Published in final edited form as:

Br J Dermatol. 2017 December ; 177(6): e321–e322. doi:10.1111/bjd.15533.

IL-17A and IL-22 producing neutrophils in psoriatic skin

Beatrice Dyring-Andersen^{1,5}, Trine Velte Honoré¹, Ann Madelung², Michael Bzorek³, Stine Simonsen¹, Stine Novrup Clemmensen⁴, Rachael A. Clark⁵, Niels Borregaard⁴, and Lone Skov¹

¹Department of Dermato-Allergology, Herlev and Gentofte Hospital, University of Copenhagen, Denmark

²Department of Pathology, Rigshospitalet, Denmark

³Department of Surgical Pathology, Zealand University Hospital, Denmark

⁴The Granulocyte Research Laboratory, Department of Hematology, Rigshospitalet, Denmark

⁵Department of Dermatology, Brigham and Women's Hospital and Harvard Medical School, Boston, US

Keywords

IL-17; IL-22; psoriasis; T cells; neutrophil granulocytes; neutrophils; skin

TO THE EDITOR

The pro-inflammatory cytokine IL-17 is recognized as playing an important role in the pathogenesis of psoriasis, an inflammatory skin disease, and the relevance of the IL-23/IL-17 axis is underlined by the recent clinical efficacy of treatments targeting this axis ¹.

The IL-17-producing T cell subset Th17 has been closely linked to and widely studied in immune-mediated human diseases. More recently, we have learned that IL-17 is not only produced by adaptive cells, including Th17 cells, CD8⁺ T cells, and $\gamma\delta$ ⁺ T cells, but also by basophils, mast cells and neutrophils in the skin ^{2–4}. Neutrophils are prominent in lesional psoriatic skin and can accumulate in the stratum spinosum as pustules of Kogoj and in the stratum corneum in Munro's microabscesses. Although not represented in all psoriatic biopsies, these are histologically pathognomonic characteristics of psoriatic skin and strongly point toward a role of neutrophils in psoriasis that remains enigmatic.

Correspondence: Beatrice Dyring-Andersen, M.D., Ph.D., Department of Dermato-Allergology, Herlev and Gentofte Hospital, Kildegaardsvej 28, DK-2900 Hellerup, Denmark, Beatrice.Dyring-Andersen@regionh.dk, www.orcid.org/0000-0002-3012-2645.

Author disclosures

Lone Skov has received consultancy and/or speaker honoraria from Abbott, Pfizer, Janssen-Cilag, MSD and Leo Pharma and is a member of the Advisory Boards of MSD, Novartis, Eli Lilly, Abbvie, Celgene, Amgen and Janssen-Cilag.

Financial disclosures

This work was supported by an unrestricted grant from Novartis. Beatrice Dyring-Andersen is supported by grants from the Novo Nordisk Foundation, the Lundbeck Foundation, the Højmossegård Grant, the Aage Bangs Foundation, the A.P. Møller Foundation for the Advancement of Medical Sciences and Else and Mogens Wedell-Wedellsborgs Foundation. None of the funding sources have been involved in the study.

IL-17A is secreted as a homodimer and binds to its receptor IL-17R on keratinocytes, leading to increased production of the cytokines TNF- α , IL-1 β , IL-8, and IL-6^{5,6}. Stimulation of keratinocytes with IL-17 also upregulates gene expression of human β -defensin 2 and other antimicrobial peptides and chemokine CCL20^{7,8}.

Confirming our unpublished observations, human and murine studies have shown that innate immune cells are important producers of IL-17 in psoriasis^{2,3,9}. The aim of this study was to broaden our understanding of the pathogenesis of psoriasis by investigating the prevalence of IL-17-producing neutrophils in psoriatic skin compared with their IL-17-producing counterparts from the adaptive immune system. We also wanted to explore the interaction between keratinocytes and neutrophils in vitro with emphasis on the IL-23/IL-17 axis.

