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Topical Prostaglandin E Analog Restores Defective Dendritic Cell–Mediated Th17 Host Defense Against Methicillin-Resistant *Staphylococcus Aureus* in the Skin of Diabetic Mice

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COMPLICATIONS

People with diabetes are more prone to *Staphylococcus aureus* skin infection than healthy individuals. Control of *S. aureus* infection depends on dendritic cell (DC)–induced T-helper 17 (Th17)–mediated neutrophil recruitment and bacterial clearance. DC ingestion of infected apoptotic cells (IACs) drive prostaglandin E_2 (PGE_2) secretion to generate Th17 cells. We speculated that hyperglycemia inhibits skin DC migration to the lymph nodes and impairs the Th17 differentiation that accounts for poor skin host defense in diabetic mice. Diabetic mice showed increased skin lesion size and bacterial load and decreased PGE_2 secretion and Th17 cells compared with nondiabetic mice after methicillin-resistant *S. aureus* (MRSA) infection. Bone marrow–derived DCs (BMDCs) cultured in high glucose (25 mmol/L) exhibited decreased *Ptges* mRNA expression, PGE_2 production, lower CCR7-dependent DC migration, and diminished maturation after recognition of MRSA-IACs than BMDCs cultured in low glucose (5 mmol/L). Similar events were observed in DCs from diabetic mice infected with MRSA. Topical treatment of diabetic mice with the PGE analog misoprostol improved host defense against MRSA skin infection by restoring DC migration to draining lymph nodes, Th17 differentiation, and increased antimicrobial peptide expression. These findings identify a novel mechanism involved in poor skin host defense in diabetes and propose a targeted strategy to restore skin host defense in diabetes.

Diabetes is a clinical syndrome associated with deficiency in insulin secretion or action. As a consequence, diabetes causes hyperglycemia, which has been associated with undermined host defense and increased susceptibility to localized and systemic infections (1). Skin and soft tissues are sites of prevalent infections primarily caused by *Staphylococcus aureus* in people with diabetes (2,3). Methicillin-resistant *S. aureus* (MRSA) is a frequent causative pathogen of complicated skin and soft tissue infections in patients with diabetes (2) that can be difficult to treat and often require hospitalization and surgery (4).

Innate immune recognition of *S. aureus* promotes proinflammatory signals that lead to neutrophil recruitment and abscess formation, a hallmark of *S. aureus* infection (5). Extensive neutrophil apoptosis is also a hallmark of *S. aureus* infections (5). An additional complication is that neutrophils from patients with diabetes have defective antimicrobial effector functions (6,7).

Clearance of apoptotic cells (efferocytosis) has long been associated with resolution of inflammation (8). However, efferocytosis of infected apoptotic cells (IACs) has been proposed to be an innate antimicrobial mechanism that results in pathogen destruction (9) and comprises a critical link to adaptive immune responses (10). Considering the increased neutrophil recruitment and apoptosis at sites of MRSA infection, we speculated that the uptake of infected apoptotic neutrophils by dendritic cells (DCs) dictate the type of host defense produced against *S. aureus* in skin infections.

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In the skin, DCs are prompted to respond to intracellular and extracellular modifications that induce a rapid change in their function and phenotype accompanied by their migration into secondary lymphoid tissue (11). During this process, DCs require maturation, a process associated with upregulation of class II MHC and costimulatory molecules (CD80, CD86, CD40) (12) and consequent migration to lymph nodes in a CC-chemokine receptor 7 (CCR7)–dependent response to its ligands CCL19 and CCL21 (13). In addition, the lipid mediator prostaglandin E_2 (PGE_2) has been shown to be a key factor for DC maturation and migration through CCR7 (14). Moreover, PGE_2 is required to initiate skin immunity by increasing maturation and migration of Langerhans cells, a subtype of skin-resident DCs (14).

PGE_2 synthesis involves activation of phospholipase A2 family members that mobilize arachidonic acid from cellular membranes. Cyclooxygenase (COX) 1 and 2 then convert arachidonic acid into prostaglandin H_2 (PGH_2), which is further metabolized by specific synthases to generate specific prostanoids. Microsomal PGE synthase 1 is induced during the inflammatory response that acts mainly downstream of COX-2 to convert PGH_2 to PGE_2. The levels of PGE_2 are regulated by the local balance between the COX-2–driven synthesis and 15-hydroxyprostaglandin dehydrogenase–mediated degradation of PGE_2 or the actions of prostaglandin transporters (15). PGE_2 acts through four G-protein–coupled receptors, designated EP1–EP4, which display different tissue distributions and deliver distinct intracellular signals (16). Moreover, efferocytosis has been shown to trigger PGE_2 production (17), although the role of PGE_2 on DC functions during efferocytosis remains to be determined.

Although efferocytosis leads to secretion of anti-inflammatory mediators that promote expansion of regulatory Foxp3 T cells, efferocytosis of *Escherichia coli*–infected neutrophils by DCs induces interleukin-6 (IL-6), IL-23, and transforming growth factor-β (TGF-β) production, which triggers T-helper 17 (Th17) differentiation (10,18).

Th17 cells are fundamental players in host defense against *S. aureus* infection (19) by activating keratinocytes and resulting in the induction of neutrophil chemoattractants and antimicrobial peptides (5). Patients with defective Th17 responses exhibit increased susceptibility to *S. aureus* skin infections (5); however, whether patients with diabetes also exhibit defective Th17 cells during *S. aureus* skin infection is unknown. Reduced frequencies of IL-17–producing memory $CD4^+$ T cells associated with elevated glucose and increased glycated hemoglobin A_{1c} have been observed in response to *Streptococcus pneumoniae* in patients with diabetes (20).

We found that localized deficient PGE_2 production impairs DC migration and maturation, which impairs the generation of Th17 cells and drives poor skin host defense against infection. The data show that topical PGE_2 treatment restores immune responsiveness and increases microbial clearance in diabetic mice.

RESEARCH DESIGN AND METHODS

Animals

Wild-type C57BL/6 mice (8–12 weeks old) were obtained from The Jackson Laboratory. Mice were maintained according to National Institutes of Health guidelines for the use of experimental animals in the Laboratory Animal Resource Center at Indiana University School of Medicine (Indianapolis, IN). Experiments were performed under a protocol approved by the Indiana University School of Medicine Animal Care and Use Committee.

Induction of Diabetes

Diabetes was induced by five daily intraperitoneal injections of low-dose (40 mg/kg) streptozotocin as previously described (21). Mice with blood glucose >300 mg/dL were considered diabetic. Experiments were performed using mice at 30 days after diabetes onset.

MRSA Skin Infection and Misoprostol Topical Treatment

Control and diabetic mice were anesthetized (ketamine/xylazine), their backs were shaved, and MRSA USA300 LAC strain (5×10^6 colony-forming units [cfu]) in 50 μL PBS was injected subcutaneously. Lesion and abscess size were monitored daily and determined by affected area calculated by the standard equation for area [area = $(\pi/2) \times$ length $\times$ width] (22). Mice were topically treated at the site of infection twice a day for 7 days with 0.03% misoprostol (prepared by emulsifying 150 μg misoprostol in 0.5 g petroleum gel or vehicle control).

In Vivo DC Migration

Control and diabetic mice were injected subcutaneously with 5×10^6 cfu of MRSA plus 50 μg of CellTrace FarRed DDAO-SE (Invitrogen) as suggested by the manufacturer. Brachial lymph nodes were harvested 36 or 48 h postinfection. Migrating DCs were identified as $FarRed^+CD11c^+$ cells detected by flow cytometry.

