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Mast Cells and Neutrophils Release IL-17 through Extracellular Trap Formation in Psoriasis

Andrew M. Lin,* Cory J. Rubin,* Ritika Khandpur,* Jennifer Y. Wang,* MaryBeth Riblett,* Srilakshmi Yalavarthi,† Eneida C. Villanueva,† Parth Shah,* Mariana J. Kaplan,† and Allen T. Bruce*

IL-17 and IL-23 are known to be absolutely central to psoriasis pathogenesis because drugs targeting either cytokine are highly effective treatments for this disease. The efficacy of these drugs has been attributed to blocking the function of IL-17–producing T cells and their IL-23–induced expansion. However, we demonstrate that mast cells and neutrophils, not T cells, are the predominant cell types that contain IL-17 in human skin. IL-17+ mast cells and neutrophils are found at higher densities than IL-17+ T cells in psoriasis lesions and frequently release IL-17 in the process of forming specialized structures called extracellular traps. Furthermore, we find that IL-23 and IL-1β can induce mast cell extracellular trap formation and degranulation of human mast cells. Release of IL-17 from innate immune cells may be central to the pathogenesis of psoriasis, representing a fundamental mechanism by which the IL-23–IL-17 axis mediates host defense and autoimmunity. The Journal of Immunology, 2011, 187: 490–500.

Although IL-17 production by T cells is widely studied, it is increasingly appreciated that diverse types of innate immune cells also can produce IL-17 (17). Recent studies demonstrate that IL-17+ mast cells and neutrophils can be found in complicated atherosclerotic plaques (18). Similarly, IL-17+ mast cells are also present in inflamed synovium of rheumatoid arthritis (19). Mast cells, particularly a subset containing tryptase and chymase (MC1R-C), are enriched in the papillary dermis of psoriasis lesions (20, 21). Mast cells frequently degranulate in early eruptive and recurring psoriasis lesions and have been described as “ghost cells” (22–24). Interestingly, mast cell numbers are decreased in psoriasis lesions after successful treatment with anthralin, psoralen plus UVA light therapy, or cyclosporine (25–27). Neutrophils also are enriched in psoriasis lesions, especially in the epidermis where they aggregate in Munro’s microabscesses (MMs) in the stratum corneum and spongiform pustules of Kogoj (SPKs) in the stratum spinosum (28). Although the precise function of neutrophils is unknown in psoriasis, a critical pathogenic role is supported by case reports of psoriasis remission during drug-induced agranulocytosis and its reappearance after the normalization of neutrophil numbers (29). Furthermore, razoxane, a drug effective against all forms of psoriasis and psoriatic arthritis, causes a dose-dependent depression of neutrophil counts (30). Very recently, a study indicated that mast cells and neutrophils contain IL-17 in psoriasis plaques of three patients (31). However, the significance of these numbers relative to normal-appearing skin and the mechanism of IL-17 release by innate immune cells in psoriasis remain unknown.

IL-17 orchestrates innate immune responses against extracellular pathogens by inducing expression of antimicrobial peptides (AMPs) and neutrophil-tropic chemokines CXCL1, CXCL2, and IL-8 (32–35). The same AMPs and chemokines are found at extremely high levels in psoriatic epidermis (36–38). Not surprisingly, mice and humans with deficits in IL-17 production or signaling are highly susceptible to infection with extracellular bacteria and fungi (39–42). Similarly, effective antimicrobial activity by neutrophils and mast cells depends on the formation of structures called extracellular traps (ETs), termed NETs and

Abbreviations used in this article: AMP, antimicrobial peptide; cf, cells per field; ET, extracellular trap; ETosis, process of extracellular trap formation; LDG, low-density granulocyte; LL-37, cathelicidin; MCET, mast cell extracellular trap; MCE-Tosis, process of MCET formation; MCTrytase/chymase+ mast cell; MM, Munro’s microabscess; MPO, myeloperoxidase; NET, neutrophil extracellular trap; NETosis, process of NET formation; NN, normal skin from control subjects; PN, normal-appearing skin from psoriasis subjects; PP, psoriasis plaque; SPK, spongiform pustule of Kogoj.

