

## INVESTIGATIVE REPORT

# Neutrophil-related Host Response in Hidradenitis Suppurativa: A Pilot Study in Patients with Inactive Disease

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**Host-defence defects in hidradenitis suppurativa patients have been suspected, but not proven. Activated neutrophils can destroy the surrounding tissues by a release of reactive oxygen species and active proteases. Peripheral neutrophils from 15 female patients (mean age 46, range 27–57 years) in an inactive state of their hidradenitis suppurativa, were studied and compared with 15 age-matched healthy female controls. There were no significant differences between patients and controls in the assessments of intracellular elastase activity, total content of antigenic elastase or release of elastase. Furthermore, no differences were found in total content and membrane expression of the receptors measured. The generation of free oxygen radicals, after stimulation with the protein kinase C activator phorbol myristate acetate, was significantly higher in the patients than in the controls, while there was no difference after Fc-receptor-mediated stimulation. Dysfunctional neutrophils might be involved in the pathogenesis of hidradenitis suppurativa, but the findings should be interpreted with caution because of the small number of observed cases. Key words: hidradenitis suppurativa; neutrophils; free oxygen radicals.**

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Hidradenitis suppurativa (HS, syn. acne inversa) (1) is a cicatrizing and frequently persistent disease of apocrine gland-bearing areas in the adult (2). It may range in severity from a few, but recalcitrant suppurating lesions to an advanced widespread and disabling disease. The aetiology is obscure, but genetic predisposition, comedones occluding the pilosebaceous apparatus, bacterial infections (3) and hormones (4) are among the factors mentioned. An association with acne has been discussed (1). Antibiotic treatment is seldom successful in the long run and the chronic forms of HS usually require surgical treatment (1, 5). The earliest inflammatory event in HS is rupture of the follicular epithelium, followed by spilling of foreign-body material such as keratin, bacteria, sebum products and hair into the dermis. The “dumping” of foreign products initiates an inflammatory response, resulting in a foreign-body granuloma. Epithelial strands form sinuses in this inflammatory tissue. Bacterial colonization, usually by coagulase-negative staphylococci, can aggravate the chronic inflammation in this milieu (6). Defects in host-defence mechanisms have been suspected, but this aspect of HS has not been adequately studied (7).

Activated neutrophils are involved in many inflammatory diseases and can damage the surrounding tissues by simultaneous release of reactive oxygen species and active proteases (8, 9). Hyperreactive neutrophils have been proposed to be of pathophysiological importance in periodontitis (10–13), which is a chronic inflammatory disease with destruction of tooth-supporting tissue. The host defence in HS may be compromised, either generally or locally. The purpose of this pilot study was to compare the release of oxygen radicals and primary granular release from *in vitro* activated peripheral neutrophils from HS patients with inactive disease and healthy controls. A further objective was to describe the degree of activation measured as receptor expression.

## MATERIAL AND METHODS

### *Patients and healthy controls*

The patients with HS consisted of 15 women (mean age 46 years, range 27–57) and the control group of 15 age-matched healthy women (mean age 46 years, range 25–56). All patients were stage II cases according to clinical staging, as adapted from Hurley (14), i.e. recurrent abscesses with tract formation and cicatrization as well as single or multiple, widely separated lesions, previously selected for surgery (15). No cases of concurrent diseases with a fistulizing tendency, such as regional enterocolitis, ulcerative colitis or rheumatoid arthritis, were included. The patients were otherwise known to be healthy. At the time of blood sampling, they had been without HS symptoms for 6 months or more. To exclude individuals with active inflammation for any reason, the numbers of white blood cells, neutrophilic granulocytes, lymphocytes and monocytes were analysed with a Coulter STKS (Coulter Electronics, Hialeah, FL, USA). The plasma concentrations of C-reactive protein (CRP),  $\alpha_1$ -antitrypsin, immunoglobulins and haptoglobin were determined with a Nephelometer-Analyzer (Behringwerke AG Diagnostica, Marburg, Germany).

