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ORIGINAL ARTICLE

Depressed production of beta-defensins from mouse splenic dendritic cells following thermal injury and its influence on susceptibility to infection

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Abstract
Purpose Beta-defensins (BDs) and dendritic cells (DC) have been described as major effectors on host antimicrobial innate immunities. In the present study, the ability of DC to produce BDs was explored using DC from normal mice and full-thickness (FT)-burned mice.
Methods DCs were isolated from spleens of mice, and 1×10^6 cells/ml of them were cultured with LPS or SAC. Culture fluids harvested 24 h after cultivation were assayed for BD1 and BD3 and antibacterial activity (colony-counting, *Pseudomonas aeruginosa*). Also, DCs were tested for BD mRNAs by RT-PCR.
Results Sixty-five percent of the bacterial killing activity was shown by the culture fluids of splenic DC from normal mice, while only 15 % killing activity was shown by the culture fluids of splenic DC from FT-burned mice. X-irradiated NOD SCID IL-2rγ^{null} mice inoculated with splenic DC from FT-burned mice showed increased susceptibility to *P. aeruginosa* infection compared to those from normal mice. Mice splenic DC expressed BD1 mRNA constitutively and expressed BD3 mRNA after stimulation. These BDs were produced by mice splenic DC. As compared with DC from normal mice, DC from FT-burned mice produced decreased amounts of BD1 and BD3 in their culture fluids.
Conclusions These results indicate that (1) DC from spleens of mice have an ability to produce BDs, and (2) the production of BDs by DC is influenced strongly by thermally injured stress. Since FT-burned mice are susceptible to *P. aeruginosa* infection, BDs produced by DC may play an important role on the host's antibacterial resistance.

Keywords Burn · Defensin · Dendritic cell · Infection · Innate immunity · Host defense

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Introduction

Despite that advances in patient care have increased survival in burned patients, infections remain a leading cause of mortality in these patients [1–3]. It is well known that immunosuppression induced by severe burn injury is associated with the development of opportunistic infections and associated complications such as sepsis and organ failure. *Pseudomonas aeruginosa* (*P. aeruginosa*) is a pathogen frequently seen in burn patients [4]. Sepsis and organ failure led by *P. aeruginosa* infection are a major cause of mortality in these patients. The very small amounts of bacteria of burn wound that escaped from topical antibacterial treatment are sufficient to spread systemically in burn victims due to their defects of host antibacterial responses [5]. The impairment in host defense of the area surrounding burn tissue could result from a defect in cell-mediated immune responses and impaired microbicidal activity by cells of the innate immune system. Many studies have demonstrated that burn injury induces a marked suppression of many immune functions, including T cell activation, proliferation, and cytokine release. Furthermore, our previous findings suggest that following burn injury, cytokine/chemokine-producing profiles of macrophage (Mø) and

polymorphonuclear neutrophils (PMN) are changed, and these alterations (M2Mø and PMN-II) are associated with decreased host antimicrobial resistance of burn victims [6]. Recent studies suggested that the defect of skin antimicro peptide production also allows *P. aeruginosa* to spread from the local sight to the whole body [7, 8].

Antimicrobial activity for the defensins has been demonstrated against a wide range of pathogens such as bacteria, fungi, protozoa, and virus [9–13]. Defensins are expressed in phagocytic leukocytes and epitherial cells at mucosal surfaces. Their antibiotic activity and widespread expression suggest that they participate in innate immune responses in a variety of tissues. Defensins have been classified into two families designated α and β. In humans, four β defensins (BDs) have been identified to date. Human BD (HBD) 1 is constitutively expressed. In contrast, HBD2, HBD3, and HBD4 expression are inducible in response to bacteria and proinflammatory cytokines. In mice, there are at least five fully characterized BDs with different, but partially overlapping, tissue distribution. Mouse BD (MBD) 1 and MBD4 are expressed constitutively, whereas MBD2, MBD3, and MBD6 are induced by cytokines or microbial stimulation. Previous studies demonstrated that BDs play an important role in host defense mechanisms to prevent infectious complication in thermally injured patients [7, 14, 15].

