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Secretory Leukocyte Proteinase Inhibitor-Competent DNA Deposits Are Potent Stimulators of Plasmacytoid Dendritic Cells: Implication for Psoriasis

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Secretory leukocyte proteinase inhibitor (SLPI) is a well-established inhibitor of serine proteinases such as human neutrophil elastase (HNE) and a NF-κB regulatory agent in immune cells. In this paper, we report that SLPI plays a previously uncharacterized role in regulating activation of plasmacytoid dendritic cells (pDCs). As the main source of IFN type I (IFNI), pDCs are crucial contributors to inflammatory and likely wound-healing responses associated with psoriasis. The mechanisms responsible for activation of pDCs in psoriatic skin are therefore of substantial interest. We demonstrate that in lesional skin of psoriasis patients, SLPI together with its enzymatic target HNE and DNA, is a component of neutrophil extracellular traps (NETs). Whereas SLPI+ neutrophils and NETs were found to colocalize with pDCs in psoriatic skin, a mixture of SLPI with neutrophil DNA and HNE induced a marked production of IFNI by pDCs. IFNI synthesis by stimulated pDCs was dependent on intracellular DNA receptor TLR9. Thus, SLPI may contribute to psoriasis by enabling pDCs to sense extracellular DNA and produce IFNI. The Journal of Immunology, 2012, 189: 1611–1617.

Secretory leukocyte proteinase inhibitor (SLPI), a cationic protein of ~12 kDa, was first described as a potent inhibitor of serine proteinases, including human neutrophil elastase (HNE) and cathepsin G (CatG) (1). SLPI is expressed by several cell types including epithelia, macrophages, and neutrophils (2–5). As one of the major agents controlling serine protease activity, SLPI is thought to play an important role in limiting protease-mediated tissue injury associated with inflammation, especially at mucosal/epithelial surfaces, as well as in wound healing (6, 7). However, its protective anti-inflammatory role is not restricted to antiprotease defense, because SLPI also exhibits antimicrobial properties and immunomodulatory activity (1). The latter is based on the ability of SLPI to interfere with the activation of transcription factor NF-κB (8, 9). Engagement of the NF-κB signaling pathway culminates in the expression of proinflammatory genes such as IL8 and TNF-α in LPS-activated monocyctic cells and class switch recombination in activated B cells. Both of these NF-κB–mediated effects are inhibited by SLPI (8, 9).

In contrast to macrophages and B cells, the role of SLPI in neutrophils remains unknown, although a recent report suggests that SLPI inhibits apoptosis of these cells (10). Neutrophils produce several inhibitors of serine proteinases, including α1-proteinase inhibitor (α1-PI), human elastase inhibitor, and minor amounts of elafin (4, 11). Nevertheless, SLPI is the major inhibitor of HNE in neutrophils (4), suggesting an important contribution by SLPI to HNE-mediated processes in these cells.

Neutrophils (also referred to as polymorphonuclear leukocytes [PMNs]) play a key role in host defense against pathogens. PMNs engulf bacteria and fuse the phagocytic compartment with cytoplasmic granules containing bactericidal compounds. Three types of cytoplasmic granules have been described in neutrophils: primary HNE-containing, secondary lactoferrin-containing, and tertiary gelatinase-containing granules (12). Activated neutrophils can also eliminate microbes extracellularly by the formation of neutrophil extracellular traps (NETs), for example. NETs, web-like structures composed of neutrophil DNA and antibacterial proteins, are thought to form via a unique cell death program called NETosis (13). Although the mechanism underlying NETosis remains largely elusive, HNE is required for chromatin decondensation, via specific histone cleavage (14). This process leads to cell rupture and release of NETs. The major structural components of NETs are DNA and HNE, although several other granular proteins have also been identified in the superstructure (15). We hypothesized that SLPI, as a controlling inhibitor of HNE, is a NET component as well.