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MCETs, respectively (43–45). ETs are formed through a specialized process of cell death termed EToxsis (46), where chromatin extends into fine, weiblike threads to which proteins are bound. In particular, NETs can contain myeloperoxidase (MPO), proteinase 3, and AMPs such as cathelicidin (LL-37) (47). The process of NET formation (NETosis) can be triggered by extracellular bacteria and fungi or their components (48, 49). MCETs contain tryptase, LL-37, and chromatin (45), forming in response to bacteria, H$_2$O$_2$, or PMA (45, 48). In humans, NETs have been visualized in physiologic host responses to infections (44, 50) and have been implicated in the pathology of anti-neutrophil cytoplasmic Ab-induced vasculitis (51). Additionally, a recent study showed that lupus nephritis is associated with an inability to degrade NETs in blood (52). Furthermore, although MCETs have been studied in a human mast cell line and mouse bone marrow– derived mast cells (45), it is unclear whether the process of MCET formation (MCETosis) occurs in human tissue under normal or pathologic conditions.

To understand the complex pathophysiology of psoriasis, it is imperative to define the precise cellular sources of IL-17 and the mechanisms mediating IL-17 release. Therefore, we investigate the production of IL-17 by innate immune cells in psoriasis and explore a potential role for ETs formed by these cells in human skin. Surprisingly, we observe that most IL-17$^+$ cells in normal and psoriatic skin are mast cells, not T cells. Additionally, neutrophils in well-developed psoriasis lesions also express IL-17. We observe that mast cells and neutrophils release IL-17 into the skin through EToxsis as well as conventional degranulation. Furthermore, a subtype of human neutrophils, low-density granulocytes (LDGs), isolated from psoriatic blood are increased in psoriatic compared with control blood. Interestingly, mast cells readily form ETs with resultant IL-17 release in control human skin explants treated with IL-23 and IL-1β, suggesting a novel mechanism driving EToxsis and IL-17 release from mast cells. These findings suggest that mast cells and neutrophils may play more prominent roles than previously appreciated in psoriasis and similar diseases by releasing IL-17 through ET formation.

Materials and Methods

Human subjects

All of the subjects provided written, informed consent. Skin punch biopsies (6 µm) were obtained from patients with normal healthy skin (NN), normal-looking symptomless psoriatic skin (PN), and psoriasis plaques (PP). Before biopsy, subjects were required to be off all systemic therapies for at least 2 wk and off topical antipsoriatic medications for at least 1 wk. Control donors were identified from respondents to advertisements in the area (Ann Arbor, MI), had no personal or family history of psoriasis, and were free of inflammatory skin disease at the time of biopsy. This study was approved by the Institutional Review Board of the University of Michigan Medical School.

Immunofluorescence

Five-micrometer sections of skin were deparaffinized on a hot plate at 65°C for 1 h. They were rehydrated by incubation and heat retrieval in CCI1 cell conditioning solution (Ventana Medical Systems, Tucson, AZ). The sections were blocked with horse serum (0.2%; Invitrogen, Carlsbad, CA) for 30 min at room temperature. Then simultaneous staining first was performed for 30 min at room temperature with goat anti-IL-17 (100 µg/ml; R&D Systems, Minneapolis, MN) and another primary Ab, either rabbit anti-CD3 (0.4 µg/ml; Ventana Medical Systems), rabbit anti-CD4 (1:25; Cell Marque, Rocklin, CA), mouse anti-CD8 (1:25; Leica Biosystems, Newcastle, U.K.), mouse anti-mast cell tryptase (1:150; Dako, Carpinteria, CA), mouse anti-mast cell chymase (1:1000; ABD Serotec, Oxford, U.K.), or rabbit anti-MPO (1:1500; Dako). Triple staining was conducted with IL-17, MPO, and mouse anti-human LL-37 Abs (1:200; Abcam, Cambridge, MA). This was followed by 30 min of incubation at room temperature with matched secondary Abs: chicken anti-goat IgG Alexa Fluor 488 (1:300; Invitrogen), chicken anti-mouse IgG Alexa Fluor 594 (1:300; Invitrogen), or chicken anti-rabbit IgG Alexa Fluor 594 (1:300; Invitrogen). For triple staining, donkey anti-rabbit Alexa Fluor 350 (1:300; Invitrogen) also was used. ProLong Gold antifade reagent with DAPI was added to the slides prior to mounting with coverslips (Invitrogen). Images were captured with a fluorescence microscope (BX50; Olympus, Essex, U.K.) using DP Controller and DP Manager (Olympus) software and a confocal macroscopic filter (FV-500; Olympus).