Defensins are expressed in phagocytic leukocytes and epithelial cells at mucosal surfaces [10, 16]. In the present study, we focused on their production by dendritic cells (DCs). DCs are the most potent antigen-presenting cells that are intimately involved in initiation of innate and adaptive immunity [17, 18]. Immature DCs are strategically located in tissues that represent pathogen entry routes. After microbial invasion and tissue destruction, immature DCs become activated and undergo maturation. Mature DCs reduce endocytic and phagocytic capacity, but they acquire an antigen-presenting capacity and migratory functions that allow antigen-loaded DCs to move to the T cell areas or lymphoid tissues such as spleen. Previous studies demonstrated that BD1 mRNA expression is detected in spleen [19, 20]. In addition, mature DC showed an increased expression of BD1 mRNA as compared to immature DC [21]. Furthermore, we previously showed that surgical stress, such as trauma-hemorrhage, induces suppressed splenic DCs maturation [22]. Previous studies demonstrated that BDs permeabilize the bacterial cell membrane by an electrostatic charge-based mechanism and show bacterial-killing activity [23–25]. However, it is still unclear whether splenic DCs also play antimicrobial roles by producing BDs.

We hypothesized that splenic DCs have a role in host defense mechanisms via producing BDs and that the producing ability of BDs by splenic DCs are suppressed in burn victims. The aim of this study was to define the relationship between the suppressed resistance to *P. aeruginosa* infection following thermal injury and the reduced ability to BDs production by splenic DCs from burn mice.

Materials and methods

Animals

Seven- to eight-week-old pathogen-free male BALB/c mice, C57BL/6 mice, and NOD-SCID IL-2rγ^{null} mice (deficient of functional T, B, NK, DC, and Mø; BALB/c origin) were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and used in this study. To deplete PMN, NOD-SCID IL-2rγ^{null} mice were exposed to whole-body X-irradiation (4 Gy, 2 days before cell inoculation). X-irradiated NOD-SCID IL-2rγ^{null} mice are immunodeficient and lack functional T cells, B cells, NK cells, DC, Mø, and PMN [26]. The Institutional Animal Care and Use Committee of The University of Texas Medical Branch and University of Occupational and Environmental Health approved all procedures utilizing animals.

Bacteria and reagents

Pseudomonas aeruginosa (strain 180) was purchased from ATCC (Rockville, MD, USA). The growth of *P. aeruginosa* was performed on brain heart infusion broth for 18 h at 37 °C in aerobic conditions. Master cultures of the strains were stored at −70 °C. Primary and secondary cultures were stored at 4 °C for up to 4 weeks.

Monoclonal antibody for MBD1 and MBD3 and recombinant MBD1 and MBD3 were purchased from Alpha Diagnostic International (San Antonio, TX, USA).

Thermal injury

Thermally injured mice were prepared according to previously reported protocol [6, 27–29]. Mice were anesthetized with pentobarbital (40 mg/kg i.p.) and electric clippers were used to shave the hair on the back of each mouse from the groin to axilla.

1. Full-thickness (FT) burned mice. FT burn injuries were created in anesthetized mice by pressing their backs into a custom-made insulated mold (with a 4 × 5 cm window) and exposing them to a gas flame for 9 s using a Bunsen burner equipped with a flame-dispersing cap [28]. This procedure consistently produced a third-degree burn on approximately 25 % of total body surface area for a 26-g mouse. Immediately after

thermal injury, physiologic saline (3 ml/mouse i.p.) was administered for fluid resuscitation. Animals were then housed until use for experiments.

2. Control mice. Control mice had their back hair shaved but no thermal injuries were created. They also received physiologic saline (3 ml/mouse i.p.).

Isolation of splenic dendritic cells

Spleens were digested by Liberase CI (Roche, Indianapolis, IN, USA) and teased apart by repeated pipetting in PBS containing 5 % FCS and 5 mM EDTA. The red blood cells were osmotically lysed and splenocytes were blocked with 1 μg/ml Fc block (clone: 93) antibody for 15 min on ice. Cell suspensions were enriched with anti-CD11c magnetic beads and positive selection columns MS + according to the manufacturer's instructions (Miltenyi Biotec, Auburn, CA, USA). Flow cytometric analysis demonstrated that cells contained >90 % CD11c-positive cells.

Detection of MBDs

To detect MBDs production and expression, splenic DCs (1×10^5 cells/well) were cultured in 96-well tissue culture plates in RPMI-1640 medium containing 10 % heat-inactivated fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin with or without Toll-like receptor (TLR) 4, the receptor for Gram-negative bacteria, agonist lipopolysaccharide (LPS; 10 μg/ml) or TLR2 agonist *Staphylococcus aureus* Cowan 1 (SAC; 0.0075 %).

To detect MBDs production by splenic DCs, the plate was centrifuged at 400 × *g* for 10 min after 24-h incubation at 37 °C, 5 % CO_2. Culture fluids were collected and frozen at −70 °C until use. The levels of MBDs in the DC culture fluids were measured by enzyme-linked immunosorbent assay (ELISA).

