Targeting neutrophil extracellular traps (NETs) ameliorates inflammation in murine psoriasiform dermatitis

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Letter to the Editor

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To the editor

Psoriasis is a chronic recurrent inflammatory disease of the skin and joints, often associated with significant morbidity. It has an immunogenic basis, centered around interactions of the innate and the adaptive immune system [1]. Neutrophils are thought to play a crucial part in the pathogenesis of this disease [2].

The discovery of neutrophil extracellular traps (NETs) as a novel immune defence mechanism and their involvement in autoimmune, malignant and cardiovascular diseases [3,4] has led to a renewed interest in these cells in psoriasis [5]. However, whether NETs play a role in the pathogenesis of psoriasis or whether they are merely a bystander phenomenon has so far remained unexplored.

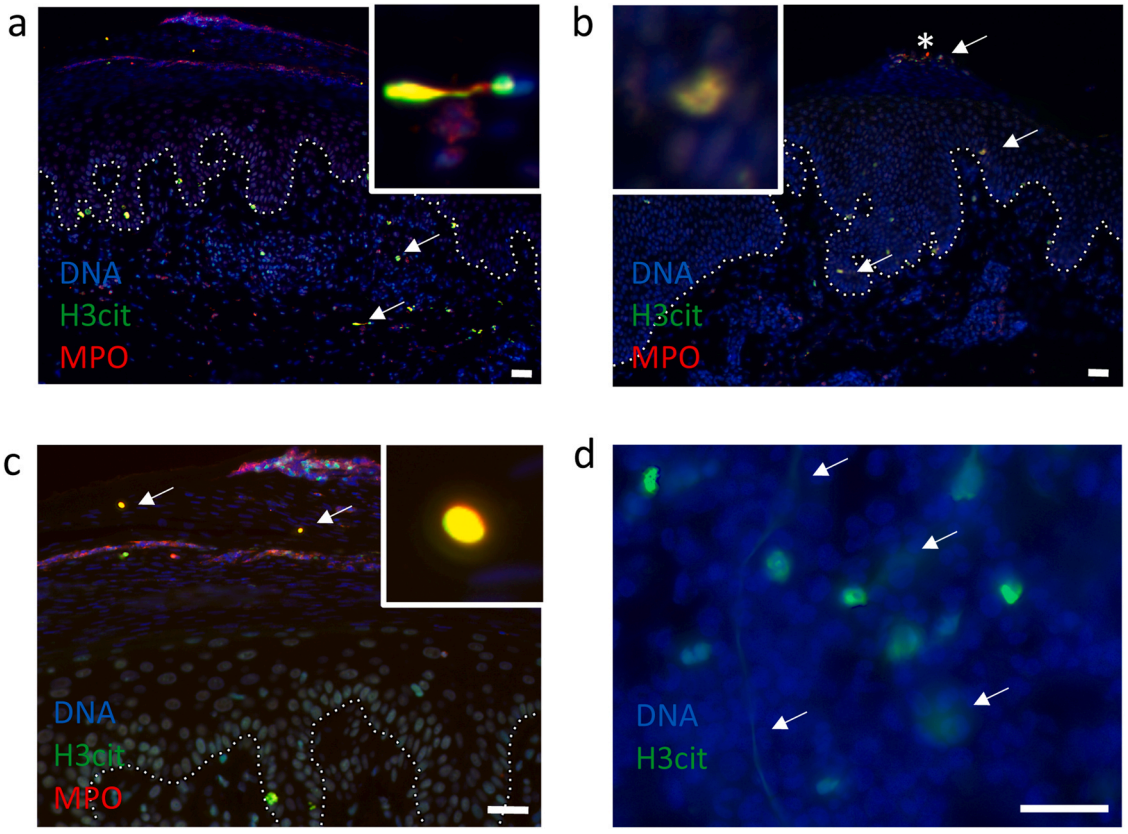

Fig. 1. Neutrophils and neutrophil extracelullar traps (NETs) are present in psoriatic skin and in pustule smears of pustular palmoplantar psoriasis (PPP). Immunofluorescence stainings of representative psoriatic skin sections with the NET-marker H3cit (green), the neutrophil marker MPO (red), and the nuclear staining Hoechst (blue): a) Psoriatic skin b) Section of psoriatic skin with Munro's microabscess in the epidermis (white star) c) Psoriatic skin, magnification (20x). Insets show nuclear morphology of NETs in more detail. d) Smears of sterile pustules of a patient with PPP (neutrophils were identified by nuclear morphology). In all sections, NETs are exemplarily indicated by white arrows and the scale bars correspond to 40 µm. All images correspond to different patients.

