Plasmacytoid dendritic cells sense skin injury and promote wound healing through type I interferons

Josh Gregorio,[1,4] Stephan Meller,[1] Curdin Conrad,[1] Anna Di Nardo,[5] Bernhard Homey,[6] Antti Lauerma,[7,8] Naoko Arai,[9] Richard L. Gallo,[5] John DiGiovanni,[10] and Michel Gilliet[1,2,3,4]

[1]Department of Immunology, [2]Department of Melanoma Medical Oncology, and [3]Department of Dermatology, the University of Texas M. D. Anderson Cancer Center, Houston, TX 77030
[4]Graduate School of Biomedical Sciences, University of Texas at Houston, Houston, TX 77030
[5]Division of Dermatology, University of California, San Diego, La Jolla, CA 92093
[6]Department of Dermatology, Heinrich-Heine University, Duesseldorf 40225, Germany
[7]Control of Hypersensitivity Diseases, Finnish Institute of Occupational Health, FIN-00250 Helsinki, Finland
[8]Skin and Allergy Hospital, Helsinki University Central Hospital, FIN-00290 Helsinki, Finland
[9]SBI Biotech Co., Ltd., Ginkgo Biomedical Research Institute, Kawasaki 216-0001, Japan
[10]Pharmacology and Toxicology, College of Pharmacy, the University of Texas, Austin, TX 78712

Plasmacytoid dendritic cells (pDCs) are specialized type I interferon (IFN-α/β)–producing cells that express intracellular toll-like receptor (TLR) 7 and TLR9 and recognize viral nucleic acids in the context of infections. We show that pDCs also have the ability to sense host-derived nucleic acids released in common skin wounds. pDCs were found to rapidly infiltrate both murine and human skin wounds and to transiently produce type I IFNs via TLR7- and TLR9-dependent recognition of nucleic acids. This process was critical for the induction of early inflammatory responses and reepithelization of injured skin. Cathelicidin peptides, which facilitate immune recognition of released nucleic acids by promoting their access to intracellular TLR compartments, were rapidly induced in skin wounds and were sufficient but not necessary to stimulate pDC activation and type I IFN production. These data uncover a new role of pDCs in sensing tissue damage and promoting wound repair at skin surfaces.

CORRESPONDENCE
Michel Gilliet:
michel.gilliet@chuv.ch

Abbreviations used: pDC, plasmacytoid DC; SLS, sodium lauryl sulfate; TLR, toll-like receptor.

Plasmacytoid DCs (pDCs) are a rare population of circulating cells specialized in the production of large amounts of type I IFNs (IFN-α/β) in response to viral infections (Cella et al., 1999; Siegal et al., 1999). This ability is linked to their unique intracellular expression of toll-like receptor (TLR) 7 and TLR9 (Kadowaki et al., 2001), which recognize single-stranded (ss) viral RNA and DNA transported into endosomal compartments by the virus infecting the cell (Hemmi et al., 2000, 2002; Diebold et al., 2004; Heil et al., 2004). IFN-α/β produced by pDCs was shown to be critical in inhibiting viral replication but also to contribute to the induction and expansion of an antiviral immune response by activating memory T cells, B cells, and NK cells (Theofilopoulos et al., 2005; Gilliet et al., 2008). Under steady-state conditions, pDCs are present in the blood stream and secondary lymphoid organs but are normally absent from most peripheral tissues including the skin (Wollenberg et al., 2002; Gilliet et al., 2004). pDCs can, however, infiltrate the skin infected by viruses including varicella zoster virus (Gerlini et al., 2006), human papillomavirus (Vanbervliet et al., 2003), and herpes simplex virus (Donaghy et al., 2009). These skin-infiltrating pDCs were found to produce IFN-α/β, which is consistent with the ability of viruses to infect pDCs and deliver their nucleic acid cargo into intracellular TLR7/9 compartments of pDCs.

Surprisingly, large numbers of pDCs have also been found in the skin of patients with psoriasis (Wollenberg et al., 2002; Gilliet et al., 2004;

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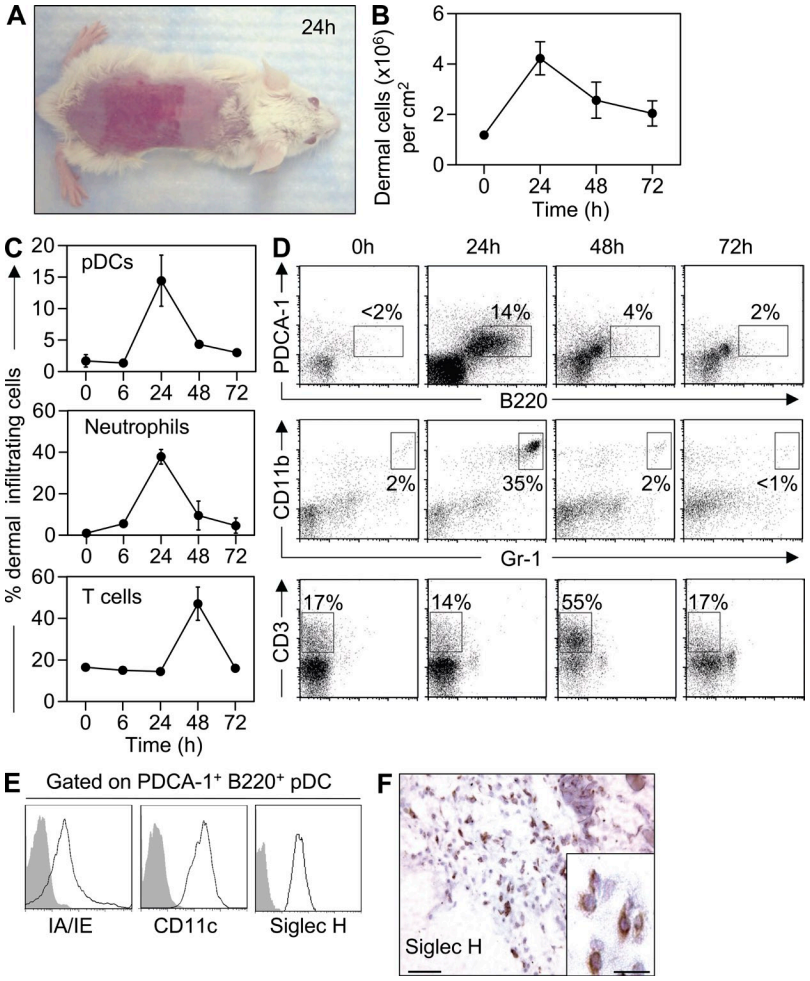

