

## INVESTIGATIVE REPORT

# Tumour Necrosis Factor-alpha and Matrix Metalloproteinase-2 are Expressed Strongly in Hidradenitis Suppurativa

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**Hidradenitis suppurativa is a chronic skin condition, characterized clinically by painful, recurrent, deep-seated nodules and suppuration, and histologically by hypertrophic scarring of apocrine gland bearing skin and sinus tracts. The overall consequence of the disease is considerable tissue remodelling and the underlying alterations in innate immunity are poorly understood. The aim of this study was to evaluate the expression of human beta-defensin 2, tumour necrosis factor (TNF)- $\alpha$  and matrix metalloproteinase-2 in skin lesions of patients with hidradenitis suppurativa. A total of 14 skin samples from patients and 2 skin samples from healthy volunteers were evaluated by immunohistochemistry. Human beta-defensin 2 was negative in 12/14 specimens. Elevated expression of metalloproteinase-2 was observed in keratinocytes, fibroblasts and inflammatory cells in dermis, sweat glands, hair follicles and sinus tracts, suggesting a key role for hidradenitis suppurativa pathogenesis. Decreased human beta-defensin 2 in the presence of inflammatory (TNF- $\alpha$ -containing) cells suggests a decreased innate immunity in hidradenitis suppurativa-affected skin. Key words: hidradenitis suppurativa; antimicrobial peptides; cytokines; innate immunity; matrix metalloproteinase-2.**

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Hidradenitis suppurativa (HS) is a chronic skin condition characterized by painful, deep-seated, recurrent nodules commonly ending in abscesses, and sinus tracts with suppuration and hypertrophic scarring of apocrine gland bearing skin (1). The histological picture varies with the severity of the disease. Perifollicular lymphocytic inflammation and small or absent sebaceous glands appear to be early changes, whereas poral occlusion, inflammation of hair follicle, apocrine and eccrine sweat glands, diffuse inflammation in dermis, abscesses and scarring occur later (2, 3). Clinically and histologically tissue remodelling occurs. A hallmark histological feature of HS is the sinus tract; an elongated cavity extending into the dermis or even subcutis with squamous epithelium

lining the walls (4). It is thought that sinus tracts represent expansive growth due to tissue remodelling/healing of the lesions based on the finding of immature keratins and stem-cell-like cells (5, 6).

HS has a complex pathogenesis that involves follicular inflammation. Genetic, endocrinological, bacterial, environmental and pharmacological factors, as well as smoking and obesity, have been described as possible pathogenic factors (7, 8). The observation that tumour necrosis factor (TNF)- $\alpha$  inhibitors may be used in the treatment of HS has led recently to increased interest in the role of the immune system and inflammation in HS (9–12). Disturbances of the innate immune system have been described (13–16).

The aim of this study was therefore to evaluate the expression of human beta-defensin 2 (HBD2), TNF- $\alpha$  and matrix metalloproteinase-2 (MMP-2) in skin lesions of patients with HS.

## MATERIALS AND METHODS

The patient group comprised 11 female and 3 male patients with HS, age range 22–59 years. All patients were Caucasian. Half of the patients had axillar and half had inguinal lesions. All specimens contained sinus tracts, as seen in preparations stained with haematoxylin and eosin. As a negative control we used healthy skin tissue from a similar region obtained from 2 volunteers, after obtaining written informed consent. The study was performed according to the principles of the Declaration of Helsinki and was approved by the local ethics committee.

Sections 4  $\mu$ m thick were prepared from the paraffin-embedded tissue. Human beta-defensin 2 (cat no. AF 2758, LOT VJU015051, obtained from goat, 1:100 dilution, R&D Systems, Germany), TNF- $\alpha$  (code ab 6671, obtained from rabbit, 1:100 dilution, Abcam, Cambridge, UK) and MMP-2 (cat no. AF902, LOT DUB034081, obtained from goat, 1:100 dilution, R&D Systems) were used in biotin–streptavidin immunohistochemistry (17) and 3 slides per biopsy were examined.

The intensity of immunostaining was graded semiquantitatively. Samples with few positive structures in the visual field were labelled +, samples with a moderate number of positive structures in the visual field were labelled ++, samples with numerous positive structures in the visual field were labelled +++, and those with an abundance of positive structures in the visual field were labelled ++++ (18).

Non-parametric statistics were used and Spearman's rank correlation coefficient was calculated to compare co-expression of the markers in the biopsies.