Cell quantification from immunofluorescence

The number of cells expressing one or both markers of interest in each 3 × 200 field was counted manually by two independent blinded observers on Adobe Photoshop. From these numbers, we calculated the proportions of cells of different populations [e.g., the proportion of IL-17$^+$ of all tryptase$^+$ cells is calculated as (number of IL-17$^+$ tryptase$^+$ cells)/(number of IL-17$^+$ tryptase$^+$ cells + number of IL-17$^+$ tryptase$^-$ cells)]. Each data point in Figs. 2 and 5 represents the average of one to six independent fields for each patient sample.

Skin explant cultures

Punch biopsies were obtained from healthy donors without psoriasis as above and were quartered longitudinally. Each divided section was incubated in R10 media (RPMI 1640 [Life Technologies] with 0.4 mM Ca$^{2+}$ and 10% FCS) with soy bean trypsin inhibitor (100 µg/ml; Sigma-Aldrich) or R10 media with soy bean trypsin inhibitor containing either Substance P (10 µM; Sigma-Aldrich), Compound 48/80 (10 µg/ml; Sigma-Aldrich), IFN-γ (200 ng/ml; Miltenyi Biotec), IL-1β (2.5 ng/ml; R&D Systems), IL-23 (20 ng/ml; R&D Systems), or both IL-1β and IL-23 together for 2 h or 3 d. The tissues were fixed in 10% neutral buffered formalin and subjected to immunofluorescence staining as above. The proportions of intact mast cells and degranulated mast cells were counted by direct visualization through the microscope by two independent observers.

Isolation of neutrophils and LDGs

Normal-density neutrophils and LDGs were as described (53). The purity of the neutrophil fraction was at least 95% and was determined by CD15, CD4, CD10, and 10% FCS. Additional expression by flow cytometry, neutrophils were sorted after dextran gradient isolation to >99.5% purity using FACSARia (BD Biosciences, San Jose, CA) gating on CD15$^+$ CD3$^-$ cells with neutrophil size and granularity characteristics as in Fig. 4A. For LDGs, PBMCs were isolated using Ficoll-Hypaque. RBCs were lysed using hypotonic/hypertonic saline, and then T and B lymphocytes, NK cells, monocytes, and residual RBCs were removed by negative selection. LDGs were identified as CD15$^+$CD4$^{ab}$ or CD10$^+$CD14$^{ab}$ (CD10$^+$ CD14$^{ab}$ CD14$^{ab}$).

Western blot analysis

Total cell lysates were obtained by Laemmlni extraction. A total of 10 µg of each sample and 40 ng of the recombinant cytokines were loaded. Samples were separated on 15% SDS-polyacrylamide gels and transferred to Immobilon-P membranes (Millipore, Billerica, MA). Western blots were blocked with PBS/0.2% Tween 20 for 1 h at RT (@ 4°C) and negative controls were washed again, and proteins were detected by chemiluminemision using ECL (Thermo Scientific, Rockford, IL) and X-OMat film (Kodak, Rochester, NY). Anti-β-actin (1:2500; Sigma-Aldrich) was used as a loading control.