Interestingly, in autoimmune and autoimmune-like diseases such as systemic lupus erythematosus (SLE), small-vessel vasculitis, and psoriasis, neutrophil accumulation is often common in affected...
sites in the absence of bacterial infection. Neutrophil migration into psoriatic skin, first observed as perivascular accumulation, is followed by an influx into the epidermis where it often manifests as microscopically detectable microabscesses (16). Although the formation of NETs has been mainly associated with antimicrobial function, recent reports suggest a role in triggering autoimmune small-vessel disease and SLE (17–20).

Psoriasis and SLE share strong dependence on chronic activation of plasmacytoid dendritic cells (pDCs) (17, 20–22). pDCs, a unique cell type of the DC lineage, specialize in sensing nucleic acid “danger signals” primarily via expression of TLR7 and TLR9, which recognize ssRNA and DNA, respectively (23). Expression of these receptors allows pDCs to uniquely respond to viral nucleic acids by production of type I IFNs, including IFN-α (24), abbreviated hereafter as IFN. Although IFN is critical in inhibiting viral replication, activating antiviral/antimicrobial functions of many cells, and promoting wound healing, IFN also plays a key role in inducing autoimmunity, including psoriasis (22). pDCs normally do not respond to host DNA. However, recent studies indicate that in addition to viral/microbial DNA, self-DNA also can initiate production of IFN in pDCs, if delivered to and retained within specific endocytic compartments to trigger TLR9 (21). In psoriasis, the delivery of self-DNA to specific endosomes is reported to require coupling of the DNA to an antimicrobial peptide of the cathelicidin family of proteins, LL37 (21). LL37 is mainly produced by epidermal keratinocytes (21). Another possible source of LL37 is neutrophils (25, 26). Keratinocyte-derived LL37 is unlikely to be solely responsible for delivery of host DNA to TLR9 in pDCs, because it is mainly detected in the epidermis, whereas pDCs primarily infiltrate the dermis.

In this work, we demonstrate that induction of IFN synthesis by skin-infiltrating pDCs may depend on SLPI, because this inhibitor is found within NETs in psoriatic skin, and together with the well-known NET constituents DNA and HNE, strongly stimulates production of IFN in pDCs. Thus, our data define a novel role for SLPI as a factor that controls the immunogenicity of extracellular DNA in damaged skin.

Materials and Methods

Materials

Recombinant SLPI was purchased from R&D Systems. Synthetic LL37 was from Emory Microchemical Facility. HNE was isolated using fresh whole human blood from healthy donors as a starting material, as described previously (27). Pig pancreatic trypsin was active-site–titrated with p-nitrophenyl guanidobenzoate (28) and used to standardize the solution of human α1-PI. Final inhibitor preparation was more than 95% pure and 80% active. This inhibitor was then used as a secondary standard to determine the activity of HNE, which was measured in 0.1 M Tris-HCl and 0.5 M NaCl (pH 7.4), using 0.5 mM MeO-Suc-Ala-Ala-Pro-Val-pNA as a substrate. Final preparation of HNE was more than 95% pure and 90% active. Azurocidin obtained during purification of HNE by ion exchange in a protein peak eluted before HNE at 0.3 M NaCl was further purified by FPLC using Pharmacia Mono S 5/5 column. Protein content in HNE and azurocidin preparation was estimated using $\varepsilon_{280}$ (1%) 9.85 and 5.0, respectively. Where indicated, serine protease inhibitor 3,4-dichloroisocoumarin was used for irreversible inhibition of HNE. After intensive dialysis such preparation of HNE with undetectable enzymatic activity was used in experiments. Human plasma α1-PI was isolated as described previously (29). SLPI was oxidized with 500 molar excess of NCS for 1 h in room temperature at pH 8, as described previously (29, 30). The oxidation was stopped by an addition of N-acetyltyrosine to final concentration of 50 mM. Oxidized SLPI was then dialyzed against 0.15 M NaCl and tested for anti-HNE activity as described above. The oxidation of SLPI resulted in 70% inactivation of its anti-NE activity, an expected value for the reversible inhibitor (30, 31).