Findings were photographed with a Leica DC 300F camera and analysed with image-processing and analysis software Image Pro Plus 6.0 (Media Cybernetics, Silver Spring, Maryland, USA).

## RESULTS

Sinus tracts and cysts, large intradermal inflammatory infiltrates containing an abundance of plasma cells, lymphocytes and epithelioid cells were observed. Arteriole sclerosis and destruction of hair follicle structures was also observed. Major secondary changes due to inflammation were seen in the morphology of apocrine sweat glands, where prominent, almost total, vacuolization in the glandular cells was observed. At the same time, we found groups of apocrine sweat glands presenting also atrophy and proliferation (Fig. 1).

HBD2 stained negative in 12/14 samples, where some weakly stained cells were detected. MMP-2 and TNF- $\alpha$  positive structures were found in all skin samples. MMP-2 was seen in keratinocytes, fibroblasts and inflammatory cells (macrophages and lymphocytes) in dermis, sweat glands, outer epithelial sheath of hair follicle and sinus tracts (Fig. 2). A moderate number of TNF- $\alpha$ -positive inflammatory cells was seen in the dermis, but the level varied from only a few cells to an abundance of strongly positive cells, correlated with the level of inflammation in the tissue. Cytokine-positive macrophages were seen both around the sweat glands and hair follicles (Fig. 3).

In comparison with skin from patients with HS, in healthy skin from controls, TNF- $\alpha$ -positive cells were not present, while a moderate number of HBD2- and MMP-2-positive structures were found in the epithelium, subepithelium and sweat glands. All semiquantitative results are summarized in Table I.

## DISCUSSION

This study suggests that mature HS lesions do not contain HBD2, while both TNF- $\alpha$  and MMP-2 are found.

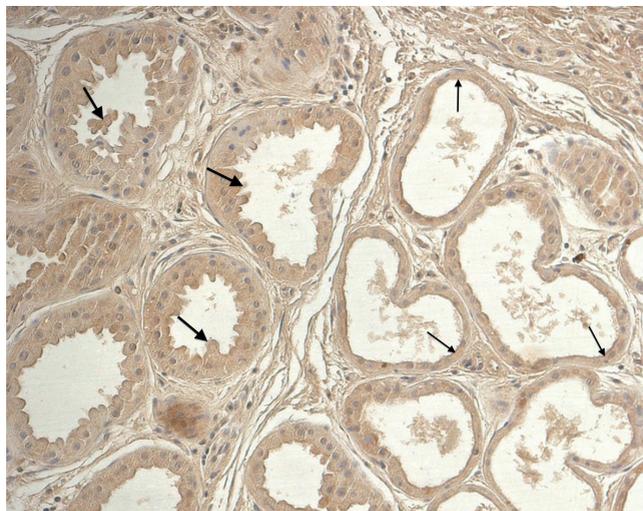


Fig. 1. Groups of apocrine sweat glands showing concomitant proliferation and atrophy. Broad arrow pointing at the proliferating glands, thin one at the atrophic ones. Matrix metalloproteinase-2 immunohistochemistry,  $\times 200$ .



Fig. 2. Metalloproteinase-2 (MMP-2)-positive terminal follicle at infra-fundibular region and surrounding large plasma cells-dominating inflammatory infiltrate. Numerous epitheliocytes, macrophages and fibroblasts expressing matrix metalloproteinase. Broad arrow at the inflammatory cells and thin at the epidermis. MMP-2 immunohistochemistry,  $\times 100$ .

HBD2 has not been extensively studied in HS, although it is one of the most common antimicrobial peptides of the skin (19). Only 2/14 lesions stained positively for HBD2, suggesting that it is either consumed or not expressed. Antimicrobial peptides are a very large and diverse part of the innate immune system. A recent study examined the levels of HBD3 in lesional skin, and found unchanged levels in very severe HS, while expression of the antimicrobial peptide ribonuclease 7 was diminished and dermcidin levels were unchanged compared with healthy skin (20). Another recent study evaluated HBD2 and HBD4 in lesional and non-lesional untreated HS skin and, in agreement with our observations, found significantly decreased expression compared with healthy skin (15).

HBD2 appears to be the first human defensin produced when epithelial cells are stimulated by, for example, microorganisms such as *Pseudomonas aeruginosa*, or cytokines such as TNF- $\alpha$  and interleukin (IL)-1 beta. The lack of HBD2 expression is therefore noteworthy in the presence of the TNF- $\alpha$ -rich infiltrate found in HS. The mechanism behind the lack of HBD2 is not clear. TNF- $\alpha$  induces further expression of HBD2, and was expressed prominently in our skin sample. However, the skin microflora was not evaluated at the same time, and could have been of importance in the induction of HBD2. As defensin plays an important role in protection against Gram-negative bacteria, we therefore cannot exclude Gram-negative bacteria as a potential causative factor of HS.