NET immunofluorescence staining and quantification

A total of 1–2 × 10$^6$ neutrophils or LDGs per milliliter were seeded in poly-l-lysine–coated coverslips and incubated at 37°C, 5% CO$_2$ for 15 min. Cells were washed with ice-cold PBS, fixed with cold 4% paraformaldehyde for 15 min, and blocked with 10% FBS, 1% BSA, 0.05% Tween 20, and 2 mM EDTA in 1 × PBS at 4°C overnight. Stimulated cells were incubated for 2 or 3 h in 1 × RPMI 1640 with glutamine and 2% BSA with 20 mM PMA (Sigma-Aldrich). For detection of NETs, fixed cells were washed with ice-cold 10% FBS in 1 × PBS for 5 min, incubated with primary Abs for neutrophil elastase (Abcam) or rabbit isotype control (Abcam) for 45 min at 4°C, washed twice, and incubated with secondary Abs for 45 min at 4°C. Nuclear material was detected by incubating cells with Hoechst 33342 (Invitrogen) for 10 min at room temperature. Coverslips were placed in slides with ProLong Gold antifade
reagent for further analysis using an inverted microscope (IX70; Olympus, Center Valley, PA). Image overlay and segmentation was performed using Metamorph software (version 7.7). Further analyses of the imaging data were performed using Photoshop, where colocalization of neutrophil elastase and nuclear staining (Hoechst) was manually counted. The percentage of NETs was calculated as the average of six (4–6) high-power fields of view normalized to the total number of cells per view.

Statistical analysis
Data are expressed as mean ± SD. NN, PN, and PP were compared using one-way ANOVA with Tukey’s post hoc test using GraphPad Prism 5. Proportions of LDGs in PBMCs were compared using an unpaired two-tailed Student t test. Mast cell explant conditions were compared with media using one-way ANOVA with Dunnett’s post hoc test. A p value <0.05 was considered to be statistically significant.

Results
T cells producing IL-17 represent a minority of all IL-17+ cells in human skin
Previous studies of IL-17 expression from human skin focused primarily on T cells stimulated ex vivo with PMA and ionomycin to increase signal intensity for subsequent flow cytometric analysis (2, 10, 11). Although this approach is very useful to determine the differentiation state of T cells isolated from tissue, it does not define the identity and anatomic localization of cells actually containing intracellular IL-17 in vivo. Thus, we analyzed tissues isolated from psoriasis plaques (PP, n = 13), normal-appearing skin from psoriasis subjects (PP, n = 10), and normal skin from control subjects (NN, n = 10). To visualize IL-17+ T cells in their anatomic setting, we performed dual-color immunofluorescence and counted the numbers of cells per field (cpf) at ×200 magnification expressing intracellular IL-17 and CD3 as a marker for T cells (Fig. 1A, Supplemental Fig. 1). Not surprisingly, the density of T cells is increased in PP skin compared with those in PN and NN skin (54 ± 35 versus 12 ± 11 and 10 ± 7 cpf; PP:PN < 0.001, PP:NN < 0.001; Fig. 2A). However, the number of CD3+ T cells containing detectable IL-17 in PP lesions compared with those in PN and NN skin was not significantly different (1.1 versus 0.8 versus 0.5 cpf, Fig. 2B). To our surprise, we found that T cells represent only a minority of all IL-17+ cells in PP, PN, and NN skin (7.3 ± 7.2, 7.3 ± 7.1, and 5.0 ± 5.0%; Fig. 2C). The proportions of IL-17 + T cells of all CD3+ T cells were calculated to be 3.4 ± 5.8 (PP), 10.1 ± 11.9 (PN), and 4.4 ± 4.9% (NN) (Fig. 2D). This finding is concordant with studies by our group and others defining the proportion of T cells capable of producing IL-17, particularly in MMs and SPKs, as 20% (28). Because neutrophils were present in superficial MMs and SPKs, analysis of the cells was by necessity more epidermally focused but also included superficial dermal regions. A significant number of IL-17+ neutrophils was seen in PP lesions but rarely present in PN skin and essentially absent in NN skin (18 ± 18 versus 1 ± 1 versus 0 ± 0 cpf; PP:PN < 0.01, PP:NN < 0.001; Fig. 2N). Because neutrophils were present in PP lesions in epidermal clusters, the proportion of neutrophils of all IL-17+ cells in PP skin shows substantial variability but is significantly higher than those in PN or NN skin (32.4 ± 20.6 versus 8.1 ± 5.7 versus 1.3 ± 4.3%; PP:PN < 0.001, PP:NN < 0.001; Fig. 2O). Neutrophils in PP or PN tended to contain more IL-17 compared with those in NN skin (33.8 ± 19.4 versus 50.7 ± 45.9 versus 0.8 ± 2.6%; PN:NN < 0.01; PP:PN < 0.001; Fig. 2P). Together, these data reveal that IL-17+ neutrophils are enriched in psoriasis lesions, primarily in focal epidermal collections.