Briefly, double immuno-fluorescence labelling studies were performed on paraffin sections from 4 mm punch biopsies collected from 25 individuals, after obtaining informed, written consent, using anti-human IL-17 (RD systems cat. no. AF-317-NA) in combination with anti-human CD4 (clone EPR6855, Epitomics cat. no. 5636-1), anti-human CD8 (clone C8/144B, Dako cat. no. M7103) or anti-myeloperoxidase (clone 59A5, Leica cat. no. NCL-MYELO). Details of the methods of this study are described online in Supplementary Materials and Methods. We found a high prevalence of CD4⁺ T cells in psoriatic skin, especially in the dermis. As expected, the prevalence of both CD4⁺ and CD8⁺ T cells was higher in psoriatic skin than in healthy skin (Figure 2A). MPO⁺ cells, the majority comprising neutrophils, were found in psoriatic skin, albeit they were less prevalent than T cells in the dermis. A higher proportion of neutrophils were IL-17 positive compared with CD4⁺ and CD8⁺ T cells in psoriatic skin (Figure 2b). These results confirm that a substantial proportion of neutrophils produce IL-17 in lesional psoriatic skin (Figure 1). No neutrophils were observed in healthy skin; this was also as we expected.

We also wanted to investigate whether co-culture of primary keratinocytes from healthy donors could induce IL-17 production in neutrophils. Keratinocytes have been shown to increase the lifespan of neutrophils in vitro and to prime them for production of superoxide anions¹⁰. We also decided to study whether neutrophils could produce IL-22. IL-22 has been shown to be produced by murine neutrophils but, to our knowledge, has not been associated with human neutrophils¹¹. IL-22 is known to induce keratinocyte proliferation and maturation, features that are also associated with psoriasis pathology¹². Surprisingly, simply co-incubating keratinocytes for 4 hours with neutrophils could induce IL-17 and IL-22 production in neutrophils. Concurrently, a subset of neutrophils upregulate CD11b and down-regulate CD62L, suggesting a state of activation (Figure 2, details of the methods in Supplementary Materials). As controls, we incubated peripheral blood mononuclear cells (PBMC) with keratinocytes and neutrophils with control medium; neither induced IL-17- and IL-22 production. The expression of IL-17A- and IL-22-mRNA was measured by real-time quantitative polymerase chain reaction, confirming the increased expression of the two genes in neutrophils co-cultured with keratinocytes compared to directly isolated neutrophils or neutrophils in control medium (Figure 1C).

CD11b mediates adherence to keratinocytes, partly through ICAM-1¹³. Blocking ICAM1 in co-culture experiments with keratinocytes and neutrophils shows a 30% decrease in

adherence to keratinocytes. On the contrary, blocking CD11b reduces adherence even more, suggesting another binding counterpart¹³. In our experiments and the timeframe of these experiments, we observed that a subset of neutrophils did up-regulate CD11b but in our analyses of the keratinocytes, no concurrent induction of ICAM1 expression was noted. IFN- γ , used as a positive control, did induce ICAM1 expression, confirming previous findings of induction of ICAM1 expression on keratinocytes by activated T cells and their pro-inflammatory cytokines. Furthermore, we noted that a subset of keratinocytes were IL-23 positive, confirming the findings of a previous study¹⁴. Both IL-17 and IL-23 are suggested to contribute to neutrophil chemotaxis¹⁵.

In summary, this study suggests that neutrophils, long known to be a part of the histological characteristics of psoriasis, contribute to the skin inflammation by producing IL-17 and IL-22. We do not know the extent of their influence in the pathology of psoriasis or in comparison with other IL-17/IL-22 producing cell types but recent publications addressing the dermato-pathology in skin diseases such as hidradenitis suppurativa, Schnitzler's syndrome and delayed-pressure urticaria suggest a broader role of neutrophil-produced IL-17 in skin inflammation^{16,17}. Another aspect of IL-17 production by neutrophils in psoriatic inflammation is that neutrophils, comprising more than 50% of leukocytes in the blood and might contribute to the cardio metabolic co morbidities associated with severe psoriasis. Increased frequency of circulating neutrophils have been observed in patients with psoriasis, concomitant with an activated state of these cells, they may provide a link between psoriasis and cardio-vascular disease¹⁸.

Future studies will address the pathways of activation of neutrophils by keratinocytes and the temporal aspects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Acknowledgement and dedication

Professor Niels Borregaard contributed to this work and approved the final manuscript, but not the revisions. We would like to dedicate this work to his memory.

We would like to thank Ahmed Gehad and Jessica Teague for technical advice and help.

