Altered innate and adaptive immune responses in patients with hidradenitis suppurativa

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Summary

Background The clinical improvement of hidradenitis suppurativa reported in a small number of patients with antitumour necrosis factor (anti-TNF-α) therapies supports the hypothesis for an altered immune response in these patients. Objectives To evaluate the state of the innate and adaptive immune responses in patients with hidradenitis suppurativa. Methods Fifty-three patients and six healthy controls were studied. Blood was sampled and subpopulations of lymphocytes were analysed by flow cytometry; monocytes were isolated and their function was evaluated from the concentrations of TNF-α and interleukin (IL)-6 in supernatants of cell cultures after triggering with endotoxins (lipopolysaccharides). TNF-α and IL-6 were estimated by an enzyme immunoassay. Results CD3/CD8 lymphocytes were lower in patients with involvement of the perineum than in controls; patients with involvement of the breast had higher levels of natural killer (NK) cells than controls. A negative correlation was found between years lapsing since initial presentation of lesions of hidradenitis and the percentage of NK cells. Monocytes isolated from healthy volunteers were more active for the secretion of TNF-α and IL-6 than those of patients with hidradenitis suppurativa. Conclusions A reduction in the percentage of NK cells over time and a lower monocyte response to triggering by bacterial components is observed in patients with hidradenitis suppurativa. Further research is needed to clarify if these changes are connected to an autoimmune mechanism in the pathogenesis of hidradenitis suppurativa.

Hidradenitis suppurativa is a skin disorder of unknown aetiology and pathogenesis. Its prevalence is reported to range from one per 300 to four per 100 in the general population.1 Current theories for its pathogenesis implicate hyperkeratosis of the follicular epithelium as the hallmark of the pathogenetic process leading to occlusion of the apocrine glands with subsequent follicular rupture, inflammation and possible secondary infection.2,3 Clinical improvement with the application of therapies targeted against tumour necrosis factor (TNF)-α may be compatible with the above theory of pathogenesis, as TNF-α is a major proinflammatory cytokine. In these studies, monoclonal anti-TNF-α infliximab antibodies4,5 or soluble TNF-α etanercept receptors6 were administered in a small number of patients. Positive responses with anti-TNF-α therapies have also addressed the question of whether any probable autoimmune predilection might contribute to the pathogenesis of hidradenitis suppurativa.8

Based on the above probability for the existence of some derangement of the activity of the host immune function, the present study aimed to evaluate characteristics of the innate and adaptive immune responses in patients with hidradenitis suppurativa. The study was designed to investigate (i) the subpopulations of lymphocytes of the adaptive immune response; and (ii) the activity of monocytes to secrete proinflammatory cytokines after triggering by bacterial components.

Patients and methods

A total of 53 patients with hidradenitis suppurativa were enrolled in the study. Diagnosis was made by clinical criteria2,3,7 comprising (i) onset after puberty; (ii) the presence of subcutaneous nodules in areas of skin rich in apocrine glands; and (iii) a compatible history of recurrent drainage of pus from the affected areas. Written informed consent was
provided by all patients. Exclusion criteria were the presence of (i) human immunodeficiency virus infection; (ii) neutropaenia, i.e. < 500 neutrophils mm$^{-3}$; (iii) any solid tumour or any haematological malignancy; (iv) intake of corticosteroids; or (v) history of rheumatoid arthritis, systemic lupus erythematosus or inflammatory bowel disease. The study was approved by the Ethics Committee of the Attikon University Hospital.

Blood (10 mL) was sampled from each patient after venipuncture of a peripheral vein under sterile conditions with a heparinized syringe; 3 mL was collected into a heparin-coated tube (Becton Dickinson, Cockeysville, MD, U.S.A.) for flow cytometric analysis of subpopulations of lymphocytes; the remaining blood was used for the isolation of monocytes. During sampling all enrolled patients had active disease, i.e. recurrent purulent discharges from several of the affected skin sites. Blood was also sampled from six healthy volunteers equally matched for age with the study population.