IL-17 is released during the formation of NETs in psoriasis lesions
At low and medium powers, we had observed that the nuclei of IL-17+ neutrophils in MMs frequently displayed the morphology of NETs. Indeed, high-power microscopic examination of dual-color immunofluorescence revealed that IL-17 and MPO were associated frequently with NET-like formations of DNA, visualized with DAPI staining. These NETs were observed frequently in psoriatic lesional epidermis (Fig. 3A), particularly in MMs and SPKs, as demonstrated by the thin extensions of nuclear material coaligning with MPO (Fig. 3B). To confirm the functional relevance of NETs, tricolor immunofluorescence staining showed that dermal NETs frequently contained both IL-17 and LL-37 (Fig. 3C). Further visualization through confocal microscopy confirmed that the morphology of NETs varied from cell to cell even within aggre-
gates (Fig. 3D), suggesting that newly arriving neutrophils may be undergoing NETosis. H&E-stained slides confirmed that not all of the neutrophils in a MM had altered nuclear morphology (Fig. 3E). The NETs with the longest chromatin projections tended to costain with MPO but often not with IL-17. In contrast, NETs with shorter extrusions often stained with IL-17, presumably representing less mature NETs. Because IL-17 is not seen in all of the NETs in PP, we hypothesize that IL-17 does not bind to extruded chromatin nearly as strongly or abundantly as MPO.

To explore the sites of IL-17 production, human neutrophils isolated using the standard dextran protocol were enriched further by flow cytometric sorting to >99.5% purity (Fig. 4A). Lysates of these flow-sorted cells contained IL-17A but not IL-17F protein, as detected using Western blot analysis (Fig. 4B).

Increased LDGs in psoriatic blood frequently undergo NETosis

Seminal studies almost three decades ago described a subpopulation of neutrophils in psoriatic blood that are polar in shape and have ruffled cell membranes (54). Similarly, a recent study

FIGURE 1. T cells, mast cells, and neutrophils contain IL-17 in psoriasis. Punch biopsies of skin from subjects without psoriasis (NN, n = 10), uninvolved skin from subjects with psoriasis (PN, n = 10), or lesional psoriasis plaques (PP, n = 10, shown) were subjected to dual-color immunofluorescence staining for IL-17 (green) and either CD3 (A), tryptase (B), chymase (C), or MPO (D, red), with DAPI counterstain. These high-power fields at ×600 magnification near the superficial dermis illustrate the colocalization of IL-17 with CD3, tryptase, chymase, or MPO (yellow, solid arrows), while simultaneously indicating the presence of other IL-17+ cells (open arrows) in the same field. Scale bar, 100 μm.
revealed a population of LDGs in the blood of patients with systemic lupus erythematosus that share some properties with activated neutrophils (53). We found increased numbers of LDGs in the blood of patients with psoriasis compared with those in control blood (34.6 ± 5.1 versus 11.0 ± 2.0%; \( p < 0.01 \); Fig. 4C).

We measured NET formation in blood neutrophils from psoriasis or controls and visualized NETosis using stains for neutrophil elastase and DAPI. In four sets from patients and controls, we observed that psoriasis LDGs tended to form NETs without any stimulation, in contrast to control or psoriasis neutrophils. After 2 h of incubation in PMA, control neutrophils, psoriasis neutrophils, or LDGs all formed NETs. However, compared with control neutrophils, psoriasis neutrophils tended to form more NETs after culture without PMA treatment although this was not statistically significant (Fig. 4D). These findings indicate that populations of circulating neutrophils in psoriasis readily undergo NETosis.