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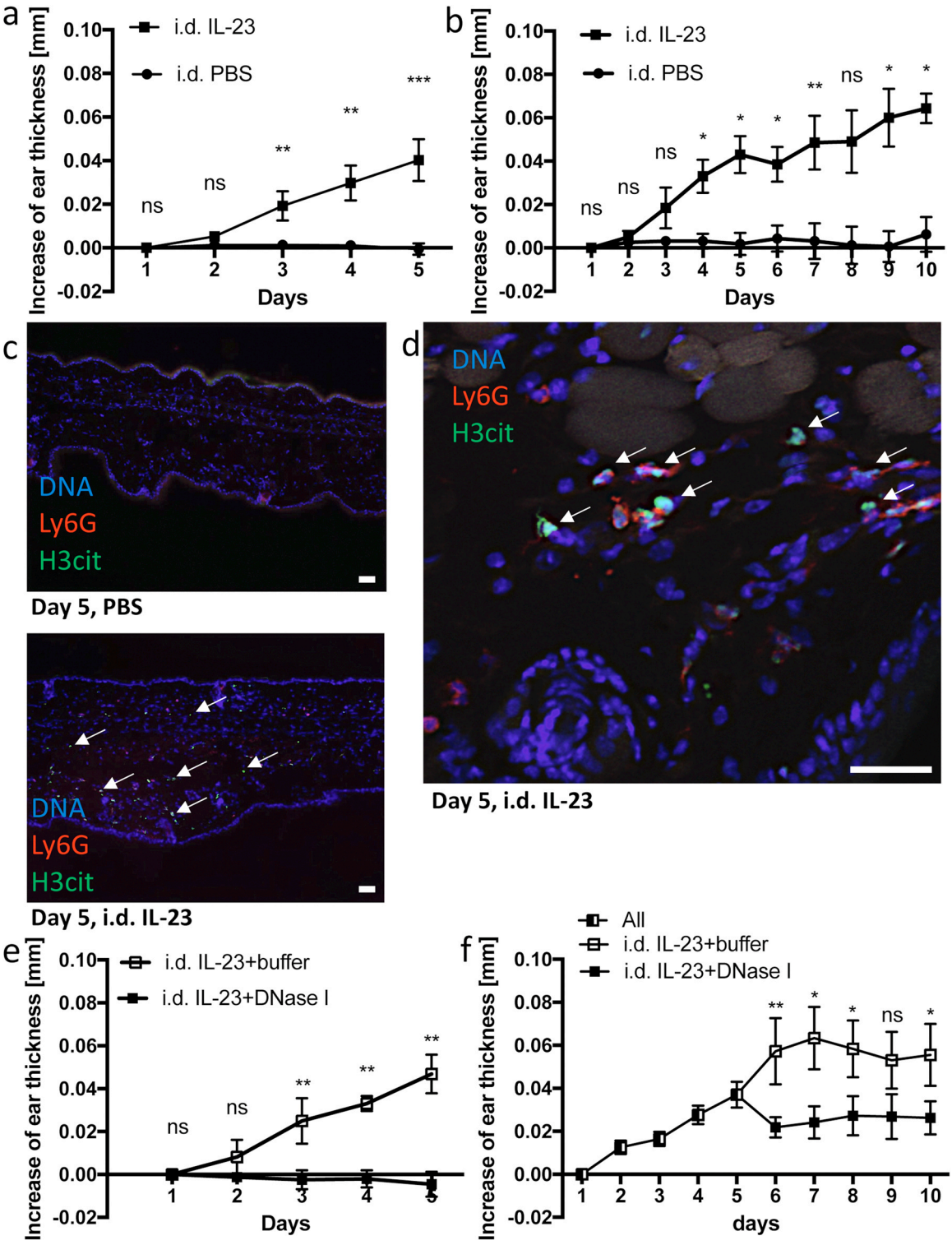

Fig. 2. NETs and NET inhibition in a murine model of psoriasiform dermatitis. Intradermal IL-23 application leads to significant ear swelling in mice after a) 5 days (n = 9–10) and b) after 10 days (n = 4–5). c) Neutrophils, identified by immunofluorescence staining against Ly6G (red) and NETs (staining against H3cit, green) were found in mice having received intradermal IL-23 for 5 days but not in the PBS control group. DNA was stained by Hoechst (blue). NETs are exemplarily indicated by white arrows. The scale bar corresponds to 40 µm. d) Magnified immunofluorescence stainings of ear sections of IL-23 treated mice. Again, the scale bar corresponds to 40 µm and NETs are exemplarily indicated by white arrows. e) Systemic DNase I application in a preventive regimen (n = 4–5) or f) as a therapeutic intervention in established psoriasiform dermatitis (n = 8–11) prevents or ameliorates ear swelling, respectively. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Values are shown as mean ± SEM.

Here, we first verified the presence of neutrophils undergoing NET formation (also termed NETosis) in sequential sections of psoriatic human skin, as detected by the presence of neutrophils (Fig. 1a–c, white arrows), stained by the neutrophil marker myeloperoxidase (MPO, anti-MPO ab25989, dilution: 1:250; Abcam, Cambridge, GB, see supplementary data for secondary antibodies) and the NET-marker citrullinated histone 3 (H3cit, anti-H3cit ab5103, dilution: 1:750; Abcam, Cambridge, GB) in addition to Hoechst (Hoechst 33342, 1.62 μM, Sigma-Aldrich, St. Louis, USA). Paraffin-embedded samples were processed according to standard histopathological protocols. Additionally, sterile pustules of patients with pustular palmoplantar psoriasis (PPP) contained H3cit-positive neutrophils as well as extracellular strands of H3cit-positive DNA, indicating NETosis (Fig. 1d, white arrows). Smears of pustule-content were fixed using 4% paraformaldehyde, NETs were stained using anti-H3cit (see above).