Figure 1. Rapid infiltration of pDCs into injured skin. (A) Representative image of shaved murine back skin 24 h after tape stripping. (B) Dermal cell suspensions isolated from skin at various times after injury and viable cells were counted. The mean number of cells ± SEM per cm^2 of injured skin is given and represent data from five mice over a 3-d time course. (C) The percentage of pDCs ($B220^+PDCA\text{-}1^+$), neutrophils ($Gr\text{-}1^+CD11b^+$), and T cells ($CD3^+$) in dermal single cell suspensions isolated from injured skin was measured by flow cytometry. Data are the mean ± SEM of five mice per each time point. (D) Flow cytometry plot of pDC, neutrophils, and T cells in injured skin over a 3-d time course. Data are representative of five mice. The percentage of each population is shown in the plots. (E) Flow cytometry for CD11c, IA/IE, and Siglec H surface expression on $B220^+PDCA\text{-}1^+$ pDC 24 h after skin injury. (F) Representative immunohistochemical staining for Siglec H in injured skin collected 24 h after tape stripping. Bars: (main image) 50 µm; (inset) 10 µm. Data in A–F are representative of two independent experiments.

Nestle et al., 2005), a chronic inflammatory disease of the skin mediated by autoimmune T cells. In psoriatic skin, pDCs are chronically activated to produce IFN-α/β, a process which triggers the activation and expansion of autoimmune T cells leading to the epidermal hyperproliferation and the formation of psoriasis (Nestle et al., 2005). We have recently found that pDC activation in psoriatic skin is driven by the human cathelicidin antimicrobial peptide known as LL37 (Lande et al., 2007). LL37 was found to convert otherwise inert extracellular host-derived (self) nucleic acids, into a potent trigger of pDC activation by forming a complex with the self-RNA and self-DNA and by transporting them into intracellular TLR7 and TLR9 compartments (Lande et al., 2007; Ganguly et al., 2009). The cathelicidin peptide is usually not expressed in healthy skin but was found to be continuously overexpressed by keratinocytes of psoriatic skin, providing an explanation for the chronic activation of pDCs in psoriasis (Lande et al., 2007). Interestingly, the expression of cathelicidin peptides can be transiently induced in keratinocytes by common skin injury (Dorschner et al., 2001; Schauber et al., 2007). However, whether skin injury is associated with pDC infiltration and activation to produce IFN-α/β is not known.

In this paper, we found that skin injury induces an early and short-lived infiltration of pDCs into skin wounds. These pDCs were activated to produce IFN-α/β through TLR7 and TLR9, indicating that they recognize self-nucleic acids released by damaged cells in skin wounds. Cathelicidin gene expression closely paralleled pDC activation and cathelicidin peptides were found to be sufficient to induce IFN-α/β production by pDC in the skin. However, cathelicidins were not required to induce IFN-α/β expression, suggesting a redundancy of this pathway for pDC activation in injured skin. Depletion of pDCs or inhibition of IFN-α/β receptor signaling significantly impaired the acute inflammatory cytokine response and delayed reepithelization of skin wounds. These data uncover a novel role of pDCs in sensing nucleic acids in wounded skin and demonstrate their involvement in the acute inflammatory response and wound healing through their production of IFN-α/β.

RESULTS

Skin injury induces a rapid infiltration of pDCs

To determine whether injury of normal skin induces infiltration and activation of pDCs, we adopted a mechanical skin injury model based on tape stripping of shaved murine skin (Sano et al., 2005; Jin et al., 2009). This procedure mechanically removes the upper epidermal layers and injures the basal layer, leading to an acute inflammatory response and reepithelization of the skin (Wojcik et al., 2000). We found that tape stripping induced a robust dermal influx of leukocytes reaching a peak between 24 and 48 h after skin injury (Fig. 1, A and B). Analysis of single cell suspensions revealed that, 24 h after skin injury, the majority of cells infiltrating the dermis were $Gr\text{-}1^+CD11b^+$ neutrophils (mean, 37.8%; range,

31–42.9%; Fig. 1 C). Interestingly, a large number of pDCs, detected as PDCA1$^+$B220$^+$ cells, also infiltrated the dermis 24 h after injury (mean, 14.4%; range, 3.7–28%; Fig. 1, C and D). The pDC identity was confirmed by showing that these cells coexpressed CD11c, MHC class II molecules, and the pDC-specific marker Siglec-H (Zhang et al., 2006; Fig. 1 E) and lacked common lineage markers CD3, CD19, and CD11b (not depicted). Furthermore, immunohistochemistry for Siglec-H showed that these cells had typical lymphocytic morphology (Fig. 1 F). The accumulation of pDCs and neutrophils in skin wounds was rapid and transient, as they accumulated at 24 h but returned to preinjury levels after 48 h. In contrast, CD3$^+$ T cells were constitutively present in uninjured skin, and increased in number at a later time point (48 h after injury; Fig. 1, C and D). Conventional DCs in the dermal compartment of injured skin were detected as CD11c$^+$PDCA1$^-$ cells, and their number did not increase but, rather, showed a tendency toward a decrease (Fig. S1), potentially reflecting their activation and migration to lymph nodes as previously reported (Holzmann et al., 2004). Thus, skin injury induces a rapid and robust infiltration of pDCs that parallels the early wound infiltration by neutrophils.

Skin injury induces a transient activation of pDCs to produce type I IFNs

Because pDCs are specialized producers of IFN-α/β, producing 100–1,000× more than any other cell type (Siegal et al., 1999), we sought to investigate whether pDCs infiltrating injured skin are activated to produce IFN-α/β. First, we isolated total skin from mice at different time points after injury and performed gene expression analysis. mRNA expression levels of both IFN-α2 (Fig. 2 A) and IFN-β (not depicted) was undetectable in normal skin before injury but rapidly induced by skin injury. Both IFN-α2 and IFN-β (unpublished data) mRNA expression levels reached a peak 24 h after injury and rapidly declined thereafter. This expression profile closely paralleled the presence of pDCs, suggesting that pDCs are the main source of IFN-α/β in injured skin (Fig. 1 C and Fig. 2 A). In contrast, the expression of the inflammatory cytokines IL-6 and TNF showed a distinct time course. IL-6 mRNA expression reached a peak 6 h after injury (Fig. 2 A), which is consistent with injured keratinocytes as an early source of this cytokine (Sehgal, 1990). TNF was induced 24 h after injury but its expression was sustained for up to 72 h (Fig. 2 A), suggesting that cell types other than pDCs are a major source of this cytokine. To confirm the role of pDCs as principal producers of IFN-α/β in injured skin, we depleted pDCs by treating mice with antibodies recognizing BST-2, a transmembrane protein specifically expressed on resting mouse pDCs (Blasius et al., 2006). Two injections of these antibodies at 48 and 24 h before skin injury efficiently depleted pDCs (Fig. S2), as previously reported (Krug et al., 2004; Yoneyama et al., 2005; Blasius et al., 2006; Kuwajima et al., 2006). pDC depletion completely inhibited the accumulation of pDC in injured skin (not depicted) and abrogated the induction of IFN-α2 (Fig. 2 B) and IFN-β (not depicted) expression at 24 h, confirming that pDCs are the principal source of IFN-α/β in injured skin. Interestingly, pDC depletion partially affected the expression of IL-6, whereas the expression of TNF was not affected at 24 h after injury. Because the infiltration of pDCs into injured skin is paralleled by the infiltration of neutrophils, we next sought to determine the role of neutrophils in the expression of these cytokines. Neutrophil depletion using a Ly6G-specific antibody did not show significant effect on the expression of IFN-α/β and IL-6 (Fig. 2 B) but significantly decreased the expression of TNF (Fig. 2 B), which is consistent with neutrophils being a main early source of this cytokine (Dubravec et al., 1990; Hübner et al., 1996). These data demonstrate that skin injury induces early infiltration of pDCs and their local activation to produce IFN-α/β.