**MCETs in psoriasis lesions are associated with IL-17 release**

In immunofluorescence studies, we noticed that MC TCs in the papillary dermis of psoriasis plaques were often not intact. On initial inspection, they appeared to be degranulated, which is consistent with scanning electron microscopy studies showing...
Neutrophils frequently undergo ETosis in psoriasis. A, Punch biopsies of skin from subjects with lesional psoriasis plaques (PP, n = 12) were subjected to dual-color immunofluorescence staining for IL-17 (green) and MPO (red), with DAPI counterstain (blue). An epidermally centered field of a mature psoriasis plaque at ×200 magnification reveals IL-17–containing neutrophils in a characteristic MM and SPK. Scale bar, 100 μm. B, NETs magnified past ×1000 were observed to extrude nuclear material (open arrows) and contained both MPO and IL-17. Scale bar, 10 μm. C, PP skin also was subjected to tricolor immunofluorescence staining (n = 3) for IL-17 (green), LL-37 (red), and MPO (blue), revealing many triple-positive NETs in the dermis (white cells, solid arrow) as well as other nonneutrophil IL-17+ cells (green, open arrow). Scale bar, 100 μm. D, NETs in a MMs were examined further by confocal microscopy at ×1000 (solid arrows) and approximately ×5000 magnification, showing elaborate distortion of nuclear material. Scale bar, 10 μm. E, H&E-stained sections of a MM at ×200 and past ×1000 magnification also revealed altered nuclear morphology resembling NETs. Scale bars, 100 μm (upper panel), 10 μm (lower panel).
FIGURE 4. Neutrophils and LDGs from the blood of patients with psoriasis exhibit frequent NET formation. A, Neutrophils isolated from human blood were sorted further by FACS Aria with forward light scatter, side scatter of light, CD15, and CD3 gates (red) to >99.5% purity. Flow cytometry plots from one representative sample are shown. B, Western blot analysis of cell lysates from postsort neutrophils with anti-IL-17A and anti-IL-17F Abs was performed to confirm the type of IL-17 contained in control neutrophils (n = 3). Staining for β-actin served as the loading control. C, A higher number of LDGs was present in blood from psoriasis patients (n = 15) compared with that in control blood (n = 6). **p < 0.01 (unpaired two-tailed Student t test). D, Neutrophils and LDGs isolated from psoriatic blood and neutrophils from control blood were stained at time 0, 2 h without stimulation, and 2 h after incubation in PMA. In vitro NETs were examined at ×400 magnification using stains for neutrophil elastase (green) and Hoechst 33342 (blue). Scale bar, 20 μm. A, rIL-17A; F, rIL-17F; γ, rIFN-γ; N, neutrophils; P, PBMCs.
FIGURE 5. IL-1β and IL-23 promote mast cell degranulation or MCET formation. A, MCETs observed in PP lesions at ×1000 magnification showed extruded nuclear material (open arrows) and contained both chymase (red) and IL-17 (green). Scale bar, 10 μm. B, MCETs were observed further in the dermis of psoriatic skin by zooming in on a confocal image past ×1000 magnification. Scale bar, 25 μm. C, Punch biopsies of skin from normal controls (NN) were incubated for 3 d in various conditions: media alone, IFN-γ, Substance P, Compound 48/80, IL-1β, IL-23, and IL-1β + IL-23 as described in Materials and Methods. Tissue sections were subjected to dual-color immunofluorescence staining for IL-17 (green) with chymase (red) or tryptase (data not shown), with DAPI counterstain (n = 3). Representative images from one NN series stained with chymase and IL-17 are shown at ×400 magnification and compared with a typical PP section. Intact cells (solid arrows) and MCETs or degranulated cells (open arrows) are highlighted. Scale bar, 100 μm. D, The proportion of MCETs or degranulated tryptase+ cells was observed to increase when exposed to IL-23 alone or IL-1β and IL-23. *p < 0.05 for media versus IL-23 or IL-1β + IL-23 (one-way ANOVA, Dunnett’s test). E, Similarly, chymase+ cells also displayed more frequent MCETs and degranulation when exposed to IL-1β and IL-23. **p < 0.01 for media versus IL-23 or IL-1β + IL-23.
degranulated MCs in developing or recurrent psoriasis plaques (22, 23). However, high-power microscopic visualization of the superficial papillary dermis revealed that although many of these MC_TCs had undergone conventional degranulation, a significant number of cells revealed mast cell tryptase and chymase colocalized with linear DNA (Fig. 5A, 5B). We identified these structures as MCETs, based on their similarity to MCETs formed in vitro (45). Although essentially all mast cells in PP contain IL-17, a minority of MCETs contained brightly for IL-17, and many were IL-17 