Human genomic DNA was isolated from peripheral blood neutrophils of healthy donors using a method based on guanidine thiocyanate. Guanidine

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Patients

All human studies were performed in accordance with guidelines established by the Jagiellonian University Institutional Bioethics Committee under approved protocols. Declaration of Helsinki protocols were followed. A total of 13 psoriasis patients (age, 34.4 ± 14 y; female/male, 6:9) and 16 healthy individuals (age, 28.6 ± 7.3 y; female/male, 9:7) were enrolled into these studies. The severity of the psoriatic skin lesions was assessed according to the Psoriasis Area Severity Index score (minimum point 0, maximum point 72) and ranged from 19.8 to 42.2 (mean ≥ SD, 32 ± 8.1). Patients on UV therapy or systemic or local corticosteroid treatment were excluded from the studies. Healthy control subjects had no clinical signs of dermatologic diseases.

Isolation of pDCs and neutrophils

Human blood was collected and PBMC and granulocyte-enriched fractions were harvested following LPS/077 (PAA Laboratories) gradient separation, as described by the manufacturer. pDCs were enriched from PBMC using negative selection with biotinylated mAbs directed against CD3, CD14, CD16, CD19, CD20, and CD56, and anti-biotin MACs Microbeads (Miltenyi Biotec), according to the manufacturer’s recommendations. Cells were separated by magnetic sorting using an Automacs. The pDCs purity was assessed as determined by CD123 and BDCA-2 immunoreactivity and flow cytometry analysis. In selected experiments, pDCs were further purified by cell sorting based on the expression of the specific BDCA-4 Ag. The pDCs purity was then ≥98%. However, because this method of pDC purification resulted in similar data, in most experiments, we used pDCs obtained by a negative selection.

Neutrophils were further purified from granulocyte-enriched fractions. The high-density fraction, containing neutrophils and erythrocytes, were mixed with a 1% solution of polyvinyl alcohol (Merck) in PBS and incubated for 20 min at room temperature. Neutrophils were harvested from the upper phase and subjected to hypotonic lysis to remove contaminating RBCs. The purity of the isolated cells was examined by flow cytometry based on forward light scatter and side scatter (of light) parameters and/or HNE immunoreactivity. Granulocytes were routinely ≥98% pure.

pDC treatment

Purified 5 $\times$ 10⁵ pDCs were seeded into round-bottom 96-well plates in 50–100 μl RPMI 1640 medium. The factors were either added directly into pDC cultures or first premixed with DNA or with each other in 10–20 μl RPMI 1640 medium. After a 15-min incubation at room temperature, the mix was diluted 10 or 5 times and added to the pDC cultures. The following factors at indicated final concentration were used: HNE, inactive HNE, azurocidin, SLPI, NCS-oxidized SLPI, α1-PI (all at 1 μM), and LL37 at 10 μM and/or human neutrophil-derived DNA at 2 μg/ml. The anti-HNE activity of SLPI was alternatively neutralized by 15-min incubation with 10 molar excess of goat anti-human SLPI Abs (R&D Systems) or goat IgG (Jackson ImmunoResearch Laboratories) as a control. The Abs treatment resulted in ~30–50% reduction of HNE inhibitory activity. SLPI plus Abs was then incubated with DNA and HNE, followed by stimulation of pDCs. The final concentration of the factors was 0.1–0.2 μM (SLPI and HNE) and 0.2–0.4 μg/ml DNA. The involvement of TLR9 in pDCs activation was studied by treatment of pDCs with the inhibitory ODNTTAGGG or control ODN (Invitrogen) both given at ~4:1 ratio (ODN: HNE/SLPI/DNA) 30 min before addition of the premixed stimuli to the pDC cultures. The pDCs were then cultured at 37°C, 5% CO₂ for 24 h in RPMI 1640 medium supplemented with 10% of FBS and human recombinant IL-3 (50 ng/ml) (Peprotech), which improves pDC survival in vitro. Conditioned media were collected, centrifuged at 500 x g for 5 min to remove the cells, and tested by ELISA.

ELISA

IFN levels were quantified by IFN-α–specific ELISA (PBL Interferon-Source) according to the manufacturer’s recommendations.