pDCs sense self–nucleic acid in injured skin

pDCs produce IFN-α/β upon recognition of ssRNA or DNA via intracellular TLR7 and TLR9, respectively (Gilliet et al., 2008). To determine the involvement of TLRs in pDC activation to produce IFN-α/β in injured skin, we used mice deficient in the adaptor molecule MyD88, which lack the ability to signal through many TLRs including

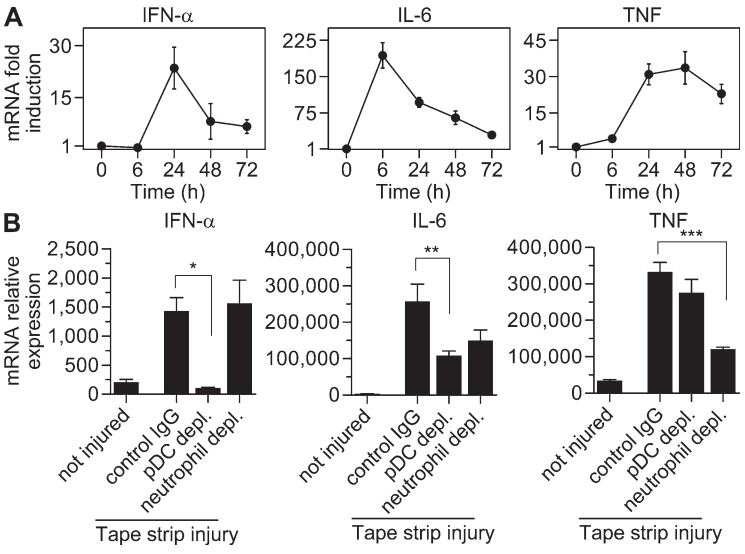

Figure 2. pDCs are transiently activated to produce type I IFNs in injured skin. (A) Time course analysis of IFN-α, IL-6, and TNF mRNA tissue expression in injured skin. The data are given as fold induction over time 0 and represent the mean ± SEM of five mice per time point. (B) Relative IFN-α, IL-6, and TNF mRNA tissue expression of uninjured skin or injured skin collected 24 h after tape stripping of pDC-depleted, neutrophil-depleted, or control IgG-treated mice. Data represent the mean ± SEM of five mice per group. *, P = 0.001; **, P = 0.02; ***, P < 0.001, unpaired Student's *t* test. Data in A and B are representative of at least two independent experiments.

TLR7 and TLR9 (Takeda and Akira, 2004). In contrast to control mice, MyD88-deficient mice failed to up-regulate expression of IFN-α2 and IFN-β mRNA in injured skin (Fig. 3 A), demonstrating the involvement of TLRs in pDC activation in wounded skin. To specifically demonstrate that pDCs recognize nucleic acids in injured skin, we used the oligonucleotide IRS 954 which selectively inhibits TLR7 and TLR9 signaling in pDCs (Barrat et al., 2005). Injection of IRS 954 before tape stripping completely abrogated expression of both IFN-α2 and IFN-β mRNA (Fig. 3 B). Because TLR7 and TLR9 were reported to have opposing inflammatory roles in a mouse model of lupus (Christensen et al., 2006), we sought to determine the specific contributions of TLR7 and TLR9 in the induction of IFN-α/β in injured skin. Expression of both IFN-α2 and IFN−β mRNA was found to be profoundly abrogated in TLR7-deficient mice (Fig. S3). Blocking of TLR9 by the specific oligonucleotide IRS 869 also resulted in a reduction of IFN-α2 mRNA expression in injured skin (Fig. S3), a finding which was confirmed in TLR9-deficient mice by our companion paper (see Guiducci et. al. in this issue). These data indicate that pDC activation to produce IFN-α/β in injured skin is dependent on both TLR7 and TLR9. Interestingly, similar to the pDC depletion data, deficiency of MyD88 and TLR7 or inhibition of TLR9 did not result in a reduction of the neutrophil-dependent TNF expression (Fig. S3 and Fig. S4), suggesting that TLR7 and TLR9 activation preferentially occurs in pDCs (Fig. S3 and Fig. S4). Because mechanical skin injury is largely sterile,

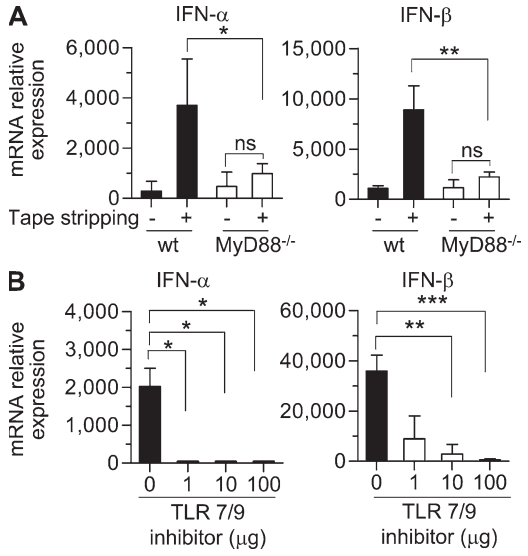

Figure 3. pDCs sense nucleic acid in injured skin. (A) Relative IFN-α and IFN-β mRNA tissue expression in injured skin collected 24 h after tape stripping of MyD88$^{-/-}$ or control mice. Data represent the mean ± SEM of five mice per group. *, P = 0.01; **, P = 0.02, unpaired Student's *t* test. (B) Relative IFN-α and IFN-β mRNA tissue expression in injured skin (24 h) of mice pretreated with 0, 1, 10, or 100 μg of TLR7 and TLR9 inhibitor IRS 954. Data represent the mean ± SEM of three mice per group. *, P = 0.002; **, P = 0.02; ***, P = 0.01, unpaired Student's *t* test. Data in A and B are representative of at least two independent experiments.