Red blood cells were lysed with 1Æ0 mmol L$^{-1}$ of ammonium chloride. White blood cells were washed three times with phosphate-buffered saline (PBS) (pH 7Æ2) (Merck, Darmstadt, Germany) and subsequently incubated for 15 min in the dark with the monoclonal antibodies (mAbs) anti-CD3 and anti-CD19 with the fluorocolour fluorescein isothiocyanate (FITC, emission 520 nm; Immunotech, Marseille, France) and with the mAbs anti-CD4, anti-CD8 and anti-CD(16+56) with the fluorocolour phycoerythrin (PE, emission 550 nm, Immunotech). The following combinations were applied: anti-CD3/anti-CD4, anti-CD3/anti-CD8, anti-CD3/anti-CD(16+56); anti-CD19 was applied singly. Cells staining positive for the above antibodies were analysed after running through the EPICS XL/MSL flow cytometer (Beckman Coulter Co, Miami, FL, U.S.A.) with gating for mononuclear cells.

For the isolation of blood monocytes, the collected heparinized venous blood was layered over Ficoll Hypaque (Biochrom, Berlin, Germany) and centrifuged. Isolated mononuclear cells were washed three times with PBS (pH 7Æ2) and incubated with RPMI 1640 (Biochrom) enriched with 10% fetal bovine serum (FBS) and 2 mmol L$^{-1}$ of glutamine in the presence of 100 U mL$^{-1}$ of penicillin G and 0Æ1 mg mL$^{-1}$ of streptomycin (Sigma Co, St Louis, MO, U.S.A.) in 75-cm$^3$ flasks. After 1-h incubation at 37$^\circ$C in 5% CO$_2$, nonadherent cells were removed; adherent monocytes were washed thoroughly with Hanks’ solution (Biochrom). Monocytes were then harvested with a 0Æ25% trypsin/0Æ02% ethylenediaminetetraacetic acid solution (Biochrom) and counted in a Neubauer plate. Their purity was more than 95% as determined after staining with the anti-CD14 mAb with the fluorocolour FITC (emission 520 nm) and reading through the EPICS XL/MSL flow cytometer. Their viability was assessed by trypan blue staining.

Table 1 Clinical characteristics of 53 patients with hidradenitis suppurativa enrolled in the study

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Details</th>
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<tbody>
<tr>
<td>Patients (n)</td>
<td>53</td>
</tr>
<tr>
<td>Age (years, mean ± SD)</td>
<td>35Æ59 ± 12Æ58</td>
</tr>
<tr>
<td>Male/female (n)</td>
<td>19/34</td>
</tr>
<tr>
<td>Years from initial diagnosis (median ± SE)</td>
<td>10Æ00 ± 1Æ39</td>
</tr>
<tr>
<td>Involved body areas (%) of patients</td>
<td>a</td>
</tr>
<tr>
<td>Axillae</td>
<td>29 (54Æ72)</td>
</tr>
<tr>
<td>Groins</td>
<td>25 (47Æ16)</td>
</tr>
<tr>
<td>Breasts</td>
<td>19 (35Æ84)</td>
</tr>
<tr>
<td>Perianal area</td>
<td>8 (15Æ09%)</td>
</tr>
<tr>
<td>Back</td>
<td>2 (3Æ78)</td>
</tr>
<tr>
<td>Thorax and back</td>
<td>1 (1Æ89)</td>
</tr>
<tr>
<td>Other sites</td>
<td>3 (5Æ66)</td>
</tr>
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</table>

Fig 1. Significant immunophenotyping alterations of lymphocytes were seen in 53 patients with hidradenitis suppurativa compared with six healthy volunteers in relation to the affected skin site. Circles denote outliers and asterisks extremes. a, Statistically significant decreases compared with controls.
Isolated monocytes were distributed in two wells of a 12-well plate; they were incubated with RPMI 1640 supplemented with 10% FBS and 2 mmol L\(^{-1}\) glutamine for 18 h at 37 °C in 5% CO\(_2\) in the absence or presence of 100 ng mL\(^{-1}\) of purified endotoxin (lipopolysaccharide, LPS) derived from *Escherichia coli* O144:H4 (Sigma). After incubation, cell supernatants were collected and kept refrigerated at −70 °C until assayed for TNF-α and IL-6.