For the analysis of BD mRNA expression, total RNA was extracted from isolated splenic DCs using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Within each experiment, each sample was normalized by the amount of isolated RNA. Total RNA was reversed to cDNA using random hexamer primers and murine leukemia virus reverse transcriptase. Polymerase chain reaction (PCR) was performed using synthesized oligonucleotide primers from Sigma-Aldrich: MBD1, 5′-CACATCCTCTCTG CACTCTGGACCC-3′ (forward) and 5′-CCATCGCTCG TCCTTTATGTCCATTC-3′ (reverse); MBD3, 5′-CTCC TGGTGCTGCTGTCTCCAC-3′ (forward) and 5′-AACT CCACAACTGCCAATCTGA-3′ (reverse). Using a thermal cycler (GeneAmp PCR System 9600), 35 cycles of PCR were performed at 94 °C for 15 s, 60 °C for 15 s, and 72 °C for 20 s. The predicted products were analyzed by electrophoresis in 2 % agarose gel containing ethidium bromide. Images of the PCR amplicons were scanned into a computer and analyzed with NIH Image 1.63 software (NIH Research Services Branch, Bethesda, MD, USA). Band intensities were normalized by dividing the corresponding β-actin values in the same lane.

Bactericidal activities of splenic DC

To test antibacterial killing activity, splenic DC (5×10^4 cells/ml), suspended in antibiotic-free RPMI 1640 medium supplemented with 10 % fetal bovine serum, were infected with 5×10^5 CFU/ml *P. aeruginosa* cells [an effector:target (E:T) ratio of 1:10] in 96-well, round-bottomed microtiter plates for 30 min at 37 °C [30]. After the elimination of unphagocytized *P. aeruginosa* by washing three times with media, the cells were recultured. Three to 24 h after recultivation, 50 μg/ml of Triton X-100 (0.1 % final concentration) was added to the cultures [30]. Serial tenfold dilutions of these fluids were plated on blood agar. The colonies were counted after being incubated for 24 h at 37 °C. As controls, *P. aeruginosa* was incubated without DC under identical conditions. To determine the killing activity of the DC, the following formula was applied to the results: killing activity (%) = (1−test group CFU/control group CFU) × 100 [30].

Infection experiments

X-irradiated NOD-SCID IL-2rγ^{null} mice lack functional T cells, B cells, NK cells, DC, Mø, and PMN [26], therefore, it is useful to define the effect of inoculated immune cells on host defense mechanisms to infections. To determine the anti-*P. aeruginosa* functions of splenic DCs, X-irradiated NOD SCID IL-2rγ^{null} mice inoculated i.v. with 1×10^4 cells/mouse of splenic DCs from normal BALB/C mice or 3 days after FT-burned mice were infected subcutaneously with 10 or 10^2 CFU/mouse of *P. aeruginosa*. All of these mice were observed every 12 h to determine mortality rates.

Statistical analysis

The survival of mice exposed to the pathogen was analyzed by the Kaplan–Meier test. Other data were expressed as mean ± standard error (SE), and the results were statistically analyzed by ANOVA. A p value < 0.05 was considered to be significant.

Results

Antimicrobial activity of splenic DC

Previous studies demonstrated that mice spleen and human mature DC express BD1 mRNA. Therefore, we experimented whether splenic DC showed antimicrobial activity to *P. aeruginosa*. In the results, splenic DCs from 3 days after FT-burned mice showed less antimicrobial activity compared with those from normal mice (Fig. 1a). To confirm the anti-bacterial activities of splenic DC, we next examined the influence of inoculation of splenic DC from FT-burned mice or normal mice on the host's resistance to

