Med* 2007;**204**(8):1849–1861
30. Catana CS, Berindan Neagoe I, Cozma V, Magdas C, Tabaran F, Dumitrascu DL. Contribution of the IL-17/IL-23 axis to the pathogenesis of inflammatory bowel disease. *World J Gastroenterol* 2015;**21**(19):5823–5830
31. Drost AC, Burleson DG, Cioffi WG, Jr., Mason AD, Jr., Pruitt BA, Jr. Plasma cytokines after thermal injury and their relationship to infection. *Ann Surg* 1993;**218**(1):74–78
32. Finnerty CC, Jeschke MG, Herndon DN, Gamelli R, Gibran N, Klein M *et al*. Temporal cytokine profiles in severely burned patients: a comparison of adults and children. *Mol Med* 2008; **14**(9-10):553–560