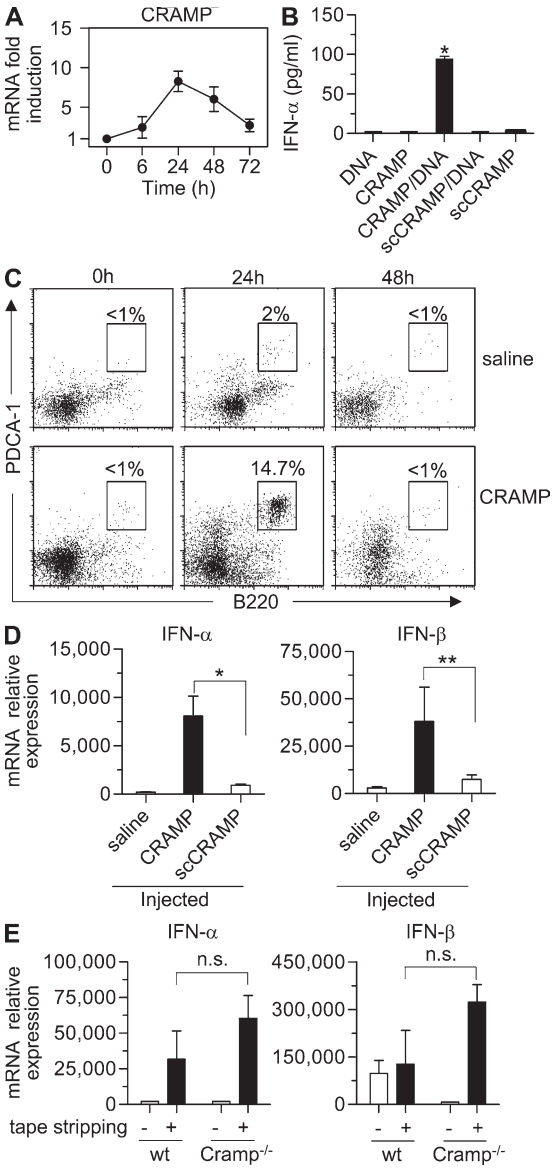

Figure 4. Cathelicidin peptides are sufficient but not necessary to induce pDC activation to produce type I IFNs in injured skin. (A) Time course analysis of cathelicidin mRNA tissue expression in injured skin. The data are given as fold induction over time 0 and represent the mean ± SEM of four mice per time point. (B) IFN-α produced by purified splenic pDC after overnight stimulation with DNA alone, CRAMP alone, scrambled (sc) CRAMP alone, CRAMP plus DNA, or scCRAMP plus DNA. Data are representative of two independent experiments. Error bars represent the SEM of triplicate wells. *, P = 0.001. (C) Flow cytometry time course analysis of pDCs (B220^{+}PDCA-1^{+}) in dermal single cell suspensions derived from skin injected with either saline or CRAMP. The percentage of each population is shown in the plots. (D) Relative IFN-α and IFN-β mRNA tissue expression in the skin injected with either saline, CRAMP, or scCRAMP and collected after 24 h. Data represent the mean ± SEM of five mice per group. *, P = 0.02; **, P = 0.1, unpaired Student's *t* test. (E) Relative IFN-α and IFN-β mRNA tissue expression in uninjured skin or injured skin collected 24 h after tape stripping of Cramp$^{-/-}$ or control wild-type mice. Data represent the mean ± SEM of five mice per group. n.s., not significant, unpaired Student's *t* test. Data in A and C–E are representative of at least two independent experiments.