Skin Biopsy Specimens and Bacterial Load

Punch biopsy specimens (8 mm) from noninfected or infected skin were harvested at different time points and used for determining bacterial counts, cytokine production, RNA extraction, and cell isolation (23). For bacterial counts, skin biopsy specimens were collected at day 7 postinfection, processed, and homogenized in tryptic soy broth media, and serial dilutions were plated on tryptic soy broth agar. Colonies were counted after incubation overnight at 37°C.

MRSA-IACs

DMSO-differentiated neutrophil-like cells were incubated with green fluorescent protein-MRSA (multiplicity of infection 50) for 2 h. We observed by FACS that 90% of neutrophil-like cells were infected after 2 h of MRSA exposure (data not shown). After phagocytosis, cells were washed, and apoptosis of infected and noninfected cells was induced by using an ultraviolet irradiation crosslinker (5 mJ) and confirmed by annexin-V and 7-aminoactinomycin staining (Supplementary Fig. 2).

Coculture of Bone Marrow–Derived DCs With IACs

Bone marrow cells from control or diabetic mice were differentiated into DCs as previously described (24) in either 5 or 25 mmol/L glucose. After differentiation, bone marrow–derived DCs (BMDCs) were cocultured with IACs (1:3) for 18 h in DMEM containing 5 or 25 mmol/L glucose. Supernatants were collected, and inflammatory mediators were detected by ELISA or enzyme immunosorbent assay (EIA); maturation of BMDCs were analyzed by FACS.

In Vitro BMDC Migration

After 18 h of coculture with MRSA-IACs, BMDCs were isolated using CD11c microbeads (Miltenyi Biotec), and cells (1×10^6) were suspended in serum-free DMEM with 5 or 25 mmol/L glucose and plated into the upper chamber of a 24-well Corning Costar Transwell plate (5 μmol/L). To study CCR7-dependent directional migration, CCL19 (100 ng/mL) and CCL21 (100 ng/mL) were placed into the lower chamber in free-serum medium with 5 or 25 mmol/L glucose. Cells that had migrated into the lower chamber 8 h later were photographed, harvested, and counted by FACS.

Skin Cell Isolation and Staining for Flow Cytometry

Skin biopsy specimens were digested with collagenase and processed to obtain a single-cell suspension. For lymphocyte staining, skin and lymph node cells were stimulated for 5 h with 0.1 μg/mL phorbol myristic acid (Sigma), 0.5 μg/mL ionomycin calcium salt, and 10 μg/mL brefeldin A. In all circumstances, before staining with antibodies, cells were treated with anti-FcγR antibodies to prevent nonspecific antibody binding. For lymphocyte staining, cells were fixed and permeabilized using Cytofix/Cytoperm (BD Biosciences) and were stained with the antibodies indicated in the figure legends. For DC staining, cells were fixed in 1% paraformaldehyde and stained with fluorescent-labeled antibodies for 20 min.

Detection of Cytokines and PGE_2

Cytokines, including IL-6, IL-1β, IL-17A, IL-10, and TGF-β (eBioscience or R&D Systems); production of PGE_2; PGE metabolites; and 6-keto prostaglandin F1α (Cayman Chemicals) were detected by ELISA or EIA in skin biopsy homogenates or supernatants from cultured BMDCs. In all circumstances, the sensitivity limit of the assays were ~4–5 pg/mL.

RNA Isolation and Quantitative Real-Time RT-PCR

Total RNA was isolated from skin biopsy specimens using lysis buffer (Buffer RLT; QIAGEN). cDNA and real-time PCR were performed as previously published (21) using primers indicated in the figure legends on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad). Primers were purchased from Integrated DNA Technologies. Relative expression was calculated as previously described (21).

Statistical Analysis

Results are shown as mean ± SEM and were analyzed with GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA). For comparisons between two experimental groups, Student *t* test was used, and for comparisons among three or more experimental groups, one-way ANOVA followed by Bonferroni multiple comparison test was used. $P < 0.05$ was considered significant.

RESULTS

Decreased Th17 Response During MRSA Skin Infection in Diabetic Mice

Initially, we aimed to characterize the events involved in enhanced susceptibility to MRSA skin infection during diabetes. Mice that were diabetic for at least 30 days were more susceptible to MRSA skin infection than nondiabetic mice as seen by increased lesion size and bacterial load measured at day 7 after infection (Fig. 1*A–C* and data not shown). We observed that MRSA skin infection in diabetic mice led to an increased number of neutrophils in the infected skin by 18 h postinfection, an event that lasted for at least 7 days (Supplementary Fig. 1). After ingestion and killing of bacteria, neutrophils become apoptotic and are eliminated by macrophages and DCs (9). When clearance is not effective, infected apoptotic neutrophils become necrotic, releasing danger-associated molecular patterns that elicit an inflammatory response. Initially, we determined the numbers of dead cells in the infected skin of diabetic and nondiabetic mice. We observed similar increased numbers of apoptotic cells among skin cells isolated 18 h or 7 days after MRSA skin infection in both control and diabetic mice (Supplementary Fig. 2*A–C*) and increased apoptosis in a human neutrophil-like cell line cultured with MRSA for 18 h (Supplementary Fig. 2*C–E*).

Bacterial infections associated with significant induction of apoptosis in host tissues preferentially induce Th17 immunity (5). Thus, we first determined the profile of the T-cell subsets in the skin of diabetic mice 7 days after infection. Diabetic mice showed decreased percentages of Th17, but no significant differences were observed in the percentage of regulatory T (Treg) or Th1 cells compared with infected nondiabetic mice (Fig. 1*D–G*).

Next, we assessed the production of PGE_2, an inflammatory mediator involved in Th17 differentiation, as well as IL-17A and IL-10 in the skin of control and diabetic mice at different time points after infection. Diabetic mice showed significantly reduced PGE_2 levels at day 2 and decreased levels of IL-17A and IL-10 in the skin at day 7 postinfection compared with infected nondiabetic controls (Fig. 1*H–J*). We then determined whether decreased PGE_2 levels in DCs cultured in low or high glucose or infected skin of diabetic mice correlated with decreased mRNA expression of enzymes involved in PGE_2 synthesis, such as *Cox-1* and *-2* and *Ptges* (which encodes microsomal PGE synthase 1). The data show that *Ptges*, but not *Cox-1* and *-2*, expression is decreased in vivo and in vitro. Indeed, another important regulatory step involved in decreased PGE_2 levels in diabetic mice could be increased PGE_2 metabolism by 15-hydroxyprostaglandin dehydrogenase or deficiency in PGE_2 transporter. We then studied the presence of PGE metabolites in the infected skin of