To elucidate whether the formation of NETs in psoriasis may contribute to the pathogenesis of the disease, we next investigated the role of NETs in a murine model of psoriasiform-inflammation. As IL-23 has been shown to be of fundamental importance in the development of psoriasis and psoriatic arthritis in mice [6], IL-23-mediated psoriasiform skin inflammation was induced by daily intradermal injections of 500 ng IL-23 (Affymetrix eBioscience, San Diego, USA) in 20 μl PBS into the right ears of 6- to 10-week-old C57BL/6 mice [7]. The left ears of these mice as well as both ears of age- and sex-matched control animals received 20 μl of PBS only. Ear swelling was measured daily by an electronic caliper in a blinded and standardized fashion and calculated as the thickness of the right ear minus that of the (control) left ear. As expected, mice receiving IL-23 developed a significant ear swelling as well as desquamation and erythema in the injected area (Fig. 2a, clinical images in Fig. S1). During this early stage of inflammation, at day 5, mice were sacrificed and ears were stained for the presence of Ly6G-positive (anti-Ly6G 1A8 127601, dilution: 1:250; BioLegend, San Diego, USA) neutrophils, as well as for H3cit (see above) to indicate NETs (Fig. 2c and d). Similar results were obtained using neutrophil elastase instead of Ly6G (NE, Alexa Flour 555 Conjugated, dilution: 1:200; Bioss, Woburn, USA) (Fig. S2). In mice that had received IL-23 intradermally, H3cit-positive neutrophils were distributed throughout the dermis and the underlying subcutis. In contrast, PBS control mice showed no infiltration of neutrophils (Fig. 2c). In a second line of experiments, the experiments were continued with daily injections of IL-23 or PBS, respectively, until day 10 (Fig. 2b). A significant increase of ear swelling was visible in mice receiving IL-23 from day 4 onward. Interestingly, though, in the histological sections from the ears of these mice NETs were no longer clearly detectable (not shown), suggesting endogenous degradation. Similarly, mice having received topical imiquimod treatment for 6 days to induce psoriasis-like dermatitis were found to have a neutrophil-rich infiltrate yet no visible NET formation (Fig. S3), once again demonstrating that mouse models of psoriasis reproduce different aspects of this disease, which must always be taken into account [7].

To investigate whether NETs played a role in the pathogenesis of psoriasis-like dermatitis, mice received 10 μg DNase I (Roche, Grenzach Wyhlen, Germany) to dissolve released NETs. The treatment was performed by daily application of DNase I, 10 μg in 100 μl DNAse buffer solution (8.77 mg/ml NaCl and 0.15 mg/ml CaCl2 in H_20) intravenously on the first day and 50 μg in 100 μl buffer solution intraperitoneally on the second to fifth day. Control mice received 100 μl buffer solution. Of note, mice that had received DNase I in this prophylactic setting neither developed any ear swelling in response to IL-23 application (Fig. 2e) nor did they show NETs or increased numbers of neutrophils in immunofluorescence staining.

In order to assess the therapeutic potential of DNase I, mice were started on DNase I from day 5 onward, when intradermal IL-23 injections had already led to ear-swelling. Compared to the group of mice that had received PBS as control, ear swelling decreased significantly (Fig. 2f).

Overall, NETs were well visible in the skin of patients with plaque psoriasis or pustular palmoplantar psoriasis as well as in a murine model of psoriasiform dermatitis. This is particularly interesting because bacterial infections are strong inducers of NET formation and often trigger psoriasis. Additionally, bacterial RNA or RNA found in human NETs can complex with LL37 (which is highly abundant in psoriatic skin) resulting in a self-propagating, vicious cycle of chronic inflammation in psoriasis [8]. Thus, NETs may indeed contribute significantly to the establishment of this inflammatory skin disease.

Disruption of NETs by DNase I in a prophylactic setting prevented the development of a murine psoriasiform dermatitis, while the same treatment in a therapeutic setting effectively reduced already established inflammation. Interestingly, an ameliorating effect for psoriasis-like lesion by NET-inhibition has also been shown in mice lacking the interleukin 36 receptor antagonist (IL36RN), a model for the rare autoinflammatory disease DITRA (deficiency of the IL36 receptor antagonist), which is associated with generalized pustular psoriasis [9].

These findings suggest that inhibitors of NET formation should be considered as therapeutic targets in psoriasis, by themselves or as add-on therapies after infections or even as topical treatments. Indeed, many current effective anti-psoriatic drugs such as dimethylfumarate [10] have been shown to inhibit NET formation, indicating that this may be a viable option to pursue more explicitly. In this context it is also interesting to note that DNase I is already approved as a drug (against cystic fibrosis) and that repurposing may be an option here.

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CRediT authorship contribution statement

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Conflict of interest

The authors have no conflict of interest to declare.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jdermsci.2023.02.006.

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