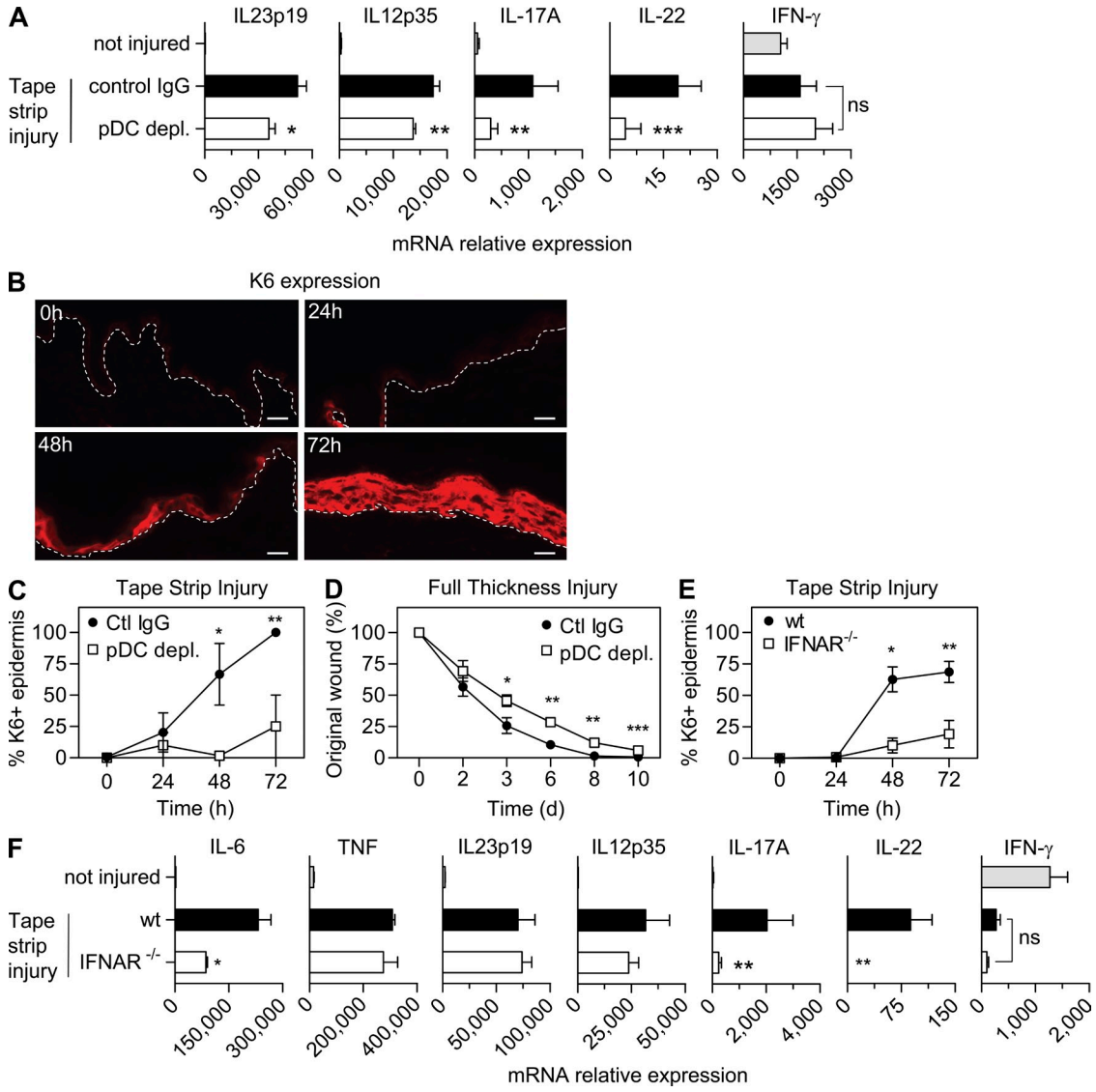

Figure 5. pDC and type I IFNs participate in the inflammatory response and reepithelialization of skin wound healing. (A) Relative IL-23p19, IL-12p35, IL-17A, IL-22, and IFN-γ mRNA tissue expression of uninjured skin or injured skin collected 24 h after tape stripping of either pDC-depleted or control IgG-treated mice. Data represent the mean ± SEM of five mice per group. *, P = 0.03; **, P = 0.05; ***, P = 0.04, unpaired Student's *t* test. (B) Representative time course of Keratin 6 (K6) expression in injured skin measured by immunofluorescence. Bar, 10 µm. (C) Percentage of K6 expression in injured skin in pDC-depleted and control IgG treated mice. Data represent the mean ± SEM of five mice per group. *, P = 0.01; **, P = 0.05, unpaired Student's *t* test. (D) Time course of wound closure after full-thickness injury of the skin pDC-depleted or IgG-treated mice (right). Data represent the mean ± SEM of at least three mice per group. *, P = 0.02; **, P < 0.001; ***, P = 0.005, unpaired Student's *t* test. (E) Percentage of K6 expression in injured skin in IFNAR$^{-/-}$ or control mice. Data represent the mean ± SEM of three mice per group for each time point. *, P = 0.002; **, P < 0.001, unpaired Student's *t* test. (F) Relative TNF, IL-6, IL-23p19, IL-12p35, IL-17A, IL-22, and IFN-γ mRNA tissue expression of uninjured skin or injured skin collected 24 h after tape stripping of IFNAR$^{-/-}$ or control mice. Data represent the mean ± SEM of five mice per group: *, P = 0.003; **, P = 0.04, unpaired Student's *t* test. Data in A–F are representative of at least two independent experiments.

these findings indicate that pDCs recognize host-derived self-nucleic acids, most likely released by keratinocytes or other cells damaged in the context of the skin injury.

Cathelicidins are sufficient but not necessary to trigger pDC activation in skin

Host-derived nucleic acids released by damaged cells are normally inert but can be converted into triggers of TLR7 and TLR9 in the presence of cathelicidin peptides (Lande et al., 2007; Ganguly et al., 2009). We therefore sought to determine whether cathelicidins are induced in our skin injury model. The expression of cathelicidin mRNA in murine skin was undetectable before injury but was found to be rapidly induced upon tape stripping, reaching a peak at 24 h and declining thereafter (Fig. 4 A). This time course closely paralleled the infiltration of pDCs into injured skin and their

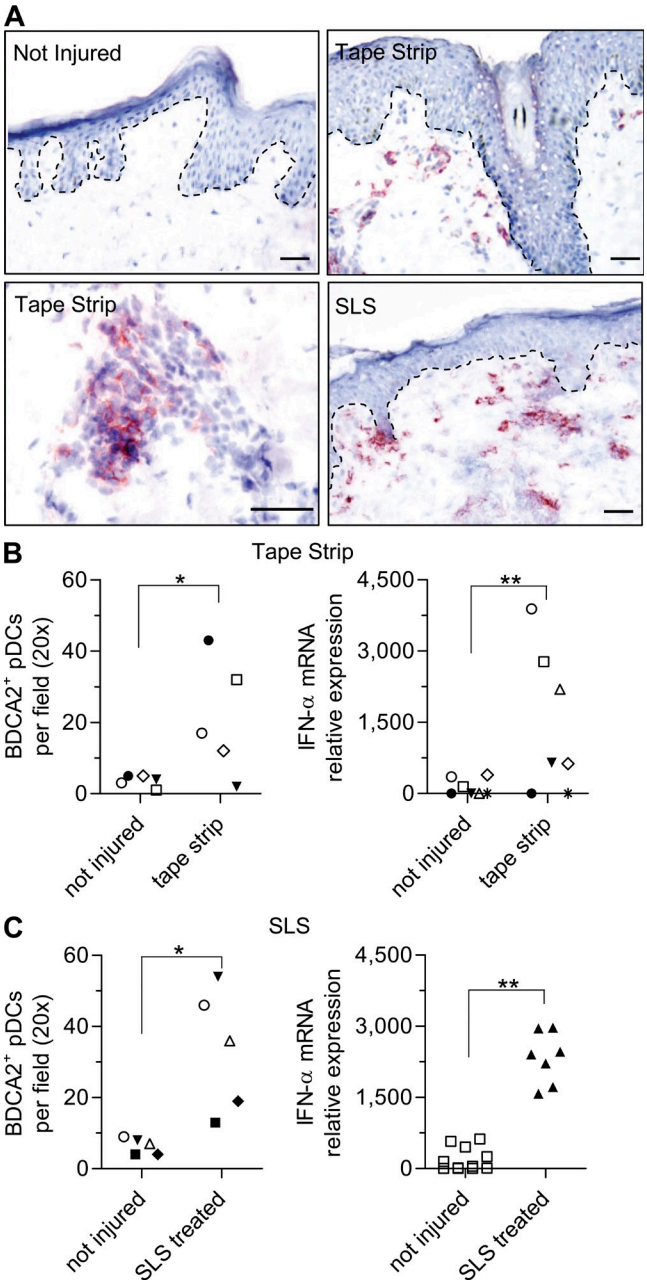

Figure 6. Human skin injury induces rapid infiltration of pDC and their activation to produce IFN-α. (A) Representative immunohistochemical staining of human skin for the pDC-specific marker BDCA2 reveals absence of pDCs in normal skin before injury (top left), the rapid infiltration of pDC in injured skin by tape stripping (top right and bottom left), or by treatment with SLS (bottom right). Bar, 10 µm. The data are representative of at least five independent healthy individuals (B) Quantification of BDCA2-positive pDC numbers in human skin before and 24 h after tape stripping of five independent healthy individuals (left), and relative IFN-α mRNA tissue expression in healthy human skin before and 24 h after tape stripping (right). Each symbol represents an independent donor. *, P = 0.05; **, P = 0.03, unpaired Student's *t* test. (C) Quantification of BDCA2-positive pDC numbers in human skin before and 24 h after chemical injury with SLS of five independent healthy individuals (left). Relative IFN-α mRNA tissue expression in human skin before and 24 h after chemical injury with SLS of at least seven independent healthy individuals (right). Each symbol represents the independent individual. *, P = 0.04; **, P < 0.001, unpaired Student's *t* test.