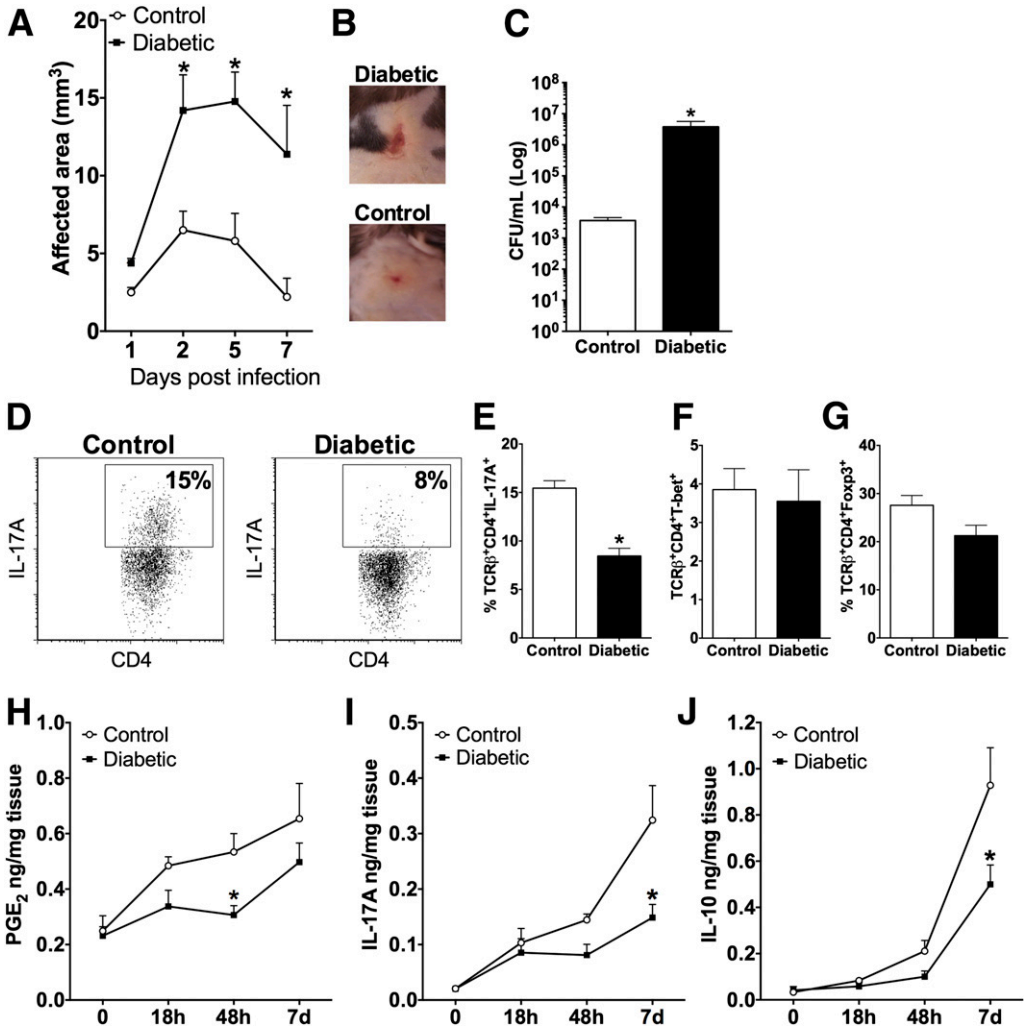

Figure 1—Uncontrolled MRSA skin infection in diabetes correlates with decreased skin PGE_2 and Th17 cells in the infected skin. *A*: Control and diabetic mice were infected subcutaneously with MRSA (5×10^6 cfu). Lesion development was monitored every other day for 7 days. *B*: Representative images of the skin of diabetic and control mice at day 7 postinfection. *C–J*: Skin biopsy specimens were collected at day 7 postinfection, and isolated cells were examined for bacterial counts (*C*), expression of CD4 and IL-17A (*D* and *E*), and expression of TGF-β (T-bet) (*F*) and Foxp3 (*G*) by $CD4^+$ lymphocytes. The lymphocyte population was gated on T-cell receptor-β–positive ($TCR\beta^+$) $CD4^+$ cells. Levels of PGE_2 (*H*), IL-17A (*I*), and IL-10 (*J*) were determined by ELISA in skin biopsy homogenates. Data are mean ± SEM of samples from 5–10 mice. *$P < 0.05$ vs. control.

diabetic and nondiabetic mice. We did not detect expression in the skin of all groups tested, and MRSA skin infection increased the production of PGE metabolites in a similar manner in both diabetic and nondiabetic mice 48 h after infection (Supplementary Fig. 5*A–C* and data not shown). Together, these findings show that decreased PGE_2 foreshadows the production of IL-17A and IL-10, suggesting a temporal relationship between PGE_2 and the generation of Th17 in the infected skin of diabetic mice.

DC Migration to Skin-Draining Lymph Nodes Is Impaired in Diabetic Mice

We next studied whether impaired DC migration to skin-draining lymph nodes is associated with the decrease in Th17 subsets during MRSA skin infection in diabetic mice. We tracked DC migration in vivo during MRSA skin infection and determined the numbers and percentages of migrating DCs ($FarRed^+CD11c^+$) and Th17 cells in the skin-draining lymph nodes. Diabetic mice exhibited decreased percentages and numbers of migrating DCs and Th17 cells in the lymph nodes compared with infected nondiabetic mice (Fig. 2*A–F*).

Langerhans cells have been described as the main cutaneous DC subset capable of inducing a Th17 response (25). Although migrating DCs were decreased in the lymph nodes, we observed an increased percentage of Langerin-positive (Langerin+) DCs in the infected skin of diabetic mice (Fig. 3*A–C*), suggesting that these cells are retained in the skin and cannot properly migrate to the lymph nodes. Although no differences were observed in numbers or expression levels of maturation markers in Langerin+ DCs (data not shown) in the naive skin of control and diabetic

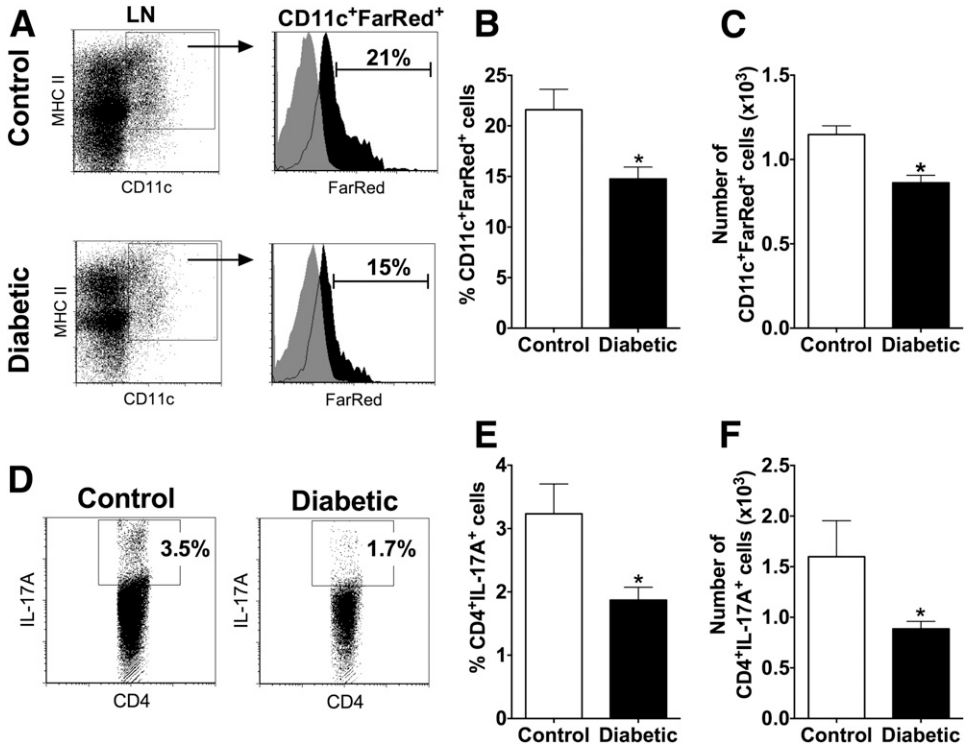

Figure 2— Impaired DC migration and Th17 generation in skin-draining lymph nodes (LN) of diabetic mice. *A*: Representative dot plots of the DC ($CD11c^+MHC\ II^+$) population and a histogram of the percentage of $FarRed^+$ DCs in the brachial lymph nodes of control and diabetic mice 36 h postinfection. *B* and *C*: Percentage and number of migrating DCs ($CD11c^+MHC\ II^+FarRed^+$) in the lymph nodes. *D*: Representative dot plots of the Th17 ($CD4^+IL\text{-}17A^+$) population in the brachial lymph nodes 36 h postinfection in control and diabetic mice. *E* and *F*: Percentage and numbers of IL-17A–producing $CD4^+$ lymphocytes. Data are mean ± SEM of samples from 5–10 mice. $^*P < 0.05$ vs. control.