Immunohistochemistry

Frozen 6-μm sections were prepared from skin biopsies. Sections were fixed in acetone, blocked with mouse IgG, and stained with the following Abs: biotin-mouse anti-human SLPI (Abcam), rabbit–anti-human HNE (Athens Research and Technology), rabbit–anti-human LL37 (Innovagen),...
or allophycocyanin–anti-BDCA2 (Miltenyi Biotec), followed by PE–
streptavidin (BD Pharmingen) and allophycocyanin–goat anti-rabbit IgG F(ab)2 (Jackson Immunoresearch Laboratories). The sections were counterstained with Hoechst 33342 (Invitrogen). Purified neutrophils were seeded on poly-l-lysine-coated coverslips (2–5 × 10^5 cells/coverslip) and incubated at 37°C, 5% CO2 for 30 min in serum-free RPMI 1640 medium. Cells were then stimulated with 20 nM PMA or 10% serum from patients suffering from acute psoriasis in RPMI 1640 medium for 3.5 h. Cells were washed with PBS, fixed with 3.7% formaldehyde for 10 min, and blocked overnight with 5% FBS, 1% BSA, 0.05% Tween, and 2 mM EDTA in PBS at 4°C. Cells were treated with 0.1% saponin in PBS for 30 min at room temperature and stained in the presence of 0.1% saponin as described above. Images were captured with a fully motorized fluorescence microscope (NIKON, Eclipse) and analyzed by NIS elements software (Nikon).

**Results**

**SLPI colocalizes with NET components in activated granulocytes**

We first asked whether SLPI is a NET component (e.g., whether SLPI colocalizes with DNA deposits). Circulating neutrophils were purified to homogeneity from healthy and psoriasis donors following stimulation with PMA, one of the strongest stimuli for NET formation. Upon treatment with PMA, ~30% of neutrophils form NETs (13). Resting neutrophils and neutrophils treated with PMA for 3.5 h were fixed and subjected to immunocytochemistry. In resting neutrophils, mainly granular staining of SLPI was observed (Fig. 1A). Following PMA activation, neutrophils from both healthy donors and psoriasis patients formed NETs composed of DNA and HNE (Fig. 1B; data not shown). Interestingly, PMA-activated neutrophils also displayed reactivity with anti-SLPI mAb. Although the pattern of SLPI distribution differed from that of DNA/HNE complexes, extracellular SLPI localized in close proximity to DNA/HNE and in some cases colocalized with emerging NETs (Fig. 1B). We did not observe a substantial difference in NET formation or in the decoration of NETs with SLPI when neutrophils from healthy or psoriasis donors were examined (data not shown).

The level of SLPI associated with NETs might depend on the mode of neutrophil activation. Neutrophils treated with sera from psoriasis patients displayed more SLPI associated with NETs compared with PMA-stimulated cells (Fig. 1C). In general, sera from normal individuals, in contrast to sera from psoriasis patients, did not cause NET release (only one of six normal versus five of six psoriatic sera caused NET release, and in each case NETs were decorated with SLPI). In some cases, SLPI colocalized with extrusions of nuclear material released by neutrophils following incubation with sera from normal donors (Supplemental Fig. 1). However, in these cases, staining for SLPI was never as intense as in neutrophils treated with psoriatic sera, suggesting that sera of psoriasis patients contains a specific factor or factors capable of triggering SLPI-competent NET release. Because the incubation of NETs formed by PMA-treated neutrophils with psoriatic sera produced no or at most weak SLPI immunoreactivity (data not shown), these data suggest that neutrophil-derived SLPI but not serum-originating SLPI is retained on NETs following stimulation of neutrophils with psoriatic sera.

Neutrophil infiltrates are a hallmark of psoriasis. Moreover, similar to SLE and small-vessel vasculitis, neutrophils were recently shown to form NETs in psoriatic skin (33). Therefore, we next investigated whether SLPI is a NET component in vivo by immunofluorescence analysis of psoriatic skin lesion sections. As demonstrated in Fig. 2A, in acute psoriasis, SLPI staining mostly colocalized with HNE^+ cells within inflammatory infiltrates. These cells were also positive for CatG (data not shown), indicating that SLPI-specific immunoreactivity is predominantly in neutrophils. In some preparations, keratinocytes stained positive for SLPI (Fig. 2A) in accordance with a previous reports (5). Notably, SLPI was also clearly present on HNE-positive extracellular DNA deposits (Fig. 2B), indicating that NETs that are formed in psoriatic skin are decorated with SLPI. Interestingly, although the distribution and intensity of SLPI and LL37 staining in neutrophils was comparable, SLPI colocalized with NETs more frequently than NETs localized with LL37 (Supplemental Fig. 2).