This suggests that IL-17 is released at low levels upon MCETosis, diffusing to a level below our threshold of detection faster than tryptase or chymase. The shapes of MCETs in skin are more compact than those formed in vitro, likely due to the physical constraints of surrounding tissue. MCETs or degranulated mast cells were not seen frequently in NN or PN samples (Supplemental Figs. 2, 3). These data demonstrate that MCETs can form in human psoriasis lesions and represent a distinct mechanism of IL-17 release by mast cells in vivo.

Treatment of normal human skin explants with IL-1β and IL-23 results in mast cell degranulation and MCETosis

Because IL-1β and IL-23 promote increased production of IL-17 from T cells, we evaluated whether these cytokines similarly might promote production or release of IL-17 from mast cells in situ in skin. Tissue sections from normal skin biopsies (n = 3) incubated for 3 d in control media contained mostly intact mast cells at a density comparable to that of tissue sections from the same patient fixed immediately after biopsy. After 3 d of treatment with IL-1β and IL-23, there was no increased expression of IL-17. However, there were significant increases in degranulated and ETosed mast cells in treated tissue, as measured by staining for tryptase, chymase, and IL-17 (Fig. 5C). Treatment of normal skin with either IL-1β or IL-23 alone led to fewer degranulated/ETosed mast cells than treatment with both compounds together as measured with tryptase |P_media:IL-1β/IL-23 < 0.05, |P_media:IL-1β/IL-23 < 0.05; Fig. 5D) or chymase (|P_media:IL-23 < 0.01, |P_media:IL-1β/IL-23 < 0.01; Fig. 5E). Treatment with Substance P or Compound 48/80, classic mast cell degranulating agents, elicited degranulation of mast cells but did not yield significant ETs. Consistent findings were observed in separate experiments from four independent healthy skin donors in the 3-d cultures. These findings indicate that IL-1β and IL-23 can rapidly induce mast cell degranulation and MCETosis in human skin, resulting in the release of IL-17 and other mast cell products (Fig. 6).

Discussion

Our findings suggest prominent roles for IL-17 released by mast cells and neutrophils in psoriasis as outlined in our model (Fig. 6). In normal skin that encounters trauma or microbial infection, mast cells detect stimuli such as necrotic tissue or microbial invasion through TLRs and other pattern recognition receptors. This detection can lead to direct release of preformed mediators including TNF-α, IL-17, CXCL2 (55), chymase, and tryptase through degranulation or MCETosis. These mediators promote endothelial activation, vascular permeability, and rapid influx of neutrophils. Encounter with extracellular bacteria or products of necrotic cells will promote NETosis with the release of mediators including IL-17, which can amplify further neutrophil accumulation by increasing CXCL1, CXCL2, and IL-8 expression. IL-17 produced by neutrophils also has been shown to be critical for neutrophil accumulation (56). Interestingly, these CXCR2 agonists are known to participate in NETosis in vivo because treatment with CXCR2 antagonists block NETosis and improve lung function in cystic fibrosis (57). Activation of CXCR2 by increased release of CXCL1, CXCL2, and IL-8 similarly may mediate NETosis in psoriasis. Although the relevant targets of IL-17 in psoriatic skin are unclear, IL-17 can increase the expression of AMPs human β-defensin-2, S100A7, S100A8, S100A9, and IL-23 in keratinocytes (33–35). These AMPs can stimulate immune cell infiltration, and NET-derived LL-37–DNA complexes may promote IFN-α release from plasmacytoid dendritic cells (58). Indeed, MCET- or NET-derived nucleic acids may be responsible for the plasmacytoid dendritic cell activation seen after tape-stripping of human skin, a model for sterile mechanical skin injury (59, 60), accounting in part for the proposed role of mast cells in the Koebner phenomenon (24). IFN-α and TNF-α can stimulate influx of inflammatory dendritic cells and macrophages, which in the presence of IFN-γ and other danger signals produce cytokines including IL-23 and IL-1β. These cytokines can also stimulate IL-23 release by T cells, neutrophils, and mast cells to further promote the cycle of inflammation.