mice, MRSA infection induced MHC II expression in about the same number of Langerin+ DCs in diabetic and control skin (Fig. 3*D*), but Langerin+ DCs in diabetic skin had lower levels (median fluorescence intensity [MFI]) than control skin (Fig. 3*E*). MRSA infection also induced a similar frequency of Langerin+ DCs expressing CD86 (Fig. 3*F*) and at a similar MFI in diabetic and control mice (Fig. 3*G*). Of note, after MRSA infection, CCR7 was expressed by fewer Langerin+ DCs with reduced MFI in diabetic skin than in control skin (Fig. 3*H–K*), indicating that Langerhans cells from infected diabetic mice fail to express the machinery necessary to efficiently activate T cells in the draining lymph node.

High Glucose Impairs BMDC Migration and Maturation After Recognition of MRSA-IACs

We investigated whether a high-glucose environment influences maturation, migration, and generation of cytokines by DCs involved in Th17 commitment after engulfment of MRSA-IACs. Initially, BMDCs from nondiabetic and diabetic mice were differentiated in the presence of 5 or 25 mmol/L glucose. We did not detect differences in spontaneous changes in the phenotype or production of cytokines by immature BMDCs from diabetic and nondiabetic mice under resting conditions (Supplementary Fig. 3*A–E*). However, after recognition of MRSA-IACs under high-glucose conditions, BMDCs showed decreased expression of the maturation markers MHC II, CD86, and CCR7 (Fig. 4*A*, *C*, and *D*) and a decreased ability to migrate through CCR7 toward a gradient of CCL19/CCL21 compared with cells under normal glucose conditions (Fig. 5*B* and *C*).

In 5 or 25 mmol/L glucose, BMDCs showed similar capacities to recognize MRSA-IACs, and no changes in efferocytosis index (Supplementary Fig. 3*F* and *G*) or cytokine production (Supplementary Fig. 3*H–J*) were observed. Moreover, BMDCs from control or diabetic mice cultured in either 5 or 25 mmol/L glucose after recognition of MRSA-IACs produced increased amounts of Th17-driving cytokines IL-6, TGF-β, and IL-1β compared with the control BMDCs before efferocytosis (Supplementary Fig. 3*K–N*). On the other hand, we observed decreased PGE_2 levels in BMDCs cultured in high glucose and challenged with MRSA-IACs (Fig. 5*A*). Efferocytosis of MRSA-IACs in BMDCs enhanced *Cox-2* but not *Cox-1* mRNA expression in cells cultured in 5 or 25 mmol/L glucose (Supplementary Fig. 4*A* and *B*). However, we observed that efferocytosis of MRSA-IACs in 25 mmol/L glucose led to decreased *Ptges* and *Ptger4* expression, and no changes were seen in *Ptger1*, *2*, and *3* expression by BMDCs after efferocytosis in 5 mmol/L glucose (Supplementary Fig. 4*C*, *E*, and *F*), suggesting that a high-glucose milieu decreases PGE_2 production and signaling in DCs.

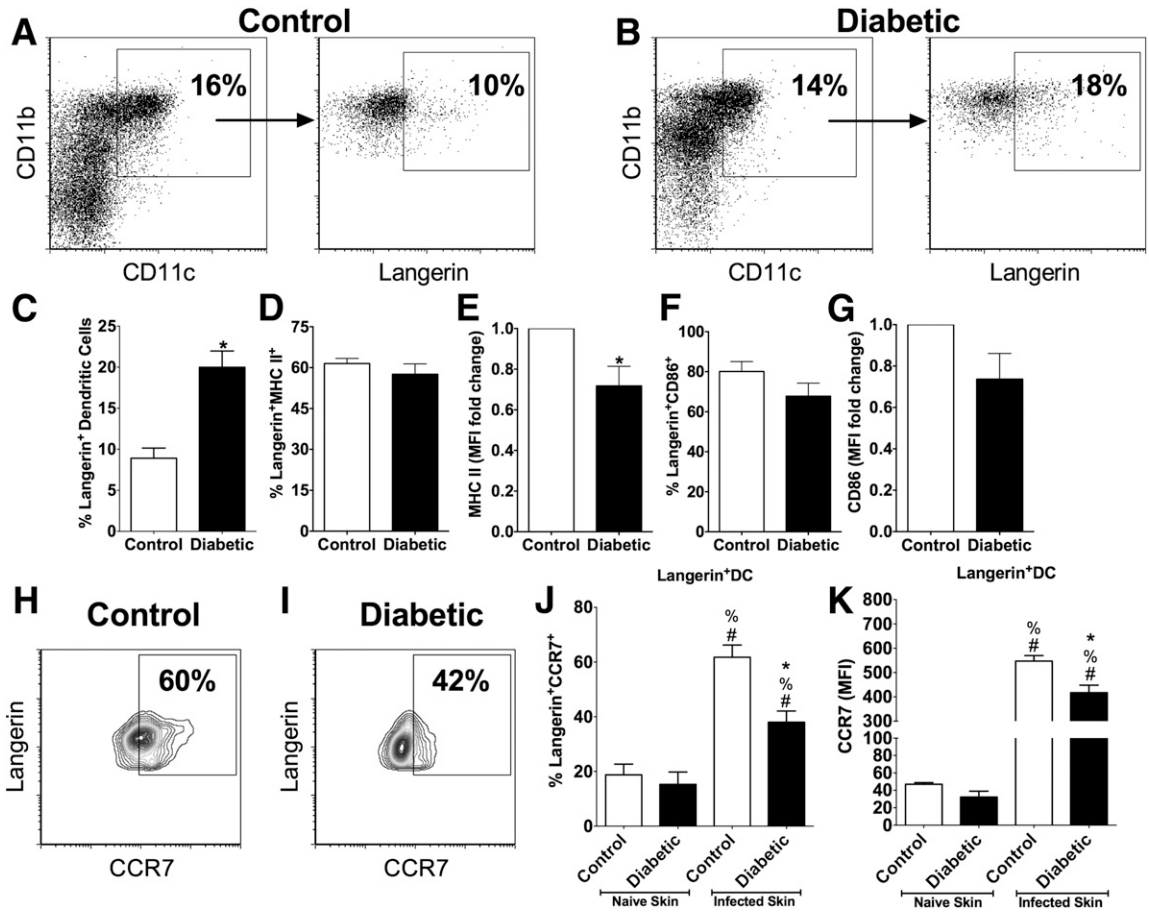

Figure 3—Decreased CCR7 and MHC II expression by Langerin+ DCs in diabetic skin during MRSA infection. *A* and *B*: Representative dot plots of $CD11c^+CD11b^+$ and Langerin+ DCs in the skin of control and diabetic mice 18 h postinfection. *C*: Percentage of Langerin+ DCs in skin of control and diabetic mice 18 h postinfection. Percentage of Langerin+ DCs expressing MHC II (*D*), CD86 (*F*), or CCR7 (*H* and *I*) 18 h postinfection. MFI of staining of MHC II (*E*) and CD86 (*G*) in Langerin+ DCs from diabetic and control mice 18 h postinfection. *J* and *K*: Percentage of Langerin+ $CCR7^+$ DCs and CCR7 MFI on Langerin+ DCs in the skin of naive control and diabetic mice or 18 h postinfection. Data are mean ± SEM of samples from 5–10 mice. *$P < 0.05$ vs. control; %$P < 0.05$ vs. diabetic naive skin; #$P < 0.05$ vs. infected control skin.