activation to produce IFN-α/β, suggesting a potential role of cathelicidins in breaking innate tolerance to self-nucleic acids injured skin. To investigate this possibility, we first stimulated purified pDCs with DNA alone, the mouse cathelicidin peptide (called CRAMP) alone, or DNA mixed with CRAMP. As a control, we also used a scrambled form of CRAMP alone or mixed with DNA. We found that only DNA mixed with CRAMP induced IFN-α production in pDCs (Fig. 4 B). To test whether CRAMP would also break innate tolerance to nucleic acids and activate pDCs in vivo, we injected CRAMP, the scrambled peptide, or saline into mouse skin. We found that CRAMP, but not the scrambled peptide or saline injection, induced a rapid and transient infiltration of pDCs and the expression of IFN-α/β (Fig. 4, C and D). These findings indicate that CRAMP is sufficient to break innate tolerance to induce activation of pDCs to produce IFN-α/β in the skin in vivo. To determine whether CRAMP is necessary for pDC activation to injured skin, we tape stripped the skin of cathelicidin-deficient mice (Nizet et al., 2001) and analyzed gene expression for IFN-α/β. Surprisingly, we found that IFN-α and IFN-β produced by pDCs were still induced in injured skin even in the absence of cathelicidins (Fig. 4 E). Thus, cathelicidins are sufficient but not necessary to break innate tolerance to nucleic acids and induce IFN-α/β production by pDCs in injured skin.

pDCs and type I IFNs promote inflammatory responses and wound healing in injured skin

Because pDCs are potent stimulators of immune responses through their production of IFN-α/β, we next sought to investigate the role of pDCs in the induction of inflammatory responses in injured skin. We found that, along with IL-6 and TNF (Fig. 2 A), skin injury induced a rapid expression of the DC-derived cytokines IL-12 and IL-23, Th1 cytokine IFN-γ, and Th17 cytokines IL-17 and IL-22 (Fig. 5 A), but not IL-4 or IL-10 (not depicted), reaching a peak between 24 and 48 h after injury. pDC depletion, which reduced the expression of IL-6 in injured skin (Fig. 2 A), was also found to decrease the expression of IL-12 and IL-23 (Fig. 5 A). Intriguingly, pDC depletion was found to profoundly inhibit the induction of IL-17 and IL-22 without affecting the expression of IFN-γ (Fig. 5 A). These findings demonstrate that skin-infiltrating pDCs play and important role in the induction of inflammatory immune responses in injured skin, in particular the induction of IL-6 and Th17 cytokines.

Because the inflammatory process is directly linked to the wound-healing response, we next sought to determine whether pDCs also play a role in the reepithelization of tape-stripped skin. Keratin 6 (K6), expressed by early differentiating and proliferating keratinocytes but not by fully differentiated keratinocytes, was used as a marker to quantify reepithelization

of skin wounds (Wojcik et al., 2000). In normal mice, K6-positive keratinocytes appeared 24 h after injury and repopulated the entire injured skin surface within 72 h (Fig. 5 B). In pDC-depleted mice, K6-positive keratinocytes appeared only around 72 h (Fig. 5 C) and complete reepithelization lagged behind control mice by 3 d (not depicted). These data indicate that the infiltration of pDCs in skin wounds plays a key role in promoting early wound reepithelization. To confirm these data, we adopted another skin injury model in which a full-thickness skin defect is placed by a 3-mm punch biopsy and the wound closure is measured over time. Similarly, pDC-depleted mice showed a significant delay in wound closure when compared with control mice (Fig. 5 D). To determine the role of pDC-derived IFN-α/β in this process, we used IFN-α/β receptor-deficient mice and repeated similar experiments. Like pDC-depleted mice, IFN-α/β receptor-deficient mice showed a significant delay in wound reepithelization (Fig. 5 E) and displayed a profound deficiency in IL-6, IL-17, and IL-22 expression levels in injured skin, without affecting the expression of IFN-γ (Fig. 5 F). A similar deficiency of IL-6, IL-17, and IL-22 expression was observed in MyD88-deficient mice (Fig. S5). These data suggest that the ability of pDCs to trigger early inflammatory responses and promote wound repair is related to their nucleic acid–mediated TLR activation and production of IFN-α/β.

Injury of human skin induces pDC infiltration and expression of type I IFNs

To determine whether pDCs also infiltrate and sense human skin wounds, we performed skin biopsies in healthy human volunteers before and 24 h after tape stripping. Immunohistochemistry for BDCA2, a specific marker for human pDCs, revealed that large numbers of pDCs infiltrate human skin 24 h after mechanical injury (Fig. 6, A and B). We also found a significant induction of IFN-α mRNA expression in injured skin (Fig. 6 B), suggesting that pDCs are activated to produce IFN-α similar to our finding in the murine models. We also confirmed these data in another model of skin injury induced by sodium lauryl sulfate (SLS) treatment, which induces a chemical disruption of the epidermal barrier by perturbing the lipid bilayer structure in the stratum corneum, leading to damages to the basal keratinocytes (Welzel et al., 1998). Like mechanical-induced skin injury, this chemical-induced skin injury–induced rapid pDC infiltration and significant induction of IFN-α mRNA expression in injured skin (Fig. 6, A and C).

DISCUSSION

Our study identifies a new physiological function of pDCs. We show that pDCs, which are normally absent from healthy skin, rapidly infiltrate common skin wounds with a surprisingly rapid kinetics, paralleling the infiltration of neutrophils. We also find that these pDCs become activated to produce IFN-α/β through endosomal TLR7 and TLR9, suggesting that they recognize nucleic acids released in the skin wounds. Finally, we demonstrate that these pDCs and their activation to produce IFN-α/β play an important role in inducing early inflammatory responses and reepithelization of skin wounds. Although pDCs have been classically considered a specialized immune cell type in sensing viral infection and initiating antiviral immunity, our study now identifies an additional important physiological function of these cells in sensing tissue damage and initiating tissue repair at epithelial surfaces.