In a typical host response to infection, TLR stimulation diminishes as pathogens and necrotic tissue are cleared, thereby interrupting the cycle of inflammation with decreased release of IL-17, TNF-α, IFN-α, IFN-γ, and IL-23. However, in people carrying polymorphisms in the psoriasis susceptibility genes IL12B, IL23A, or IL23R, IL-23 signaling may be dysregulated, resulting in inappropriate sustained release of IL-17 and other mediators from mast cells, neutrophils, and T cells. Likewise, polymorphisms in genes modifying signaling downstream of IL-17 (TRAF3IP2), TNF-α, and TLRs (TNFAIP3 and TNIP1) may fail to appropriately dampen or extinguish these pathways. Either scenario would result in prolonged inflammation in the absence of infection.

Although our Western blot and immunofluorescence studies indicate the presence of IL-17A protein, mRNA from human neutrophils from several subjects showed either low or undetectable levels of IL-17 using real-time RT-PCR and Affymetrix gene array experiments (M.J. Kaplan, unpublished observations). This may be explained by early production and storage of IL-17A by developing neutrophils prior to their release from bone marrow. Given their short life spans, upon entering the circulation neutrophils may downregulate transcription of many mRNAs. An
alternative explanation is that neutrophils and mast cells may bind and accumulate IL-17A protein produced by other cell types instead of directly producing IL-17. However, although analysis of mRNA expression from skin mast cells is hindered by limited numbers, in vitro studies have shown that mast cells release IL-17 protein in response to multiple stimuli in a retinoic acid receptor C-dependent manner (19).

IL-17 release from innate immune cells may provide a biologic basis for systemic features of psoriasis. Similar to its role in inflamed synovium of rheumatoid arthritis patients (19), mast cell-derived IL-17 also may be involved in psoriatic arthritis. The presence of IL-17+ mast cells in psoriasis plaques and complicated atherosclerotic plaques (18) suggests that mast cell release of IL-17 may contribute to increased atherosclerosis and increased mortality associated with severe psoriasis (61). Indeed, inhibition of IL-17 attenuates both psoriasis and atherosclerotic plaque development (62).

Neutrophils, mast cells, and eosinophils form ETs on exposure to bacteria, fungi, bacterial products, activated platelets, or IL-8 through activation of TLRs and cytokine and Fc receptors (47, 63). These receptors stimulate protein kinase C and ultimately result in the generation of reactive oxygen species through the activation of the NAPDH oxidase complex (64). The ETosis-promoting activities of IL-23 and IL-1β were not suspected previously, and the precise signaling mechanisms regulating this process remain to be defined. On the basis of our results, we suggest that modulation of mast cell and neutrophil ETosis and promotion of IL-23 and IL-1b may contribute to increased atherosclerosis and increased mortality associated with severe psoriasis (61). Indeed, inhibition of IL-17 attenuates both psoriasis and atherosclerotic plaque development (62).

In conclusion, this study demonstrates that increased numbers of mast cells and neutrophils in psoriasis lesions contribute to the release of the pathogenic cytokine IL-17 through the formation of ETs and that IL-23 and IL-1β provide a novel mechanistic stimulus for this phenomenon. These observations support a model in which mast cells and neutrophils play significant roles in the pathophysiology of psoriasis and potentially other autoimmune diseases driven by the IL-23–IL-17 axis. This model presents opportunities to better understand current treatments and develop novel therapies that inhibit ETosis and release of IL-17 is a novel therapeutic mechanism of action for drugs targeting IL-23.

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