Next, we sought to determine whether decreased PGE_2 levels in high glucose is responsible for impaired maturation and migration of BMDCs. To test this hypothesis, we added PGE_2 in BMDC cocultures in high glucose and observed that exogenous PGE_2 restored BMDC migration (Fig. 5*B* and *C*) and CD86 and CCR7 (Fig. 4*C*, *D*, *G*, and *H*) but not CD80 (Fig. 4*B*) expression. Together, these data show that deficient PGE_2 production accounts for inadequate DC maturation and chemotaxis during efferocytosis of IACs, which influences later events involved in MRSA skin infection in diabetes.

Topical Treatment With PGE Analog (Misoprostol) Improves DC Migration to Skin-Draining Lymph Nodes in Diabetic Mice

Considering that exogenous PGE_2 in vitro improved BMDC migration in diabetic mice, we investigated whether topical treatment with PGE_2 in vivo improved DC migration to skin-draining lymph nodes as well as Th17 differentiation and host defense after MRSA skin infection in diabetic mice. For these studies, we used misoprostol, which is a PGE_1 analog with agonist activity for EP2, EP3, and EP4 receptors (26) used therapeutically in humans (27).

At 2 days postinfection, we detected increased percentages of migrating DCs ($FarRed^+CD11c^+$) in lymph nodes from misoprostol-treated diabetic mice compared with untreated diabetic mice (Fig. 6*A* and *C*). We also observed that misoprostol treatment in diabetic mice increased the Th17 population in skin-draining lymph nodes (Fig. 6*B* and *D*) but did not significantly alter Treg and Th1 cells (Fig. 6*E* and *F*).

Misoprostol Attenuates Infection and Improves Host Defense Against MRSA Skin Infection in Diabetic Mice

Given that misoprostol improved DC migration and increased differentiation of Th17 in the lymph nodes, we speculated that misoprostol topical treatment could restore the skin host defense in diabetic mice. Diabetic mice treated with misoprostol had smaller lesion and abscess size (Fig. 7*A*) and lower bacterial load (Fig. 7*B*) than vehicle-treated diabetic mice. Indeed, the treatment of diabetic mice with misoprostol promoted an increased percentage (Fig. 7*C*

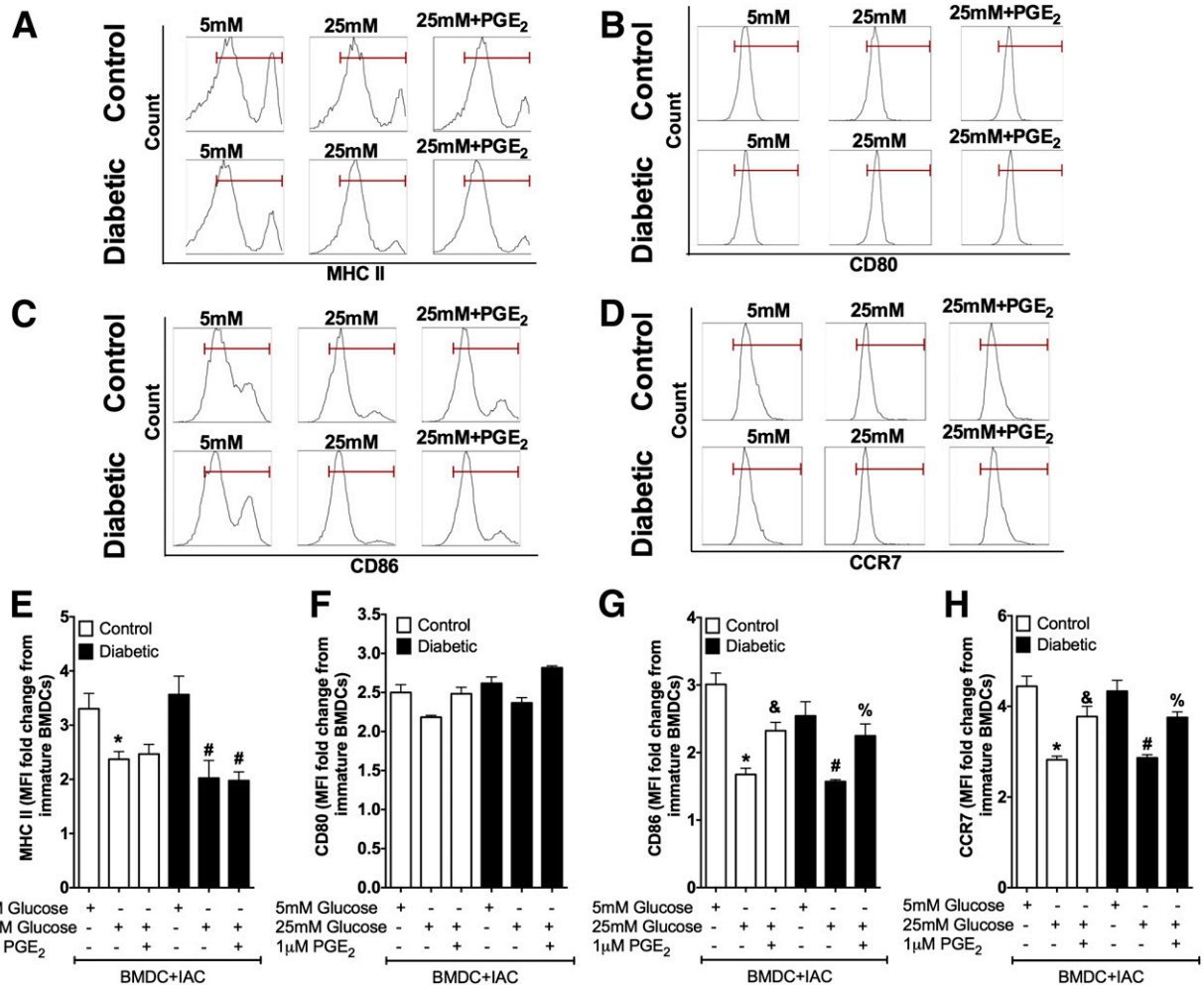

Figure 4—Exogenous PGE_2 restores CD86 and CCR7 expression in BMDCs cultured in high glucose and incubated with MRSA-IACs. Representative histograms of expression (MFI) of MHC II (*A*), CD80 (*B*), CD86 (*C*), and CCR7 (*D*). Fold change in expression (MFI) of MHC II (*E*), CD80 (*F*), CD86 (*G*), and CCR7 (*H*) by BMDCs from control or diabetic mice after efferocytosis of MRSA-IACs compared with inactivated BMDCs from control or diabetic mice. The analyzed populations were gated on $CD11c^+CD11b^+$. Data are mean ± SEM of at least three independent experiments performed in triplicate. **P* < 0.05 vs. control 5 mmol/L glucose; &*P* < 0.05 vs. control 25 mmol/L glucose; #*P* < 0.05 vs. diabetic 5 mmol/L glucose; %*P* < 0.05 vs. diabetic 25 mmol/L glucose.

and *D*) and number (Fig. 7*E*) of Th17 cells in the skin compared with vehicle-treated diabetic mice but not Treg and Th1 percentages (Fig. 7*F* and *H*) or numbers (Fig. 7*G* and *I*) compared with vehicle-treated diabetic and control mice.