TNF- $\alpha$  in HS has been studied previously, partly due to the observation that TNF- $\alpha$  inhibitors may be used to treat the condition. We found a wide distribution of TNF- $\alpha$ -positive cells in the tissue, providing broad contact with epithelia, where HBD2 expression could have

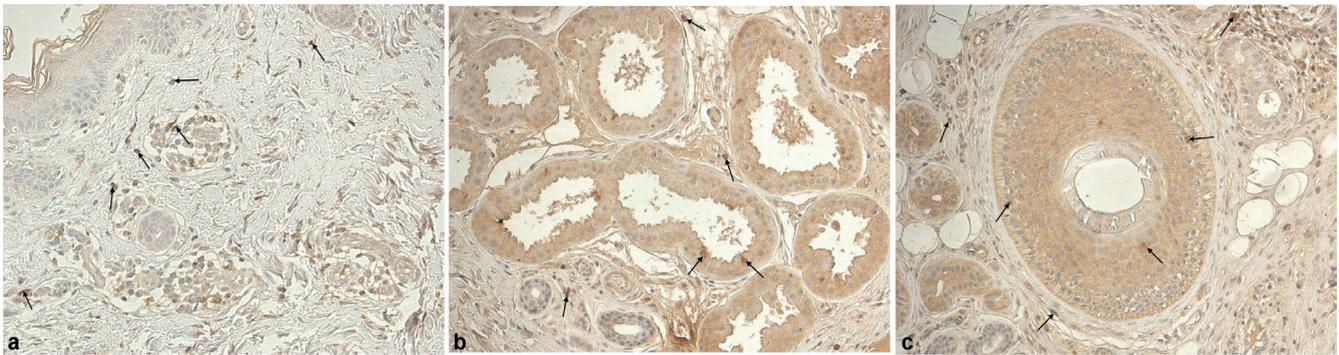


Fig. 3. (a) Tumour necrosis factor (TNF)- $\alpha$  positive inflammatory cells (mainly macrophages and lymphocytes) in subepithelium (arrows). (b) TNF- $\alpha$  positive structures in apocrine sweat glands (arrows). (c) Outer epithelial root sheet of hair follicle (arrows). TNF- $\alpha$  immunohistochemistry,  $\times 200$ .

been induced. A previous study detecting serum TNF- $\alpha$  levels has also shown significantly higher levels in HS patients compared with healthy controls (11). This was supported by a study suggesting that isolated monocytes from the peripheral blood of HS patients were unable to produce more TNF- $\alpha$  on stimulation, suggesting that they were completely stimulated *in vitro* (21). TNF- $\alpha$  has also been found previously to be significantly elevated in HS lesional and perilesional skin, together with interleukin-1- $\beta$  and interleukin-10 (9). Our study focused on TNF- $\alpha$  expression in inflammatory cells in HS-affected tissue. The results support the theory of a notable presence of TNF- $\alpha$ , and thus probably support the rationale for therapy with anti-inflammatory blockers in patients with HS.

Finally, we detected vigorous expression of MMP-2 in HS-affected skin. While it is important to mention that MMP matrilysin has been found to participate in the normal function of dermal exocrine glands (22), we speculate that MMP-2, which is a tissue degradation enzyme, is a common key event for HS due to the expansive growth of the lesions, which links to the theory that HS is due to dysregulated repair of unspecific

tissue damage. In addition, the excessive expression of MMP-2, providing a boundless proteolytic environment, may be the inactivator of HBD2 and accordingly limit antimicrobial defence in patients with HS. Interestingly, MMP-2 has been detected previously in significantly higher levels in neutrophilic dermatoses, and various studies report a role of MMP-2 in cancer (23–29). It may therefore be speculated that the increased MMP-2 expression is related to the histological picture of HS, with its proliferating epithelial strands and sinus tract formation (30). Elevated expression of MMP-2 observed in keratinocytes, fibroblasts and inflammatory cells in dermis, sweat glands, outer epithelial sheath of hair follicle and sinus tracts suggests its key role in HS pathogenesis.

A decrease in the antimicrobial protein HBD2 on the background of presence of inflammatory cytokines (TNF- $\alpha$ -containing cells) indicates a decreased innate immunity in HS-affected skin.