### *Monoclonal antibodies*

Elastase, CD15 (Lewis x) and CD16 (Fc $\gamma$ RIII) were measured with monoclonal antibodies (mAbs) from Dakopatts a/s (Glostrup, Denmark). Antibodies against CD 11b (complement receptor 3) and CD35 (complement receptor 1) were obtained from Immunotech (Marseille, France). The antibodies against the specific phenotypes of Fc $\gamma$ RIIIb-Na1 (CD16b) and Fc $\gamma$ RIIIb-Na2 (CD16) were purchased from CLB (Amsterdam, The Netherlands) and Harlan Sera-Lab (Loughborough, UK), respectively. As negative controls, unconjugated mouse immunoglobulin G (IgG) and fluorescein isothiocyanate (FITC)-conjugated mouse IgG and IgM (Dakopatts a/s, Glostrup, Denmark) were used.

### *Cell preparation*

A neutrophil preparation from venous ethylenediamine tetraacetic acid (EDTA) blood was made by lysing the red blood cells, followed by density centrifugation on a 2-layer discontinuous Percoll gradient (16). After counting, the cells were stored for a maximum of 90 min

in phosphate-buffered saline (PBS), pH 7.4, with 0.25% human serum albumin at +4°C, until used.

#### Chemiluminescence

Analysis of radical generation using luminol-enhanced chemiluminescence (CL) was done on  $0.25 \times 10^6$  neutrophils after Fc $\gamma$ -receptor stimulation with opsonized *Staphylococcus aureus* (bacteria:cell ratio = 300:1) or with 150 pmol phorbol myristate acetate (PMA, Sigma Chemical Co., St Louis, MO, USA). The procedure of bacteria opsonization with commercial  $\gamma$ -globulin and the method of CL have been described elsewhere (16). The CL reaction was followed until maximal light intensity was reached and recorded as  $mV_{peak}$ . Unstimulated neutrophils gave no measurable CL.

#### Degranulation and release of elastase after Fc $\gamma$ -receptor stimulation

The method has been described elsewhere (13). In brief, neutrophils ( $1.0 \times 10^6$ ) were incubated with the opsonized *Staphylococcus aureus* (bacteria:cell ratio = 20:1) or without the bacteria as a control in Hanks' balanced salt making up a final reaction volume of 1 ml for 1 h during horizontal agitation (100 oscillations/min). The reaction was stopped by centrifugation at 1000 g for 5 min, and the supernatant was removed and stored at -70°C. The content of elastase was measured, using 100  $\mu$ l of the supernatants with 67  $\mu$ l neutrophil elastase substrate S-2484 (1-pyroglyutamyl-1-prolyl-1-valine-p-nitroaniline; Chromogenix AB, Mölndal, Sweden) on 96-well microtitre plates after 5 h incubation at +37°C using a spectrophotometer (Millenia Kinetic Analyzer; Diagnostic Product Corporation, Los Angeles, CA, USA). The elastase activity was expressed as the instrument's units of absorbance at 405 nm, mAbs.

#### Flow cytometry

Neutrophils (250,000 cells) were incubated with 5  $\mu$ l of undiluted antibody for the indicated receptor molecule at room temperature for 15 min, fixed for 15 min and washed once with PBS containing EDTA (40 mg/1000 ml PBS). The cell samples to be analysed for total receptor content (intracellular and membrane-bound, elastase, CD16 and CD11b) were also incubated with permeabilization fluid (Fix and Perm; Caltag, Burlingame, CA, USA) for 15 min at room temperature, while the samples to be analysed for membrane expression alone were incubated with PBS in the same manner. All samples were stained once again with the respective antibody and the unconjugated antibodies against CD16 Na1 and CD16 Na2 were also stained with a FITC-conjugated goat antimouse IgG (Dakopatts a/s, Glostrup, Denmark). All samples were washed as above and resuspended in PBS + EDTA. Immunofluorescence was measured on an Epics®-profile II Flow Cytometer (Coulter Corporation, Hialeah, FL, USA), as described earlier (17).

Intracellular elastase activity was studied with the Ala-Ala-Pro-Val elastase enzyme substrate (AAPV-Elastase, Cell Probe™, Miami, FL, USA) used in accordance with the manufacturer's instructions, and the fluorescence measured flow cytometrically.

#### Statistics

The significance of the differences between the means in patients and controls was calculated with the Mann-Whitney U-test.  $p > 0.05$  was considered not significant (ns).