Next, we determined whether increased skin host defense in misoprostol-treated diabetic mice was accompanied by changes in production of antimicrobial peptides, which are key mediators involved in controlling MRSA skin infection (28). We found that misoprostol-treated diabetic mice expressed significantly increased *mbd2* and *mbd3* in the skin compared with vehicle-treated diabetic and nondiabetic mice (Fig. 7*K* and *L*) and expressed increased, albeit not significantly, *mbd1*, *4*, *5*, and *Cramp* (Fig. 7*J* and *M*–*O*). Together, these data suggest that misoprostol treatment improves skin host defense in diabetic mice by increasing Th17 generation and expression of antimicrobial peptides.

DISCUSSION

Skin and soft tissue infections are the most important chronic complications of diabetes and may lead to hospitalization, amputation, osteomyelitis, and death (29). The higher incidence of infections in patients with diabetes is related to hyperglycemia, which is known to cause immune dysfunction (30). In the current study, we provide evidence that reduced PGE_2 in the skin of diabetic mice compromises DC maturation and migration to lymph nodes, which inhibits the development of Th17 cells and host defense. These effects correlate with inefficient bacterial clearance and progressive skin infection in diabetic mice (Fig. 8). These findings suggest new therapeutic opportunities for treating skin infections in patients with diabetes.

After being activated by infectious or inflammatory stimuli, DCs upregulate the expression of maturation marks

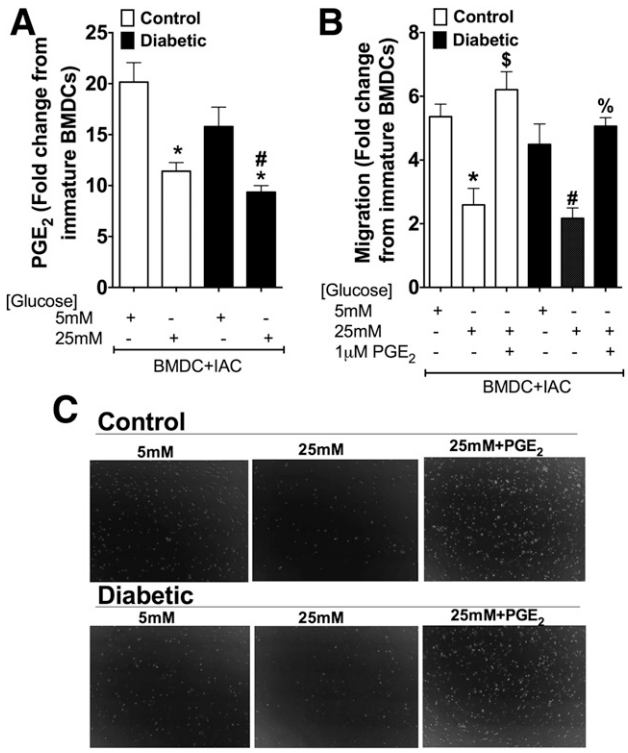

Figure 5—Exogenous PGE_2 enhances BMDC migration in a CCR7-dependent manner after recognition of MRSA-IACs. *A*: Fold change of PGE_2 levels in the supernatants of BMDC + MRSA-IAC cocultures after 18 h determined by EIA. *B*: Migratory BMDCs using flow cytometry acquisition software. *C*: Representative images of migrating BMDCs. Data are mean ± SEM of at least three independent experiments performed in triplicate. *$P < 0.05$ control vs. 5 mmol/L glucose; \$$P < 0.05$ control vs. 25 mmol/L glucose; #$P < 0.05$ diabetic control vs. diabetic 5 mmol/L glucose; %$P < 0.05$ diabetic control vs. diabetic 25 mmol/L glucose.

as they migrate to lymph nodes in a CCR7-dependent manner (12). Migrating mature DCs initiate adaptive immune responses through presentation of peripherally acquired antigens to T cells. PGE_2 signaling in Langerhans cells promotes skin immune responses by supporting maturation and migration to skin-draining lymph nodes (14). Of note, initiation of skin immune responses and DC homing is severely impaired in mice lacking the PGE_2 receptor EP4 (14), highlighting the importance of PGE_2 in DC-mediated skin immunity.

The influence of hyperglycemia on inhibition of PGE_2 by endothelial cells and defective maturation and function of DCs in diabetes has been reported (31,32). As well, during wound healing, diabetic mouse skin exhibits a decreased capacity to produce PGE_2 (15). Moreover, diminished production of COX products of arachidonic acid metabolism have been described as an abnormality of polymorphonuclear leukocytes in diabetes (33). In the current study, we determined whether such effects were responsible for decreased skin DC actions and inefficient host defense in diabetes and found that at a late time point, diabetic mice maintained an open lesion, higher bacterial load, and diminished Th17 population in the infected skin compared with nondiabetic mice.

The finding of increased apoptosis during MRSA skin infection directed us to investigate whether efferocytosis by DCs was involved in PGE_2 production and Th17 differentiation during infection. Efferocytosis induces PGE_2 production (17) and is a condition for restricting *Mycobacterium tuberculosis* replication (9). In addition, in the absence of microbial signals, efferocytosis induces the differentiation of Treg cells, whereas the phagocytosis of *E. coli*–infected apoptotic neutrophils by DCs induces Th17 cell differentiation (10). Thus, apoptosis during bacterial infection has been shown to create a proper cytokine microenvironment promoted by efferocytosis that triggers Th17 (34). Here, we extend these findings to show that efferocytosis of MRSA-infected skin cells in a hyperglycemic environment impairs PGE_2 production, which affects maturation and migration of DCs, and consequently prevents Th17 development.

However, after efferocytosis of MRSA-IACs, we observed a specific reduction of PGE_2 levels in the supernatant of BMDCs cultured in high glucose, which correlated with decreased *Ptges* expression but not COX enzymes. We did not detect any differences in the production of prostacyclin (another prostanoid that exerts immunoregulatory properties) or PGE_2 metabolites in the skin of diabetic mice infected with MRSA. These data contrast with previous reports that showed that hyperglycemia enhances prostaglandin degradation by increasing the expression and activity of PGE_2 transporter, leading to diminished PGE_2 levels and angiogenic signaling in diabetic skin (15). The reasons for such discrepancies are unknown, but it could be due to differences in animal models, cells involved in the inflammatory response during angiogenesis, and infection. In addition to reduced PGE_2 production, decreased expression of EP4 by BMDCs after efferocytosis under hyperglycemic conditions has been observed. This effect may be related to the capacity of PGE_2 to enhance its own receptor expression because PGE_2 increases the levels of both *Pgter4* mRNA and protein expression in monocytoid cell lines (35) and bone marrow stromal cells, pointing to a mechanism of autoamplification of PGE_2 action (36).