The ability of pDCs to sense host-derived nucleic acids has been recently uncovered in psoriasis (Lande et al., 2007; Ganguly et al., 2009). Keratinocytes of psoriatic skin lesions are continuously activated to produce cathelicidin peptides, a family of cationic antimicrobial peptides with the ability to break innate tolerance to extracellular nucleic acids released by dying cells. This occurs by forming complexes with the released self-DNA and self-RNA and by promoting their transport into intracellular compartments containing TLR7 and TLR9, leading to chronic pDC activation and IFN-α/β production (Lande et al., 2007; Ganguly et al., 2009). In this study, we show that in contrast to psoriatic skin, the expression of cathelicidin peptides in injured skin is short lived and associated with the transient IFN-α/β expression. We also show that injection of cathelicidin peptides into healthy skin is sufficient to trigger pDC infiltration and activation, suggesting a potential role for these peptides in pDC activation in the skin. However, we demonstrate that cathelicidin peptides produced in injured skin are not required for pDC activation, suggesting redundancy of this pathway and the presence of additional factors that control the immunogenicity of extracellular self-nucleic acids. It is unlikely that this process is driven by autoantibodies and the formation of immune complexes, as we did not find an increase in anti-nuclear antibodies after skin injury (unpublished data). Potential factors that drive this process are HMGB1 (Tian et al., 2007), heat shock proteins (Okuya et al., 2010), and other cationic antimicrobial peptides (unpublished data), as they are all expressed in damaged skin and they have the ability to form self-nucleic acid–containing complexes that activate pDCs.

pDC migration to the skin has been attributed to the effect of chemerin, an agonist for CMKLR1 (chemokine-like receptor 1) which is specifically expressed by pDCs (Vermi et al., 2003; Zabel et al., 2005; Albanesi et al., 2009). Chemerin is constitutively expressed in healthy skin by endothelial cells and fibroblasts as an inactive propeptide that requires activation through C-terminal cleavage by serine proteases. It is possible that during skin injury the release of proteases by damaged keratinocytes allows the formation of the active chemerin that recruits pDCs to the injury site. In addition, skin injury induces the expression of CXCR3 ligands (unpublished data), a set of chemokines which are typically induced in structural cells of the skin as a result of IFN-α/β expression and which have been shown to promote recruitment pDCs into sites of their activation (Vanbervliet et al., 2003).

We demonstrate that skin-infiltrating pDCs are important for the induction of early inflammatory responses that promote reepithelization of skin wounds. pDCs significantly contribute to the expression of IL-6, an inflammatory cytokine which indirectly stimulates reepithelization of skin

wounds (Gallucci et al., 2004). pDCs also affect the production of Th17 cytokines, as the induction of IL-17 and IL-22 is abrogated in pDC-depleted mice. This is consistent with recent studies showing that pDCs can drive the differentiation of IL-17– and IL-22–producing T cells (Duhen et al., 2009; Isaksson et al., 2009; Yu et al., 2010) and that IL-6 is implicated in this process (Duhen et al., 2009). IL-22 appears to be particularly important in epidermal regeneration, as this cytokine directly promotes keratinocyte migration and proliferation (Boniface et al., 2005; Zheng et al., 2007; Eyerich et al., 2009). Surprisingly, mice deficient in IFN-α/β receptors display a similar inhibition in IL-6 and Th17 cytokine induction with delayed reepithelization of skin wounds. Because IFN-α and IFN-β are potent stimulators of immune responses but do not exert a direct activity on keratinocytes (van der Fits et al., 2004), these findings suggest that IFN-α/β in skin wounds promotes epidermal regeneration and wound repair through the induction of Th17-biased inflammatory responses. The exact mechanism that links IFN-α/β production to pDC-mediated Th17 responses is still unclear. However, there is additional evidence that IFN-α/β produced by pDCs drives Th17 responses and epidermal proliferation in a therapeutic model of skin treated with the TLR7 agonist imiquimod (van der Fits et al., 2009). Furthermore, IFN-α/β produced by pDCs triggers psoriasis (Nestle et al., 2005), a disease characterized by large numbers of pathogenic Th17 cells which trigger epidermal hyperproliferation (Zaba et al., 2007).

The new pathway for the induction of inflammation and wound healing described in this paper appears to complement the recent finding that skin injury triggers keratinocyte activation via TLR3 (Lai et al., 2009), a TLR which recognizes double-stranded (ds) RNA and signals independently of MyD88. A likely scenario is that skin injury induces TLR3 activation of keratinocytes with the production of factors that control the recruitment of pDCs and the ability of pDCs to sense nucleic acids. Future studies will have to test this possibility and further elucidate the interplays between keratinocytes and pDCs in the wound-healing response.

In conclusion, our study identifies a role of pDCs in recognizing nucleic acids released in injured skin and promoting early inflammatory responses and reepithelization of the wounds. These findings provide a paradigm shift in understanding the function of pDCs from the classical view of a specialized cell type in the recognition of viral infections to important sensors of tissue damage at epithelial surfaces.

MATERIALS AND METHODS

Mice. All animal experiments were approved by the Institutional Animal Care and Use Committee of the University of Texas M.D. Anderson Cancer Center. Wild-type BALB/cJ, C57BL/6, or 129SV/J mice and TLR7$^{-/-}$ (B6.129S1-Tlr7tm1Flv/J) mice were purchased from The Jackson Laboratory. MyD88$^{-/-}$ mice were provided by S. Akira (Osaka University, Osaka, Japan; Adachi et al., 1998) and IFNAR$^{-/-}$ mice (Müller et al., 1994) were provided by W. Overwijk (University of Texas M.D. Anderson Cancer Center, Houston, TX). Cramp$^{-/-}$ mice were from the laboratory of R.L. Gallo (Nizet et al., 2001). All animal experiments were conducted on 6–14-wk-old mice. Animals were maintained and bred in pathogen-free facilities.

Reagents. The synthetic mouse cathelicidin peptide CRAMP (GLLRKGGEKIGEKLKKIGQKIKNFFQKLVPQPEQ) and the corresponding scrambled control (LLGQNGKFKIREPPIQKVKGIQEKEFGLKQKLKG) were obtained from AnaSpec. For in vivo experiments, 200 μg of the peptides were injected into the dermis of the upper dorsum of shaven and depilated mice. For in vitro pDC stimulation, 30 μM of the peptides were used. The TLR 7/9 inhibitor IRS 954 was a gift from F. Barrat (Dynavax Technologies, Berkely, CA). The TLR9 inhibitor IRS 869 (5′-TCCTGGAGGGGTTGT-3′, phosphorothioate oligodeoxynucleotide) was purchased from Integrated DNA Technologies. For inhibition of TLR9 or TLR7 plus TLR9 in vivo, intradermal administration of IRS 869 or IRS 954 was injected 24 and 4 h before the skin injury.