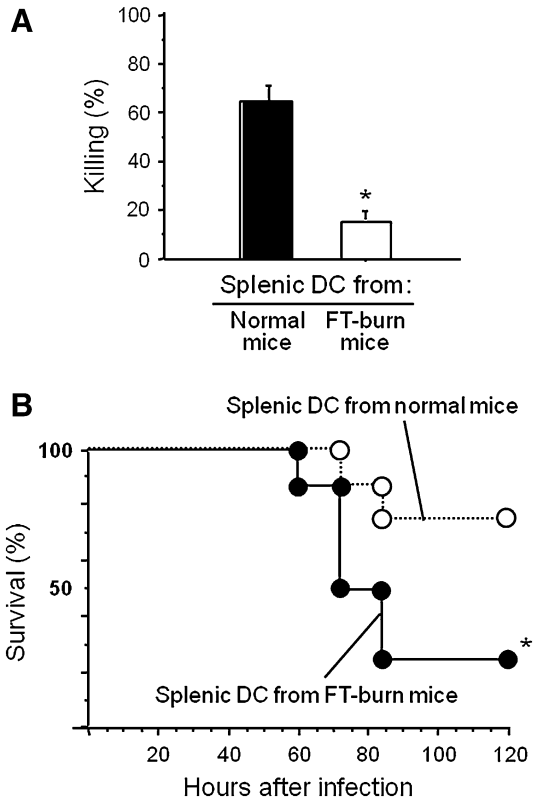

Fig. 1 **a** Antimicrobial activity of splenic DC. Splenic DC (5×10^4 cells/ml) from FT-burn mice or normal mice were infected with 5×10^5 CFU/ml *P. aeruginosa* cells [an effector:target (E:T) ratio of 1:10] for 30 min at 37 °C. Unphagocytized bacteria were washed out and the fate of phagocytized bacteria 2.5 h after the cultivation was determined by a colony counting methods. As controls, *P. aeruginosa* was incubated without DC under identical conditions. To determine the killing activity of the DC, the following formula was applied to the results: killing activity (%) = (1−test group CFU/control group CFU) × 100. Data are shown as mean ± SEM of six experiments in each group. $*p < 0.05$ compared to normal mice. **b** Susceptibility of NOD SCID IL-2rγ^{null} Mice inoculated with splenic DC from FT-burn mice or normal mice to *P. aeruginosa*. X-irradiated NOD SCID IL-2rγ^{null} mice after inoculation with splenic DC were infected 100 CFU/mouse of *P. aeruginosa* subcutaneously. All of these mice were observed to determine their mortality rates ($n = 8$ in each group). $*p < 0.05$ compared to normal mice

P. aeruginosa infection using X-irradiated NOD SCID IL-2rγ^{null} mice. NOD SCID IL-2rγ^{null} mice are mice deficient of functional T cells, B cells, NK cells, DC, and monocytes/macrophages, but functional neutrophils are intact in these mice. After being X-irradiated, there are no functional immune cells in these mice. Splenic DCs were prepared from 3 days after FT-burned mice or normal BALB/c mice, then these cells were inoculated into X-irradiated NOD SCID IL-2rγ^{null} mice. One day after inoculation, mice were infected with various CFU/mouse of *P. aeruginosa* subcutaneously (s.c.). All of these mice were observed every 12 h to determine their survival. In our previous study [31], 10 CFU/mouse of *P. aeruginosa* s.c. infection was shown to be 1 LD_{50} in X-irradiated NOD SCID IL-2rγ^{null} mice without splenic DC inoculation. Figure 1b shows the susceptibility of X-irradiated NOD SCID IL-2rγ^{null} mice to *P. aeruginosa* infection; 75 % of mice inoculated with splenic DC from FT-mice infected with 100 CFU/mouse of the pathogen died within 4 days of infection. However, 75 % of the X-irradiated NOD SCID IL-2rγ^{null} mice inoculated with splenic DC from normal mice survived when they were infected with 100 CFU/mouse of the pathogen. These results indicate that splenic DC from FT-burned mice increased susceptibility to bacterial infections compared to those from normal mice.

Production of MBD1 and MBD3 by splenic DC from normal and thermally injured mice

We investigated whether splenic DCs produce BDs. So far, there are no papers that showed mice splenic DCs produce MBDs. Splenic DCs (1×10^5 cells) from normal mice were stimulated with or without TLR4 agonist LPS or TLR2 agonist SAC for 1, 3, 6, 12, and 24 h. Then, MBD mRNA expression in splenic DC was analyzed by RT-PCR. In the results, MBD1 mRNA was expressed constitutively in splenic DC. MBD3 mRNA was not expressed without stimulation; however, this expression was increased upon activation with LPS or SAC for 24 h (Fig. 2). Next, splenic DCs (1×10^5 cells) from normal mice were stimulated with or without LPS or SAC for up to 48 h. In vitro production of MBD by splenic DC was analyzed by ELISA. Figure 3 shows the kinetics of MBD1 and MBD3 production by splenic DC. A small amount of MBD1 were found in culture fluids of splenic DCs even without stimulation. The production of HBD1 by splenic DCs was significantly increased after LPS or SAC stimulation. In contrast, production of MBD3 by splenic DCs was not detected without stimulation. After LPS or SAC stimulation, MBD3 production was significantly increased. These results of MBD1- and MBD3-producing profiles were consistent with previous studies showing that MBD1 is expressed constitutively, whereas MBD3 are induced by