Taken together, these data suggest that NETs decorated by SLPI may contribute to the pathogenesis of psoriasis.

**pDCs colocalize with neutrophils and SLPI-decorated NETs in psoriatic skin**

pDCs are key effectors of the innate immune system important in the initiation and/or maintenance of psoriasis (22). Although pDCs and neutrophils are both known to infiltrate psoriatic skin, their location relative to one another in situ in psoriatic skin and thus their potential for cross-talk remain unknown. Therefore, we analyzed biopsies of neutrophil-infiltrated psoriatic skin for pDCs. As demonstrated in Fig. 2C, pDCs were found associated with SLPI^+ neutrophils in lesional skin, in some cases with obvious colocalization of NETs decorated with SLPI. SLPI^+ cells were also positive for HNE and/or CatG, confirming their identification as neutrophils (data not shown). Generally, neutrophils were more abundant than pDCs. Nearly half of all skin-infiltrating pDCs were located in close proximity to SLPI^+ neutrophils, whereas one of...
five pDCs were in direct contact with SLPI+ neutrophils (Table I; n = 5 independent donors). Thus, neutrophil-derived, SLPI-competent NETs are in many cases well-positioned to activate adjacent pDCs in psoriatic lesions.

Production of IFNγ by pDCs is stimulated by a complex of DNA with HNE and SLPI via TLR9

pDCs are considered key players in psoriasis via their production of IFNγ. To determine whether SLPI is capable of triggering pDCs to synthesize IFNγ, we used magnetic bead-selected blood pDCs from normal donors and treated the cells for 24 h with SLPI or complexes of the SLPI with HNE and DNA isolated from human blood neutrophils. As demonstrated in Fig. 3A, when tested independently, SLPI, HNE, and DNA did not effectively stimulate IFNγ production by human blood pDC. Furthermore, SLPI in complex with either HNE or DNA did not trigger pDC activation. However, the trio complex of SLPI, HNE, and DNA triggered a significant increase in IFNγ production by pDCs (∼64-fold increase over untreated pDCs). As low as 0.1 μM HNE and SLPI in combination with 2 μg DNA effectively stimulated pDCs, but the combination of HNE and SLPI was most efficient when both components were used at 1–3 μM together with 2 μg DNA (Fig. 3A; data not shown).

Interestingly, HNE/SLPI/DNA complexes were more potent compared with LL37/DNA tested under similar experimental conditions (Fig. 3A). This was manifested in the stronger response elicited by the HNE/SLPI/DNA mixture compared with LL37/DNA complexes used at optimal concentration (1 μM SLPI and HNE versus 10 μM LL37). Moreover, there was no obvious synergistic or additive effects between LL37 and HNE/SLPI in the stimulation of IFNγ production in complex with DNA (data not shown), suggesting that LL37 and SLPI may operate independently of each other.

We next asked whether particular structural and functional features of HNE and SLPI were required for pDC activation by replacing the components with functional or nonfunctional analogs. HNE was replaced with human neutrophil-derived azurocidin, which is structurally similar to HNE but lacks proteinase activity because of mutations in the catalytic triad (34). SLPI was replaced with α1-PI, an inhibitor of HNE also expressed by neutrophils (4, 35). To discriminate between the enzymatic and nonenzymatic

Table I. pDCs interact with SLPI+ neutrophils in psoriatic skin

<table>
<thead>
<tr>
<th>Patient</th>
<th>% of pDC in Close Proximity to SLPI+ Cells</th>
<th>% of pDC in Direct Contact with SLPI+ Cells</th>
<th>% of pDC in Close Proximity to SLPI+ Cells</th>
<th>% of pDC in Direct Contact with SLPI+ Cells</th>
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<tr>
<td>1</td>
<td>60</td>
<td>20</td>
<td>39.4</td>
<td>18.8</td>
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<tr>
<td>2</td>
<td>30.3</td>
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<td>3</td>
<td>47.8</td>
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<td>4</td>
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<td>5</td>
<td>45</td>
<td>25</td>
<td>Mean ± SD: 41.2 ± 14.7</td>
<td>23.1 ± 10.2</td>
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</table>