The current results show that a high-glucose environment causes similar maturation and migration defects in BMDC from control and diabetic mice, suggesting that high glucose is the sole driver of the PGE_2/CCR7/Th17 axis during MRSA infection in diabetes. The role of PGE_2 in CCR7 expression has been controversial. Recently, PGE_2 has been shown to induce CCR7 receptor oligomerization rather than expression, resulting in an efficient signaling pathway that enhances migration (13). However, together with other soluble mediators such as tumor necrosis factor-α, IL-1β, and IL-6, PGE_2 has been shown to increase CCR7 expression (37). The current data show that exogenous addition of PGE_2 to medium containing high glucose restored BMDC migration to CCL19/CCL21

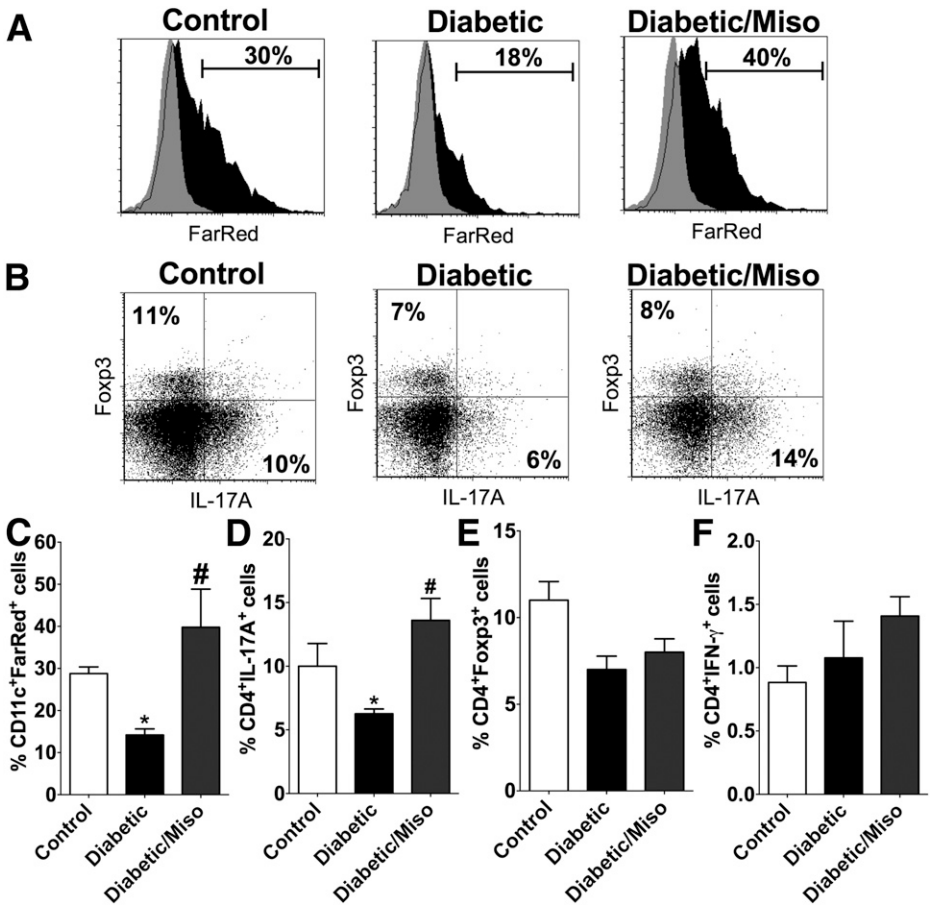

Figure 6— Misoprostol (Miso) improves DC migration and Th17 development in skin-draining lymph nodes in diabetes. *A*: Representative histograms of the percentage of migrating DCs in the brachial lymph nodes of MRSA-infected control or diabetic mice treated or not treated with misoprostol. *B*: Representative dot plots of the percentage of Foxp3- and IL-17A–expressing T lymphocytes in the brachial lymph nodes of control and diabetic mice treated or not treated with misoprostol. *C*: Percentage of migrating DCs ($CD11c^+FarRed^+$) in the brachial lymph nodes. The percentage of intracellular IL-17A– (*D*), Foxp3- (*E*), and interferon-γ (IFN-γ)–producing (*F*) T-cell receptor-β–positive $CD4^+$ T lymphocytes determined by FACS analysis. Data are mean ± SEM of samples from 5–10 mice. *$P < 0.05$ vs. control; #$P < 0.05$ vs. untreated diabetic mice.

chemoattractants and induced CCR7 and CD86 expression, which prompted us to investigate the in vivo effects of PGE_2 treatment on host defense against MRSA skin infection in diabetes.

Because of their roles in angiogenesis and wound healing, PGE_2 analogs have been used to treat patients with diabetic neuropathy (38). We found that diabetic mice receiving topical misoprostol treatment had decreased lesions and bacterial counts as a consequence of improved DC migration to skin-draining lymph nodes and enhanced Th17 immunity.

Th17-derived cytokines, such as IL-17A and IL-22, induce secretion of antimicrobial peptides by neutrophils and keratinocytes (39). We found that topical misoprostol treatment in diabetic mice increased expression of *mbd2* and *mbd3* antimicrobial peptides, which correlates with increased skin Th17 cells after infection. Although we did not explore the mechanism by which misoprostol promotes higher antimicrobial peptide expression or determine the cell source, keratinocytes have shown a stronger dependence on IL-17 for expression of antimicrobial peptides than other cell types (39). On the other hand, misoprostol might directly upregulate antimicrobial peptide expression on keratinocytes without a particular dependence of IL-17. Therefore, further investigations are needed to fully elucidate the role of PGE_2 in the antimicrobial peptide machinery. Furthermore, COX-2 activity is important for optimal production of human β-defensin 1 and 2 by human keratinocytes in vitro (40). Therefore, we suggest that improving Th17 responses in diabetic mice promotes skin host defense against MRSA through induction of antimicrobial peptide expression. Indeed, in considering the known effects of hyperglycemia in impairing macrophage and neutrophil phagocytosis and killing, which are associated with resistance to antibiotics, endogenous antimicrobial peptides are attractive sources of more sustainable antimicrobial agents.

The current data show that PGE_2 equilibrium is a key player in skin host defense. Reduced PGE_2 in diabetic skin impairs DC activation and migration to skin-draining