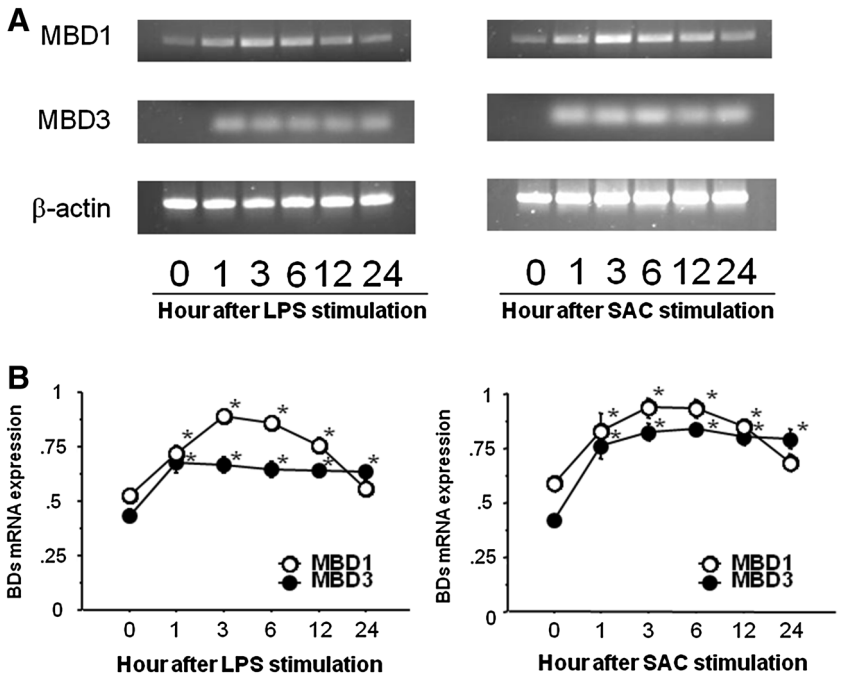

Fig. 2 Expression of MBD1 mRNA and MBD3 mRNA by splenic DC. Splenic DC from normal mice were stimulated with LPS (10 μg/ml) or SAC (0.0075 %) for various hours. **a** The expression of MBD1 and MBD3 mRNAs by splenic DC were analyzed using RT-PCR. **b** The mRNA signals obtained RT-PCR shown in panel **a** were measured by densitometry and normalized to β-actin. The value of β-actin was set to 1. Data are shown as mean ± SEM of six experiments in each group. $^{*}p < 0.05$ compared to control (0 h)

cytokines or bacterial stimuli. Therefore, the results of the present study indicate that mice splenic DCs also show the ability to produce MBDs.

Figure 4 shows the profile of SAC-induced MBD1 and MBD3 production by splenic DC from burn mice or normal control mice. Splenic DCs (1×10^5 cells) from burn mice or normal mice were stimulated with SAC for 24 h. The SAC-induced production of MBD1 by splenic DCs from burn mice was significantly decreased compared to that from normal mice. Splenic DCs from 3 days after thermally injured mice showed the most potent suppressing property of MBD1 production. Furthermore, SAC-induced production of MBD3 by splenic DCs was also decreased in burn mice. As well as MBD1, splenic DCs from 3 days after thermally injured mice showed the most potent suppressing property of MBD3 production. In consistent with SAC-induced MBD1 and MBD3 production, LPS-induced MBD1 and MBD3 production by splenic DCs from burn mice was significantly decreased compared to that from normal mice (Fig. 5). Splenic DCs (1×10^5 cells) from burn mice or normal mice were stimulated with LPS for 24 h. The most potent suppressing property was also shown in splenic DC from 3 days after thermally injured mice. Therefore, the results of the present study indicate that the suppression of the profile of MBD1 and MBD3 production by splenic DCs from burn mice is one of the causes of increased susceptibility to infections in thermally injured mice.

In the next experiments, we investigated whether MBDs were also produced by splenic DCs in another strain of mice. We used BALB/c mice in the above experiments; therefore, we used C57BL/6 mice in this study. Splenic DCs (1×10^5 cells) from 3 days after burn C57BL/6 mice or normal C57BL/6 mice were stimulated with SAC or LPS for 24 h. In the results, MBD1 and MBD3 were also produced by splenic DCs from C57BL/6 mice (Fig. 6). As well as BALB/c mice, MBD1 and MBD3 production was significantly suppressed after thermal injury. These results indicate that thermal injury attenuates MBD's producing ability of splenic DCs in two different strains of mice.