References

1. Dyring-Andersen B, Skov L, Zachariae C. Ixekizumab for treatment of psoriasis. *Expert Rev Clin Immunol*. 2015; 11:435–42. [PubMed: 25748485]
2. Res PCM, Piskin G, de Boer OJ, et al. Overrepresentation of IL-17A and IL-22 producing CD8 T cells in lesional skin suggests their involvement in the pathogenesis of psoriasis. *PLoS One*. 2010; 5:e14108. [PubMed: 21124836]
3. Lin AM, Rubin CJ, Khandpur R, et al. Mast Cells and Neutrophils Release IL-17 through Extracellular Trap Formation in Psoriasis. *J Immunol*. 2011; 187:490–500. [PubMed: 21606249]
4. Senra L, Stalder R, Alvarez Martinez D, et al. Keratinocyte-Derived IL-17E Contributes to Inflammation in Psoriasis. *J Invest Dermatol*. 2016; 136:1970–80. [PubMed: 27329229]

5. van Beelen AJ, Teunissen MBM, Kapsenberg ML, de Jong EC. Interleukin-17 in inflammatory skin disorders. *Curr Opin Allergy Clin Immunol*. 2007; 7:374–81. [PubMed: 17873575]
6. Teunissen MBM, Koomen CW, de Waal Malefyt R, et al. Interleukin-17 and Interferon- γ Synergize in the Enhancement of Proinflammatory Cytokine Production by Human Keratinocytes. *J Invest Dermatol*. 1998; 111:645–9. [PubMed: 9764847]
7. Nograles KE, Zaba LC, Guttman-Yassky E, et al. Th17 cytokines interleukin (IL)-17 and IL-22 modulate distinct inflammatory and keratinocyte-response pathways. *Br J Dermatol*. 2008; 159:1092–102. [PubMed: 18684158]
8. Cho K-A, Suh JW, Lee KH, et al. IL-17 and IL-22 enhance skin inflammation by stimulating the secretion of IL-1 β by keratinocytes via the ROS-NLRP3-caspase-1 pathway. *Int Immunol*. 2012; 24:147–58. [PubMed: 22207130]
9. Reich K, Papp KA, Matheson RT, et al. Evidence that a neutrophil-keratinocyte crosstalk is an early target of IL-17A inhibition in psoriasis. *Exp Dermatol*. 2015; 24:529–35. [PubMed: 25828362]
10. Guérard S, Allaeys I, Martin G, et al. Psoriatic keratinocytes prime neutrophils for an overproduction of superoxide anions. *Arch Dermatol Res*. 2013; 305:879–89. [PubMed: 23974213]
11. Tecchio C, Cassatella MA. NEUTROPHIL-DERIVED CYTOKINES: FACTS BEYOND EXPRESSION. *Front Immunol*. 2014; :5.doi: 10.3389/fimmu.2014.00508 [PubMed: 24478774]
12. Aujla SJ, Chan YR, Zheng M, et al. IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. *Nat Med*. 2008; 14:275–81. [PubMed: 18264110]
13. Barker JN, Groves RW, Allen MH, MacDonald DM. Preferential adherence of T lymphocytes and neutrophils to psoriatic epidermis. *Br J Dermatol*. 1992; 127:205–11. [PubMed: 1356409]
14. Piskin G, Sylva-Steenland RMR, Bos JD, Teunissen MBM. In Vitro and In Situ Expression of IL-23 by Keratinocytes in Healthy Skin and Psoriasis Lesions: Enhanced Expression in Psoriatic Skin. *J Immunol*. 2006; 176:1908–15. [PubMed: 16424222]
15. Lemos HP, Grespan R, Vieira SM, et al. migration in inflammation by inhibiting IL-12 and IFN γ production. *Pnas*. 2008:1–6.
16. Lima AL, Karl I, Giner T, et al. Keratinocytes and neutrophils are important sources of proinflammatory molecules in hidradenitis suppurativa. *Br J Dermatol*. 2015:514–21. [PubMed: 26436522]
17. de Koning HD, van Vlijmen-Willems IMJJ, Rodijk-Olthuis D, et al. Mast cell interleukin-1 beta, neutrophil interleukin-17 and epidermal antimicrobial proteins in the neutrophilic urticarial dermatosis in Schnitzler's syndrome. *Br J Dermatol*. 2015:448–56. [PubMed: 25904179]
18. Naik HB, Natarajan B, Stansky E, et al. Severity of Psoriasis Associates with Aortic Vascular Inflammation Detected by FDG PET/CT and Neutrophil Activation in a Prospective Observational Study. *Arterioscler Thromb Vasc Biol*. 2015; 35:2667–76. [PubMed: 26449753]

Figure 1. Neutrophil granulocytes co-express IL-17 in psoriatic skin

Double immunohistochemical stainings for IL-17 (green) and myeloperoxidase (MPO, red) of lesional psoriatic skin. Double positive cells were observed in both dermis and epidermis and the lobed nuclei of the neutrophils are visualized (inset) with DAPI (blue).