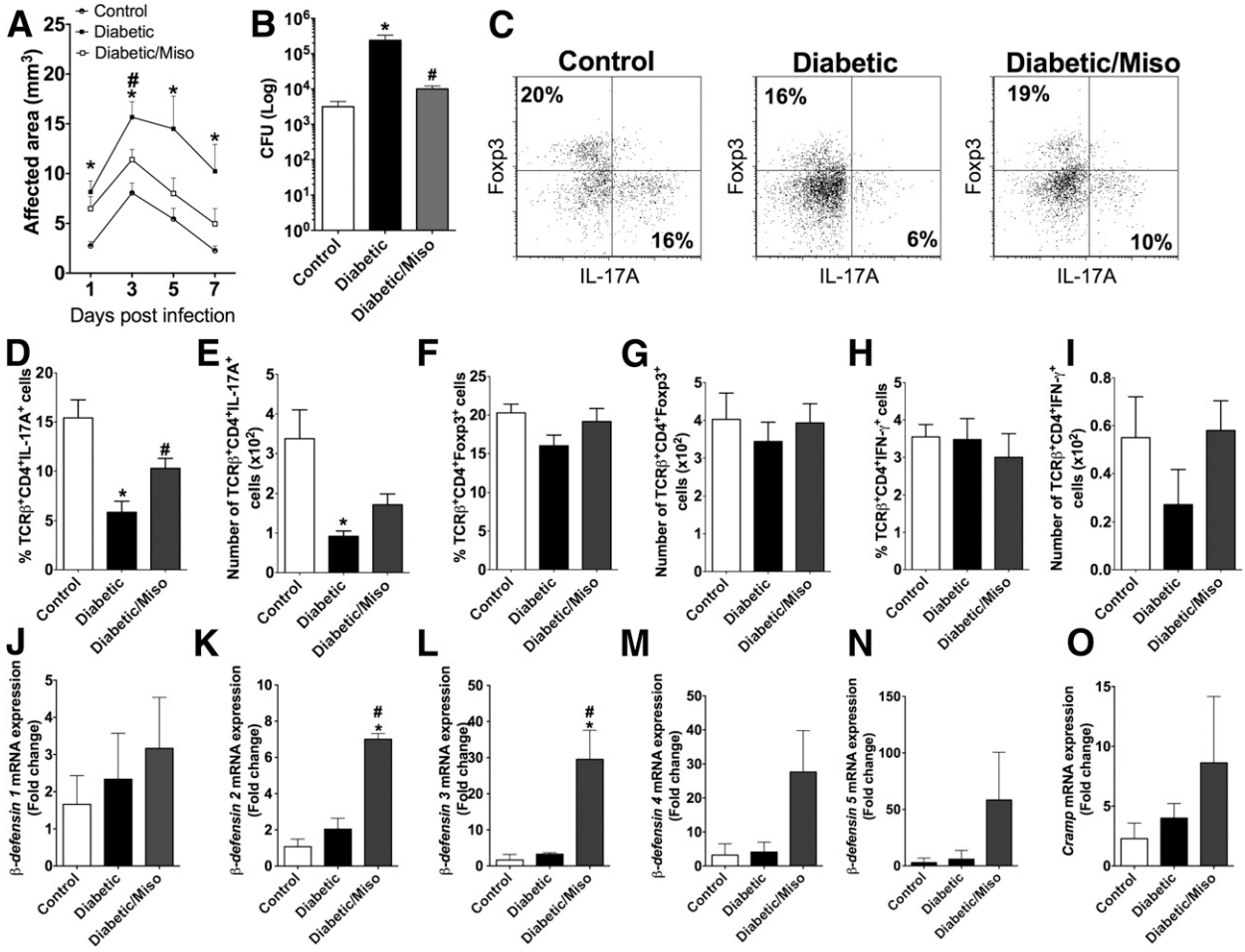

Figure 7—Topical misoprostol (Miso) decreases MRSA skin infection in diabetes. *A*: Lesion and abscess size are represented by affected area in mm^3 as described in RESEARCH DESIGN AND METHODS. *B*: Bacterial load determination in the skin of diabetic mice treated or not treated with Miso at day 7 after infection. *C*: Representative dot plots of the percentage of Foxp3- and intracellular IL-17A–expressing T lymphocytes determined by FACS analysis. Percentage and number of IL-17A– (*D* and *E*), Foxp3- (*F* and *G*), and interferon-γ (IFN-γ)–expressing (*H* and *I*) T-cell receptor-β–positive ($TCR\beta^+$) $CD4^+$ lymphocytes determined by FACS analysis. mRNA expression of *β-defensin 1* (*J*), *β-defensin 2* (*K*), *β-defensin 3* (*L*), *β-defensin 4* (*M*), *β-defensin 5* (*N*), and *Cramp* (*O*). Data are mean ± SEM of samples from 5–10 mice. *$P < 0.05$ vs. control; #$P < 0.05$ vs. untreated diabetic mice.

lymph nodes, compromising the development of adaptive immunity. Delayed priming of Th1 responses against *M. tuberculosis* in pulmonary infections has been previously demonstrated in diabetic mice, although no association with decreased PGE_2 levels or DC migration were observed (41). In addition, PGE_2 has been shown to direct IL-23 secretion by DCs and to be crucial for Th17 development (42).

PGE_2 may have both positive and negative immune regulatory roles, showing opposite effects depending on its concentration, microenvironment, cell type, and receptor subtype (16,43,44). PGE_2 affects mainly macrophage phagocytosis and killing of bacteria and fungus (45). On the other hand, the effects of PGE_2 on macrophage functions in the skin microenvironment are still unclear. Indeed, neutrophils and macrophages in patients with diabetes have already poor phagocytosis and killing capacities, which lead to defective bacterial clearance compared with cells of control subjects (46,47). Thus, enhancing the endogenous antimicrobial machinery could be an attractive strategy to control bacterial growth when effector functions of innate cells are impaired. Thus, the use of a PGE analog may have some limitations.

In summary, this study provides evidence that PGE_2 production was compromised during recognition of MRSA-IACs by DCs in a hyperglycemic environment. Moreover, topical treatment with misoprostol improved the host defense against MRSA by enhancing DC-mediated Th17 responses and expression of antimicrobial peptides. On the basis of these results, targeting pathways to restore PGE_2 balance in diabetic skin may be a strategy to improve innate and adaptive immune responses in patients with diabetes and skin infections. Identifying and delineating levels and mechanisms by which PGE_2 improves the skin host defense

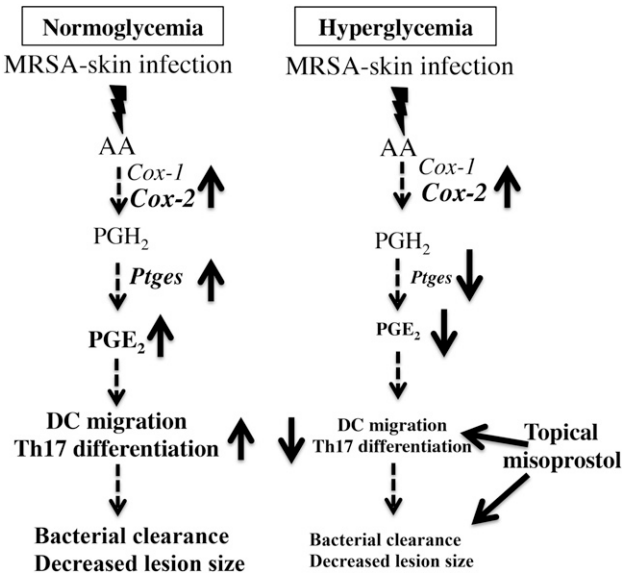

Figure 8—Proposed model of PGE_2 regulation of DC migration and Th17 generation during MRSA skin infection in diabetic mice. During MRSA skin infection, PGE_2 is produced in the skin and enhances CCR7-dependent DC migration to lymph nodes and Th17 generation. Increased Th17 enhances defensin generation and bacterial clearance in infected nondiabetic mice. In diabetic mice, hyperglycemia decreases the *Ptges* mRNA expression that culminates in low PGE_2 production and deficient CCR7-dependent DC migration and Th17 generation in the lymph nodes, which impairs skin host defense. Adding back a topical PGE analog restores the DC/Th17 axis and improves host defense in diabetic mice. AA, arachidonic acid.

in patients with diabetes may provide an avenue toward development of a therapeutic target.

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