## RESULTS

The analysis of acute-phase reactants and blood cell counts showed that all values were within the normal reference range and there was no difference between the 2 groups, with the exception of higher haptoglobin mean concentrations in the HS group (Table I). However, all the patients were well within the normal range for haptoglobin.

The generation of free oxygen radicals in the neutrophils after activation of their Fc $\gamma$ -receptors was measured as

Table I. Blood counts and acute-phase reactants (mean  $\pm$  SD) from patients with hidradenitis suppurativa and controls, all within the normal range.

	Patients (n = 15)	Controls (n = 15)
White blood cells (cells/ $1 \times 10^9$ )	6.2 $\pm$ 1.0	6.6 $\pm$ 1.8
Neutrophils (cells/ $1 \times 10^9$ )	3.6 $\pm$ 0.8	4.1 $\pm$ 1.5
Lymphocytes (cells/ $1 \times 10^9$ )	2.0 $\pm$ 0.5	1.8 $\pm$ 0.5
Monocytes (cells/ $1 \times 10^9$ )	0.32 $\pm$ 0.08	0.39 $\pm$ 0.10
Eosinophils (cells/ $1 \times 10^9$ )	0.51 $\pm$ 0.08	0.20 $\pm$ 0.12
Basophils (cells/ $1 \times 10^9$ )	0.04 $\pm$ 0.01	0.04 $\pm$ 0.02
$\alpha_1$ -antitrypsin (g/l)	1.33 $\pm$ 0.22	1.35 $\pm$ 0.31
C-reactive protein (g/l)	2.5 $\pm$ 3.1	3.6 $\pm$ 4.8
Haptoglobin (g/l)	1.34 $\pm$ 0.44	0.97 $\pm$ 0.38
Immunoglobulin G (g/l)	9.5 $\pm$ 2.3	10.7 $\pm$ 1.8

luminol-enhanced CL. The mean CL was slightly increased in the patients (about 25%), but not significantly different from that in controls. However, another stimulation mode, activation of intracellular protein kinase C (PKC) by PMA, resulted in a significantly higher CL value in the patients (about 50%,  $p = 0.021$ ) (Fig. 1). There was no significant difference between patients and controls in primary granular elastase release during Fc $\gamma$ -mediated activation ( $1165 \pm 626$  mAbs and  $1086 \pm 725$  mAbs, respectively).

No significant difference was noted between patients with HS and controls in the intracellular elastase activity and the total content of antigenic elastase when measured by a flow-cytometric method with elastase substrate and a FITC-conjugated antibody, respectively. Similar results were found in the flow-cytometric analysis of the various receptor molecules, even though a slight increase in membrane expression was detected in the patients with HS (Table II). No difference was found between patients and controls in the distribution of the 2 Fc $\gamma$ RIIIb phenotypes, Na1 and Na2 (data not shown).

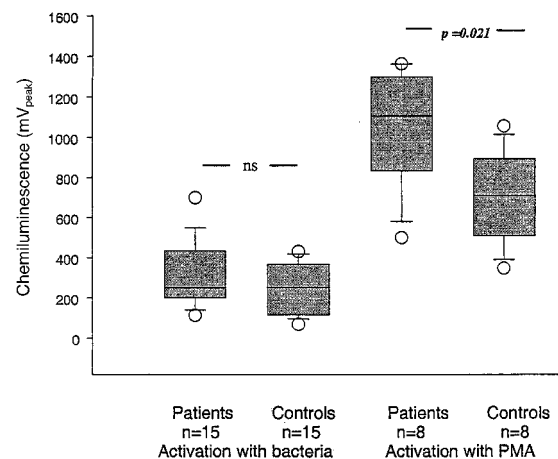


Fig. 1. Maximal production of free oxygen radicals after stimulation with opsonized bacteria and phorbol myristate acetate (PMA), respectively. The box plot shows 25th, median and 75th percentiles, error bars indicate 10th and 90th percentiles, and circles are the extremes. The differences between patients with hidradenitis suppurativa and controls were calculated with the Mann-Whitney U-test.  $p > 0.05$  is considered not significant (ns).