Discussion

In this study, splenic DC from burn mice showed less antimicrobial activity to *P. aeruginosa* compared with those from normal mice. We also showed that mice splenic DC have the ability to produce MBD1 and MBD3 using RT-PCR and ELISA. MBD1 and MBD3 production by splenic DC was suppressed following burn injury. It is well documented that MBDs have potent antibiotic activities, therefore, the suppressed resistance to *P. aeruginosa* infection following burn injury might be due to the reduced ability to MBDs production by splenic DC.

The contribution of DC on host antimicrobial immunity has been described. Enhancement of DC production by Fms-like tyrosine kinase-3 ligand increased the resistance of mice to a burn wound infection with *P. aeruginosa* [32]. DC migration is necessary for induction of protective immunity against *Leishmania donovani* [33]. Furthermore, NKDC are defined as innate immune responders to bacterial infection with *Listeria monocytogenes* [34]. Previously

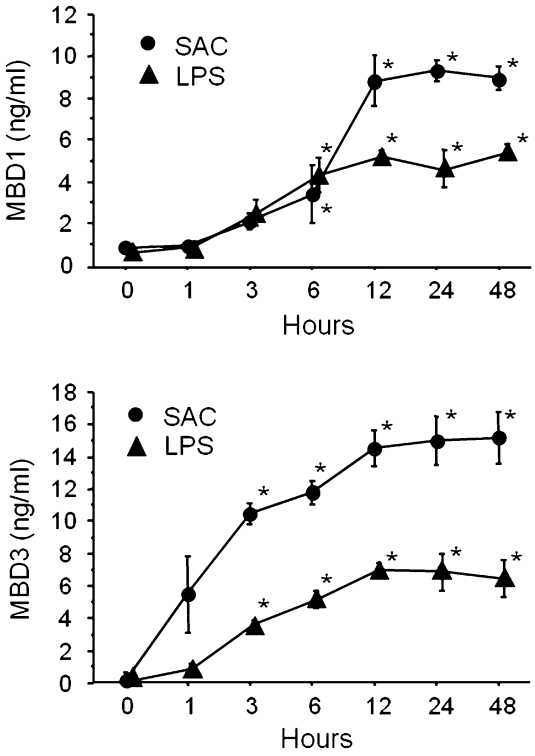

Fig. 3 Kinetics of MBD1 and MBD3 production by splenic DC. Splenic DC (5×10^5 cells/ml) from normal mice were stimulated with LPS (10 μg/ml) or SAC (0.0075 %) for various hours. The amounts of MBD1 and MBD3 in their culture fluids were measured by ELISA. Data are shown as mean ± SEM of six experiments in each group. *$p < 0.05$ compared to control (0 h)

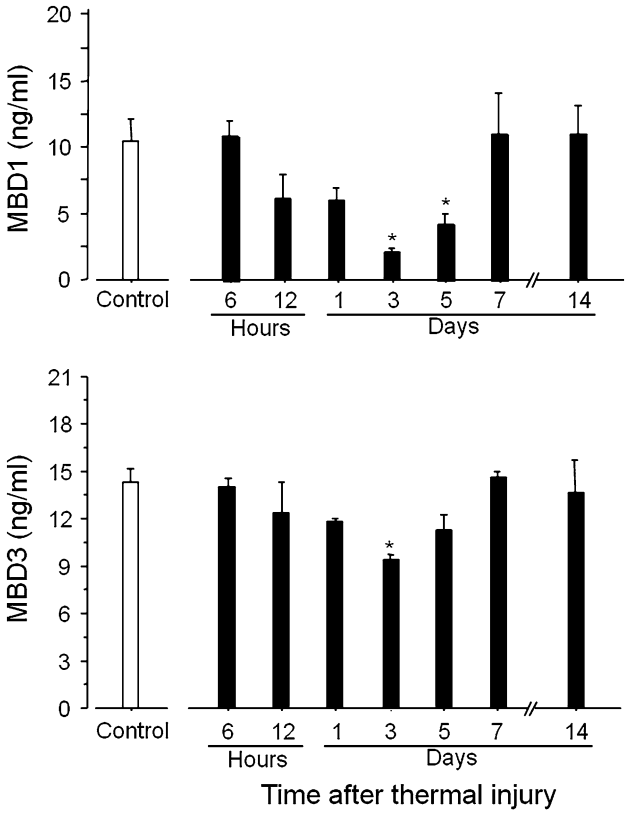

Fig. 4 SAC-induced production of MBD1 and MBD3 by splenic DC from thermally injured mice. Splenic DC (5×10^5 cells/ml) from FT-burn mice or normal mice were stimulated with SAC (0.0075 %) for 24 h at 37 °C. The levels of MBDs in the DC culture fluids were measured by ELISA. Data are shown as mean ± SEM of six experiments in each group. *$p < 0.05$ compared to control