For each patient, 5–20 different high-power fields from one section were analyzed. The total number of BDCA-2–expressing cells, the number of BDCA2+ cells in close proximity to SLPI+ cells (e.g., cells indicated by arrows in left panel of Fig. 1C), or BDCA2+ cells in direct contact with SLPI+ cells (e.g., cells indicated by arrows in right panel of Fig. 1C) were counted for each field. The indicated fractions of pDCs are shown as a percentage of total BDCA2+ cells with mean ± SD in the last row.
3–9 is indicated by the horizontal line. Student data point represents one experiment, and the mean value in each group levels determined by ELISA. IFNI synthesis is displayed as pg/ml. Each described above. Supernatants of stimulated pDCs were collected and IFNI RPMI 1640 medium containing a HNE/SLPI/DNA mixture prepared as pDCs at 7.5

TLR9 inhibitor ODNTTAGGG or a control ODN were preincubated with activated HNE (dicHNE), azurocidin (Azuro), and SLPI, and 10 μM LL37. TLR9 inhibitor ODNTTAGGG or a control ODN were preincubated with pDCs at 7.5 μM for 30 min followed by addition of an equal volume of RPMI 1640 medium containing a HNE/SLPI/DNA mixture prepared as described above. Supernatants of stimulated pDCs were collected and IFNI levels determined by ELISA. IFNI synthesis is displayed as pg/ml. Each data point represents one experiment, and the mean value in each group n = 3–9 is indicated by the horizontal line. Student t test. **p < 0.01, *p < 0.05.

effects of HNE, the proteinase was pretreated with a selective inhibitor (3,4-dichloroisocoumarin) followed by dialysis. To distinguish the inhibitory and non-inhibitory role of SLPI, SLPI was oxidized with N-chlorosuccinimide (NSC), which decreases its HNE inhibitory capacity (30). Alternatively, SLPI was pretreated with 10 molar excess of neutralizing anti-SLPI Abs. Although HNE/SLPI/DNA stimulated significant IFNI production by pDCs, a mixture of DNA with azurocidin and SLPI, or α1-PI and HNE, had no effect. Likewise, replacement of active HNE with enzymatically inactive HNE did not stimulate pDC production of IFNI (Fig. 3B). Conversely, the reduction of anti-HNE function of SLPI, either by inhibitor oxidation with NSC or by treatment with neutralizing Abs, did not impair IFNI production by the stimulated pDCs (data not shown). Because the indicated treatment reduced the inhibitory activity of SLPI against HNE by 50–70%, these data suggest that SLPI does not require HNE inhibitory activity to stimulate pDCs. However, it is possible that the remaining inhibitory activity of SLPI against HNE was necessary and sufficient to contribute to pDC-stimulated IFNI production.

Taken together, these results likely suggest that enzymatically active HNE, noninhibitory SLPI, and DNA are required for pDC-stimulated IFNI production.

One of the DNA sensors expressed by pDCs and critically involved in the production of IFNI is TLR9 (21). To determine whether the effect of SLPI IFNI production was TLR9 mediated, the cells were pretreated with a TLR9 antagonist, inhibitory oligonucleotide ODNTTAGGG, which acts by disrupting the colocalization of stimulatory oligonucleotides with TLR9 in endosomal vesicles. As demonstrated in Fig. 3C, ODNTTAGGG but not control ODN significantly inhibited the effect of the HNE/SLPI/DNA complexes, indicating that TLR9-mediated signaling accounts for the majority of the complex activity. Thus, TLR9 is required for SLPI-dependent activation of pDCs.