Table 1. Semiquantitatively evaluated expression of human beta-defensin 2 (HBD2), tumour necrosis factor (TNF)- $\alpha$  and metalloproteinase-2 (MMP-2) in hidradenitis suppurativa lesions

| Pat. no.  | Age/sex | Lesion   | HBD2 | TNF- $\alpha$ | MMP-2    |
|-----------|---------|----------|------|---------------|----------|
| 1         | 30/F    | Axillar  | -    | +             | ++/+++   |
| 2         | 42/F    | Inguinal | -    | ++            | +++      |
| 3         | 25/F    | Inguinal | -    | ++            | +++/++++ |
| 4         | 22/F    | Axillar  | -    | ++/+++        | +++      |
| 5         | 45/F    | Inguinal | -    | ++            | +++/++++ |
| 6         | 27/F    | Axillar  | -    | +++           | +++      |
| 7         | 38/M    | Axillar  | -/+  | +             | +++      |
| 8         | 34/M    | Axillar  | -    | +++/++++      | ++       |
| 9         | 59/F    | Inguinal | +    | ++            | +++      |
| 10        | 41/F    | Axillar  | -    | +             | +++      |
| 11        | 33/F    | Axillar  | -    | +             | +++      |
| 12        | 46/F    | Inguinal | -    | +++           | +++/++++ |
| 13        | 49/F    | Inguinal | -    | ++++          | ++       |
| 14        | 31/M    | Inguinal | -/+  | ++            | +++/++++ |
| Median    |         |          | -    | ++            | +++      |
| Control 1 | 35/F    | Inguinal | ++   | -             | ++       |
| Control 2 | 44/M    | Axillar  | ++   | -             | ++       |

REFERENCES

1. Jemec GB. Hidradenitis suppurativa. *N Engl J Med* 2012; 366: 158–164.
2. Boer J, Weltevreden EF. Hidradenitis suppurativa or acne inversa. A clinicopathological study of early lesions. *Br J Dermatol* 1996; 135: 721–725.
3. Kamp S, Fiehn AM, Stenderup K, Rosada C, Pakkenberg B, Kemp K, et al. Hidradenitis suppurativa: a disease of the absent sebaceous gland? Sebaceous gland number and volume are significantly reduced in uninvolved hair follicles from patients with hidradenitis suppurativa. *Br J Dermatol* 2011; 164: 1017–1022.
4. Jemec GB, Hansen U. Histology of hidradenitis suppurativa. *J Am Acad Dermatol* 1996; 34: 994–999.
5. Kurokawa I, Nishijima S, Suzuki K, Kusumoto K, Sensaki H, Shikata N, et al. Cytokeratin expression in pinoidal sinus. *Br J Dermatol* 2002; 146: 409–413.
6. Gniadecki R, Jemec GB. Lipid raft-enriched stem cell-like keratinocytes in the epidermis, hair follicles and sinus tracts in hidradenitis suppurativa. *Exp Dermatol* 2004; 13: 361–363.
7. Alikhan A, Lynch PJ, Eisen DB. Hidradenitis suppurativa: a comprehensive review. *J Am Acad Dermatol* 2009; 60: 539–561.
8. Sartorius K, Emtestam L, Jemec GB, Lapins J. Objective scoring of hidradenitis suppurativa reflecting the role of tobacco