we showed that NKDC were more effective at preventing *P. aeruginosa* infection than NKDC depleted DC [31]. In the current study, we showed that splenic DC from normal mice showed more antimicrobial activity to *P. aeruginosa* compared with those from burn mice. The protective effect was due to superior production of MBD and potent killing activity of *P. aeruginosa* by DC. This protective effect of DC was proven with DC-inoculated X-irradiated NOD SCID IL-2rγ^{null} mice, which have no active immune cells except for DC. Taken together, these findings clearly demonstrate that DC plays a role in the antibacterial immunities against *P. aeruginosa* infection following thermal injury.

The BDs are mainly expressed by epitherial cells, although HBD1, but HBD2, was previously detected in mature DCs, monocytes, and macrophages in response to LPS [21]. Yin et al. [35] also showed that both HBD1 and HBD2 are expressed in DCs with basal level in immature DCs approximately 100-fold lower than in epithelial cells. However, in contrast to epithelial cells, in which HBD1 is constitutively expressed and HBD2 and HBD3 are inducible, both HBD1 and HBD2 were inducible in DCs by bacterial exposure, while HBD3 was only weakly expressed. Furthermore, previous studies demonstrated that BDs influence DC properties. Both HBD1 and HBD3 influence DC maturation with the up-regulation of co-stimulatory molecules [36, 37], and HBD1 also stimulates expression of pro-inflammatory cytokines [37]. MBD1 is a functional homolog of HBD1 [38]. MBD1 is expressed constitutively in airways and epithelia of many mucosal surfaces. MBD1 and HBD1 show tremendous similarities; they are salt sensitive and active against an array of bacteria [16]. MBD3 is a homolog of HBD2 [16]. These have significant similarities in structure, function, and regulation. MBD3 is expressed throughout epithelia of multiple mucosal surfaces, with relatively higher levels found in the gastrointestinal tract [16]. MBD3 expression is significantly up-regulated in response to infection and inflammation. In this study, we demonstrated that MBD1 and MBD3 are expressed in mice DC as well as HBD1 and HBD2 are expressed in human DC. These BDs had a protective effect on *P. aeruginosa* infection consistent with the results of previous studies that showed that recombinant and synthetic forms of MBD3 peptide are active

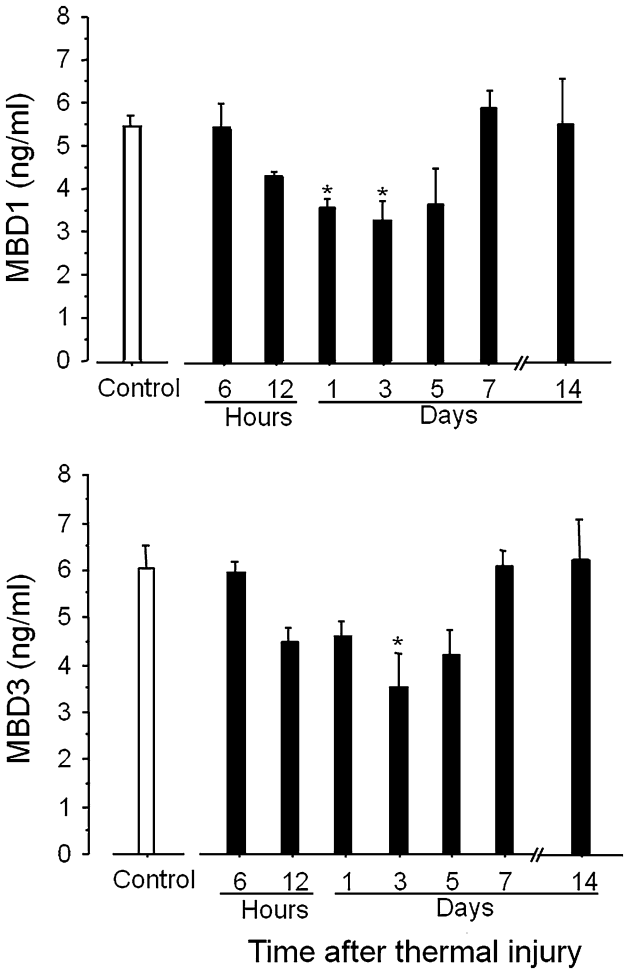

Fig. 5 LPS-induced production of MBD1 and MBD3 by splenic DC from thermally injured mice. Splenic DC (5×10^5 cells/ml) from FT-burn mice or normal mice were stimulated with LPS (10 μg/ml) for 24 h at 37 °C. The levels of MBDs in the DC culture fluids were measured by ELISA. Data are shown as mean ± SEM of six experiments in each group. $*p < 0.05$ compared to control