Mechanical injury of mouse skin. Mice backs were shaven and depilated (Veet; Reckitt Benckiser) immediately before injury. Mechanical injury was then applied by tape stripping, using 20 strokes of transparent tape (3M; Scotch) across the back. For full-thickness injury, mice dorsal skin was shaven and cleaned with 70% ethanol, and a 3-mm punch biopsy (Acuderm) was applied to remove skin (care was taken to ensure excision was restricted to a depth of the fascia layer). Calipers were used to monitor wound closure over a 14-d period.

Depletion experiments. PDC depletion was performed using a combination of two anti-BST antibodies (PDCA1; clone JF05-1C2.4.1; Miltenyi Biotec) and mAb 927 (provided by M. Colonna, Washington University School of Medicine, St. Louis, MO; Blasius et al., 2006). 0.5 mg of each antibody was injected, intraperitoneally, 48 and 24 h before injury. Rat IgG antibodies were injected into control mice. Depletion of pDCs was monitored in the spleen by flow cytometry and was found to be most efficient when the combination of the antibodies was used. Neutrophil depletion was conducted using an anti-Ly6G–specific antibody (clone 1A8; BioXell). 1 mg anti-Ly6G or whole rat IgG was injected, intraperitoneally, into mice at 24 and 4 h before injury.

Mechanical and chemical injury of human skin. Mechanical injury of human skin was induced by application of 10 strokes of cellophane tape across healthy skin of seven human volunteers. Biopsies were taken before (uninjured) and 24 h after (injured) tape stripping. The specimen was snap frozen and stored at −80°C before immunohistochemical and gene expression analysis was performed.

Chemical injury of human skin was induced by treatment of healthy skin of human volunteers with the chemical irritant SLS (Merck) at 1% in water as described previously (Meller et al., 2007). In brief, SLS was applied in large Finn Chambers to the skin on the back of the patients before biopsies were taken. Specimens were immediately snap frozen and stored at −80°C before immunohistochemical and gene expression analysis was performed. All human studies were performed at the Skin and Allergy Hospital at Helsinki University Central Hospital (Helsinki, Finland) and approved by the local ethics committee (Helsinki-Uusimaa Hospital District Ethics Committee).

Generation and analysis of dermal single cell suspensions. Injured skin was excised, minced, and digested with 1 mg/ml Dispase (Sigma-Aldrich) for 1 h at 37°C and the epidermis was manually removed with forceps. The dermis was removed to a clean culture plate and incubated with 1 mg/ml collagenase (Invitrogen) for 2 h to generate a single cell suspension. Leukocytes were counted using trypan blue exclusion. Cells were treated for 20 min with anti-CD16/CD32 to block nonspecific binding, followed by the addition of the following antibodies at 10 μg/ml final concentration for 20 min: anti–PDCA-1 FITC (Miltenyi Biotec), anti–Siglec-H-FITC (eBioscience), anti–CD11c-PE, anti–B220-APC, anti–IA/IE-PE, anti–CD11b-FITC, anti–Gr-1-PerCp-Cy5.5, and anti–CD3e-APC (all BD). Cells were washed twice and acquired on a FACSCalibur (BD) and analyzed using FlowJo software (Tree Star, Inc.).

Immunohistochemistry and immunofluorescence. Mouse skin tissue was excised, embedded in OCT (Tissue-Tek), and frozen immediately on

dry ice. 8-μM frozen sections were cut and stained with anti-Siglec H (3H3 clone; Kamogawa and Arai, personal communication) followed by horseradish peroxidase–labeled goat anti–rat IgG and a color development step with aminoethylcarbazole. For K6 staining, frozen sections were stained with a purified rabbit anti–mouse keratin 6 antibody (Covance) and subsequently stained with Alexa Fluor 546–labeled goat anti rabbit IgG (H+L; Invitrogen). For detection of pDCs in human skin tissue we used an anti-BDCA2 antibody (Miltenyi Biotec) according to the previously described protocol (Lande et al., 2007).

Real-time PCR analysis. All excised tissue was immediately saturated in RNAlater (Applied Biosystems) and stored at −20°C until RNA was isolated. RNA was isolated using a tissue homogenizer (Thermo Fisher Scientific) along with a commercial kit (RiboPure; Applied Biosystems), followed by RNA clean up with an additional kit (RNAqueous; Applied Biosystems). All isolated RNA had an A260/A280 value of ≥1.7. 2 μg RNA was used to generate cDNA using a commercial kit (High Capacity cDNA; Applied Biosystems). 40 ng cDNA was used for each individual gene expression analysis using Taqman-based amplification on an ABI 7500 Fast system using the default Standard protocol. Mouse Taqman probes used were: Gapdh, Ifna2, Ifnb, Cramp, Il6, Tnf, Il23p19, Il12p35, Il12p40, Il22, Il17a, and Ifng. Human Taqman probes used were Gapdh and Ifna2.

pDC isolation and in vitro stimulation. pDCs were isolated from spleens of 6–12-wk-old female BALB/cJ mice using the pDC Isolation kit II (Miltenyi Biotec) with a purity of >90%. 50,000 cells were cultured overnight in RPMI-based media (10% FCS, 50 μM β-mercaptoethanol, pen/strep, glutamine, sodium pyruvate, and Hepes). The supernatants were used to determine IFN-α protein secretion using a commercial Elisa kit (PBL). As a source of DNA, we used synthetic phosphodiesteric CpG-containing oligonucleotides hybridized to its complementary strand to mimick natural mammalian DNA fragments. 0.3 μM dsDNA was mixed with 30 μM cathelicidin peptides in a volume of 20 μl for 30 min at room temperature.

Online supplemental material. Fig. S1 shows the kinetics of mDCs in the skin after skin injury. Fig. S2 shows the efficiency of pDC depletion in the spleen over a 5-d period using BST-specific monoclonal antibodies. Fig. S3 shows the role of TLR7 and TLR9 in the expression of type I IFN, IL-6, and TNF induced by skin injury. Fig. S4 shows the role of MyD88 and TLR7/9 in the induction of IL-6 and TNF in injured skin. Fig. S5 shows the role of MyD88 in the induction of T cell–derived IL-17A, IL-22, and IFN-γ in injured skin. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20101102/DC1.

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