Table II. Results of flow-cytometric analysis

	Patients (IFL/cell)	Controls (IFL/cell)	n (pairs)
<i>Elastase activity</i>			
Intracellular elastase	157 ± 146	140 ± 124	12
Total elastase	68 ± 34	58 ± 26	12
<i>Fcγ-receptors</i>			
Membrane CD16	216 ± 48	202 ± 39	12
Total CD16	270 ± 93	320 ± 127	9
Membrane CD64	8 ± 2	8 ± 1	5
<i>Complement receptors</i>			
Membrane CD35	30 ± 10	29 ± 10	5
Membrane CD11b	54 ± 39	46 ± 26	12
Total CD11b	87 ± 22	84 ± 13	10
<i>Diapedes receptor</i>			
Membrane CD15	50 ± 19	45 ± 14	5

The mean ± SD of immunofluorescence (IFL) per cell is given. Differences between the means of patients with hidradenitis suppurativa and controls were evaluated with the Mann-Whitney *U*-test. No significant differences ( $p > 0.05$ ) were found.

## DISCUSSION

To the authors' knowledge, only one study has been conducted on the host response in HS (7). Over 20 years ago, Dvorak et al. (7) measured the percentage of engulfed bacteria opsonized with autologous serum and plasma immunoglobulins and found no differences between patients and healthy controls. In this study the acute-phase reactants and blood cell counts were similar in both groups and within the normal ranges in all patients. The HS patients were selected after surgical treatment, when they were healed, without symptoms of HS and with normal routine laboratory analyses. The patients and their negative controls had no detectable general inflammation that would have affected the activities of the neutrophils. Thus, the systemic effects of lesions in this postoperative quiescent state of HS were considered negligible.

The Fcγ-receptor-mediated CL was moderately increased in the patients, as was the membrane expression of FcγRIII (Table II), but not related to a difference in receptor avidity, since the distributions of the phenotypes of the Na1 and Na2 receptors were similar in patients and controls (data not shown). The results suggest that local stimulation of these receptors in the lesions may not have conclusive importance in HS. The membrane expression of CR3 (CD11b) involved in complement-mediated phagocytosis also showed a tendency to increase. However, CL induced by this type of stimulation was not studied.

There was an increased radical generation in HS patients after PMA stimulation. PMA is a well-known direct activator of PKC without any need for membrane receptors. It mimics the proinflammatory agent bradykinin, which has vasodilatory properties and also increases the permeability of the microvessels (18). It seems unlikely that this higher sensitivity of PKC to PMA and possibly also bradykinin stimulation in the patients with HS was induced by the disease and its local lesions, since the systemic effects are minor in the quiescent state (Table I). Thus, the sensitivity may be constitutional and associated with the disease, but its pathological relevance is unclear. An increased neutrophil activity, expressed as increased generation of oxygen radicals, has been shown in

other chronic inflammatory diseases, such as peridontitis and regional enterocolitis (17, 19). Furthermore, results of biopsies from HS lesions suggest both local and general host defects related to neutrophils, i.e. a diffuse dermal infiltration of polymorphonuclear cells and in some cases intraluminal infiltration of the apocrine gland (20, 21).

At least two limitations should be noted in the interpretation of the present findings. Firstly, the small number of cases included in this pilot study makes the statistical estimates highly sensitive to the effects of chance. Secondly, because the study included only patients with HS of stage II, graded according to Hurley (14), but neither the more severe cases, graded as stage III, nor the milder cases, with the clinical presentation of Hurley stage I, no information was obtained on the neutrophil function of the 2 latter stages of the disease.

Another way to study host-defence factors is to characterize human leukocyte antigen (HLA) genotypes in patients with HS and controls. There is no evidence of immunogenetic involvement in the pathogenesis since, compared with healthy controls, the frequencies of HLA-A, -B and -DR specificities were normal in 42 unrelated Swedish patients with HS using molecular biology-based typing (22).

In general, no significant differences were found in the membrane expression of the receptor molecules, but all of them had an obvious tendency to be more up-regulated in the patients, which may indicate that the cells are more activated.

In conclusion, the key finding of this study was that the generation of free oxygen radicals, after PMA stimulation, was significantly higher in the patients than in the controls, while there was no difference after Fc-receptor-mediated stimulation. Therefore, it is suggested that a defect in the function of neutrophils might be of pathogenic importance in HS, but further functional studies are needed to elucidate the merits of the case.

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