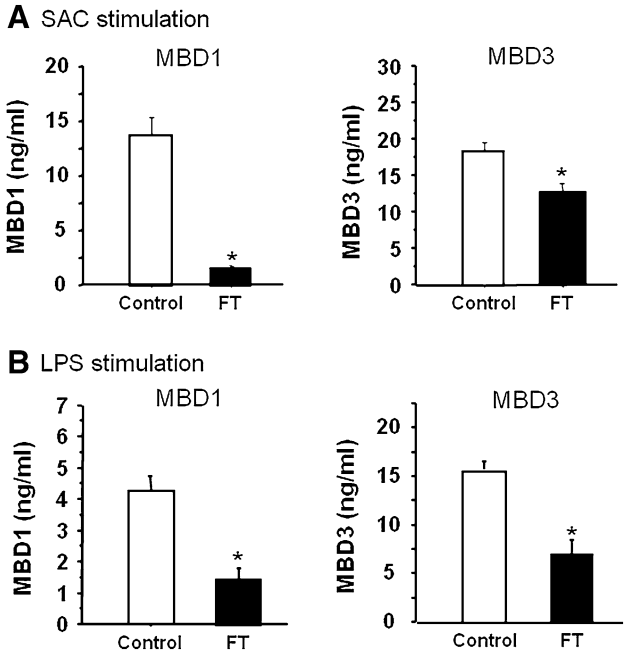

Fig. 6 MBD1 and MBD3 production by splenic DC from C57BL/6 mice. Splenic DC (5×10^5 cells/ml) from C57BL/6 mice 3 days after FT-burn or normal C57BL/6 mice were stimulated with **a** SAC (0.0075 %) or **b** LPS (10 μg/ml) for 24 h at 37 °C. The levels of MBDs in the DC culture fluids were measured by ELISA. Data are shown as mean ± SEM of six experiments in each group. $*p < 0.05$ compared to control

against *P. aeruginosa* and *Escherichia coli, P. aeruginosa, Staphylococcus aureus*, and *Candida albicans* infection [39, 40].

In this study, TLR4 agonist LPS or TLR2 agonist SAC induced MBD1 and MBD3 production by splenic DCs from burn mice were most suppressed 3 days after thermal injury. Our previous study also showed that LPS-induced CCL3 and IL-12 production by splenic DCs from 3 days after FT-burn mice were significantly depressed as compared with those from control mice [31]. These suppressive effects were recovered 5–7 days after thermal injury. Therefore, in the mice-burn injury model, splenic DC function is most suppressed 3 days after thermal injury. Indeed, survival study demonstrated that FT-burn mice infected with *P. aeruginosa* died within 3 days of infection [31]. Furthermore, IL-12 enhances HBD2 production in human keratinocytes [41]. MBD3 is a homolog of HBD2, therefore, IL-12 and MBD3 may act synergistically for antimicrobial defense. Our results are supported by a previous study by Wu et al. [42] that showed that MBD3 promotes resistance to *P. aeruginosa* by modulating bacterial load and TLR activation. Mice splenic DCs express TLR2, TLR4, TLR7, and TLR9 on their surface [43]. TLRs play an essential part in the recognition of bacterial components, therefore, modulation of expression and function of TLRs via MBDs may be a potential therapeutic strategy for antibacterial resistance in burn patients.

We should argue the limitations of this study. We only examined the expression kinetics and the subsequent analysis of mortality rate with respect to MBD1 and MBD3. Therefore, it is not clear whether treatment with MBD1 or MBD3 restores bactericidal activity of the FT-burned mice. Furthermore, other factors may also contribute to our results, such as MBD14, a murine counterpart of HBD3. Further studies are required to solve these suppositions.

In conclusion, our data suggest that decreased MBD1 and MBD3 production in splenic DCs contributes to the susceptibility of *P. aeruginosa* infection in the mice-burn model. These findings may suggest new therapies specifically targeting MBDs, which act on antimicrobial defense. However, the mechanism through which MBDs production were up-regulated remains unclear and further studies are needed to examine this issue.

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