**FIGURE 3.** SLPI-competent DNA structures stimulate pDC to express IFNI in a TLR9-dependent manner. Purified pDCs were stimulated with the indicated factors, which were added directly into pDC cultures, or first premixed with DNA or with each other. Final concentrations of the factors was as follows: 2 μg/ml DNA, 1 μM HNE, 3,4-dichloroisocoumarin-inactivated HNE (dicHNE), azurocidin (Azuro), and SLPI, and 10 μM LL37.

In this paper, we identified a previously unappreciated role for SLPI in converting inactive HNE-decorated extracellular DNA into a trigger for TLR9-dependent IFNI production by pDCs. We also demonstrated colocalization of neutrophils and pDCs in situ in skin of psoriasis patients and identified neutrophils as a source of SLPI-competent DNA structures in lesional skin. The presence of endogenous DNA in the vicinity of immune sensors of nucleic acids, such as pDCs, might have profound consequences for initiating psoriasis-relevant cell responses, provided that the DNA can reach intracellular TLR9. On the basis of our findings, we propose a model in which: 1) NETs formed by activated neutrophils and decorated by SLPI are deposited in involved skin; and 2) SLPI as a NET component stimulates the proinflammatory function of pDCs, leading to the initiation/augmentation of psoriasis through production of IFNI. Recent data indicate that the ability of pDCs to either trigger a proinflammatory response or promote wound repair are both linked to production of IFNI (21, 36). Therefore, activation of pDCs by SLPI-decorated NETs might lead to development of skin lesions and/or support healing of injured skin. Alternatively or in addition to a pathogenic role in psoriasis, SLPI-competent NETs may contribute to wound repair in damaged psoriatic skin by stimulating pDCs to produce IFNI.

Several studies implicate pDC-derived IFNI as important contributors to psoriasis. Psoriatic skin has a strong IFNI transcriptional fingerprint (e.g., activation of numerous IFNI-inducible genes (37, 38)). Moreover, psoriasis patients receiving IFNI therapy for unrelated conditions can develop exacerbated psoriasis symptoms (39, 40). Direct evidence for a pathogenic role for pDCs and IFNI in psoriasis is provided by a human/mouse skin xenograft model in which uninvolved skin of psoriasis donors is transplanted onto AGR129 mice that are deficient in recombinant interleukin-12 (IL-12) and IFNI. In this model, the spontaneous conversion of uninvolved skin into psoriatic lesions is prevented by blocking IFNI signaling or inhibiting pDC production of IFNI, indicating that pDC-derived IFNI is critical to drive the development of this skin condition (22). Therefore, by activating pDCs to produce IFNI, SLPI-decorated NETs might contribute to breaking tolerance to self-DNA and induce autoimmune-like psoriasis.

In contrast, in a mechanical skin injury model based on tape-stripping of mouse skin, wound reepithelialization of injured skin...
is significantly impaired following depletion of pDCs, or in mice deficient in IFNα receptor (36), identifying an important role for pDC-derived IFNα in promoting wound repair. Because skin of psoriasis patients shows features of skin injury accompanied by skin healing, SLPI-competent NETs might play a role in regulating wound repair in psoriatic skin. Notably, pDCs in both lesional skin of psoriasis patients and mechanically-wounded skin seem to be activated by a similar mechanism. Recognition of released nucleic acids by intracellular TLR7 and TLR9, a process facilitated by cationic peptides such as LL37, is reported to underlie activation of pDCs to produce IFNα (21, 36). However, although cationic peptides are sufficient to stimulate pDCs they also seem to play a redundant role in this process, because the production of IFNα by pDCs is induced in wounded skin in the absence of cathelicidins (36). Other factors might convert extracellular DNA into a stimulator for TLR9 in skin-recruited pDCs. On the basis of our findings, it is tempting to speculate that SLPI plays a compensatory role in stimulation of TLR9 by DNA in pDCs in damaged skin.

Interestingly, cathelicidin hCAP-18 (cationic antimicrobial peptide of 18 kDa), the LL37 precursor protein, is stored in secondary granules of PMNs similar to SLPI (2, 25). SLPI and LL37 peptide of 18 kDa, the LL37 precursor protein, is stored in secondary granules of PMNs. Although our data clearly demonstrate that extracellular trap degradation is associated with lupus nephritis. Sci. Transl. Med. 3: 73ra20.