- smoking and obesity. *Br J Dermatol* 2009; 161: 831–839.
9. Van der Zee HH, de Ruyter L, van den Broecke DG, Dik WA, Laman JD, Prens EP. Elevated levels of tumour necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\beta$  and IL-10 in hidradenitis suppurativa skin: a rationale for targeting TNF- $\alpha$  and IL-1 $\beta$ . *Br J Dermatol* 2011; 164: 1292–1298.
  10. Haslund P, Lee RA, Jemec GB. Treatment of hidradenitis suppurativa with tumour necrosis factor-alpha inhibitors. *Acta Derm Venereol* 2009; 89: 595–600.
  11. Matusiak L, Bieniek A, Szepietowski JC. Increased serum tumour necrosis factor-alpha in hidradenitis suppurativa patients: is there a basis for treatment with anti-tumour necrosis factor-alpha agents? *Acta Derm Venereol* 2009; 89: 601–603.
  12. Van der Zee HH, Laman JD, de Ruyter L, Dik WA, Prens EP. Adalimumab (anti-TNF-alpha) treatment of hidradenitis suppurativa ameliorates skin inflammation: an in situ and ex vivo study. *Br J Dermatol* 2012; 166: 298–305.
  13. Nazary M, der Zee HV, Prens E, Folkerts G, Boer J. Pathogenesis and pharmacotherapy of hidradenitis suppurativa. *Eur J Pharmacol* 2011; 672: 1–8.
  14. Brunasso AM, Massone C. Rotational therapy with TNF-alpha blockers for recalcitrant hidradenitis suppurativa. *Eur J Dermatol* 2010; 20: 644–646.
  15. Dréno B, Khammari A, Brocard A, Moysé D, Blouin E, Guillet G, et al. Hidradenitis suppurativa: the role of deficient cutaneous innate immunity. *Arch Dermatol* 2012; 148: 182–186.
  16. Schlapbach C, Haani T, Yawalkar N, Hunger RE. Expression of the IL-23/Th17 pathway in lesions of hidradenitis suppurativa. *J Am Acad Dermatol* 2011; 65: 790–798.
  17. Hsu S, Raine L, Fanger H. The use of antiavidin antibody and avidin-biotin peroxidase complex in immunoperoxidase techniques. *Am J Clin Pathol* 1981; 75: 816–821.
  18. Pilmane M, Luts A, Sundler F. Changes in neuroendocrine elements in bronchial mucosa in chronic lung disease in adults. *Thorax* 1995; 50: 551–554.
  19. Schroder JM, Harder J. Human beta-defensin-2. *Int J Biochem Cell Biol* 1999; 31: 645–651.
  20. Hofmann SC, Saborowski V, Lange S, Kern WV, Bruckner-Tuderman L, Rieg S. Expression of innate defense antimicrobial peptides in hidradenitis suppurativa. *J Am Acad Dermatol* 2012; 66: 966–974.
  21. Giamarellos-Bourboulis EJ, Antonopoulou A, Petropoulou C, Moukhtaroudi M, Spyridaki E, Baziaka F, et al. Altered innate and adaptive immune responses in patients with hidradenitis suppurativa. *Br J Dermatol* 2007; 156: 51–56.
  22. Saarialho-Kere UK, Crouch EC, Parks WC. Matrix metalloproteinase matrilysin is constitutively expressed in adult human exocrine epithelium. *J Invest Dermatol* 1995; 105: 190–196.
  23. Marzano AV, Cugno M, Trevisan V, Lazzari R, Fanoni D, Berti E, et al. Inflammatory cells, cytokines and matrix metalloproteinases in antimicrobial pustulosis of the folds and other neutrophilic dermatoses. *Int J Immunopathol Pharmacol* 2011; 24: 451–460.
  24. Orimoto AM, Neto CF, Pimentel ER. High numbers of human skin cancers express MMP2 and several integrin genes. *J Cutan Pathol* 2008; 35: 285–291.
  25. Vaisanen A, Kuvaja P, Kallioinen M, Turpeenniemi-Hujanen T. A prognostic index in skin melanoma through the combination of matrix metalloproteinase-2, Ki67, and p53. *Hum Pathol* 2011; 42: 1103–1111.
  26. Patel S, Sumitra G, Koner BC, Saxena A. Role of serum matrix metalloproteinase-2 and -9 to predict breast cancer progression. *Clin Biochem* 2011; 44: 869–872.
  27. Peng ZH, Wan DS, Li LR, Chen G, Lu ZH, Wu XJ, et al. Expression of COX-2, MMP-2 and VEGF in stage II and III colorectal cancer and the clinical significance. *Hepato-gastroenterology* 2011; 58: 369–376.
  28. Li J, Lau GK, Chen L, Dong SS, Lan HY, Huang XR, et al. Interleukin 17A promotes hepatocellular carcinoma metastasis via NF-kB induced matrix metalloproteinases 2 and 9 expression. *PLoS One* 2011; 6: e21816.
  29. Reno F, Rocchetti V, Migliario M, Rizzi M, Cannas M. Chronic exposure to cigarette smoke increases matrix metalloproteinases and Fillagrin mRNA expression in oral keratinocytes: role of nicotine stimulation. *Oral Oncol* 2011; 47: 827–830.
  30. van der Zee HH, de Ruyter L, Boer J, van den Broecke DG, den Hollander JC, Laman JD, et al. Alterations in leukocyte subsets and histomorphology in normal-appearing perilesional skin and early and chronic hidradenitis suppurativa lesions. *Br J Dermatol* 2012; 166: 98–106.