Concentrations of TNF-α and IL-6 were estimated by an enzyme immunoabsorbent assay (Diaclone, Paris, France). The lowest limits of detection were 0.5 pg mL\(^{-1}\) for TNF-α and 6.25 pg mL\(^{-1}\) for IL-6. Concentrations of TNF-α and of IL-6 were expressed as pg 10\(^{-4}\) monocytes. The monocyte function was determined after subtracting the concentrations of TNF-α and IL-6 yielded after incubation in the presence of LPS from respective concentrations yielded after incubation in the absence of LPS.

Patients were divided into subgroups depending on the affected skin areas. Results were expressed as median ± 95% confidence interval (CI). Comparisons were performed using
the Mann–Whitney U-test after Bonferroni correction. Patients with disease of the back and chest were excluded from analysis. Statistical correlations were assayed after assessment of the nonparametric Spearman coefficient (\(r_s\)). \(P < 0.05\) was considered statistically significant.

**Results**

The clinical characteristics of patients enrolled in the study are shown in Table 1. Significant differences were seen in the subpopulations of mononuclear cells of patients with perianal disease and breast disease compared with those of healthy controls (Fig. 1). More precisely, patients with perianal disease had lower CD3/CD8 lymphocytes than healthy controls (\(P = 0.045\)) and patients with breast disease had higher percentages of NK cells than healthy controls (\(P = 0.049\)). Similar differences were not encountered for patients with involvement of the other skin areas. A negative correlation was found between years lapsing since initial presentation of hidradenitis lesions and the percentage of NK cells (\(r_s = -0.376, P = 0.049\)).

Figure 2 shows the potency of monocytes for the release of TNF-\(\alpha\) after triggering with LPS in relation to the affected skin...
site. Monocytes isolated from healthy volunteers were more active for the secretion of TNF-\(\alpha\) than those isolated from patients with involvement of the axillae (\(P = 0.002\)), of the groin (\(P = 0.002\)) and of the breast (\(P = 0.004\)), but not of the perianal area.

The potency of monocytes for the release of IL-6 after triggering with LPS in relation to the affected skin site is shown in Figure 3. Monocytes isolated from healthy volunteers were more active for the secretion of IL-6 than patients without involvement of the perianal area (\(P = 0.036\)).

Discussion

Clinical improvement of patients with hidradenitis suppurativa after administration of infliximab and etanercept directed against TNF-\(\alpha\) has led to the assumption that significant alterations of the immune responses may exist in these patients. The present study was designed to evaluate the characteristics of both the innate and adaptive immune responses of patients with hidradenitis suppurativa.

Alterations in the subpopulations of lymphocytes were found only for patients with disease of the breast and perianum. These patients had elevated percentages of NK cells, i.e. CD3(-)/CD16(16+56)(+) cells and lower percentages of CD3/CD8(+) cells than their respective controls (Fig. 1). Furthermore, it was found in the entire study population that the percentage of NK cells was decreased as the history of the disease was prolonged.

Another major finding of the present study was the decreased capacity of monocytes of patients with hidradenitis suppurativa to secrete proinflammatory cytokines upon triggering by LPS compared with healthy controls. These findings involved mainly TNF-\(\alpha\) and to a lesser extent IL-6 (Figs 2, 3).

These findings of decreased innate and adaptive immune responses in hidradenitis suppurativa should be considered in the light of the existing data in the literature. The latter are characterized by considerable discrepancies as two previous studies did not reveal any defects of the immune functions in patients with hidradenitis suppurativa, whereas two other studies have identified some defects of the patients’ immune systems. More precisely, adequate function of neutrophils has been reported in seven and 15 patients, respectively. A defective bactericidal effect associated with low intracellular levels of cyclic guanosine monophosphate has been described in one patient, whereas decreased counts of T lymphocytes were reported in seven other patients. However, to our knowledge no study exists such as the one performed herein, focusing on the activity of blood monocytes in relation to hidradenitis suppurativa.

The lack of a specific mechanism for the pathogenesis of hidradenitis suppurativa has led to the application of a variety of therapeutic approaches such as antibiotics and immunosuppressive therapies; all were proved of limited or no benefit. Anti-TNF-\(\alpha\) strategies appear to be promising in the treatment of this disorder. Their effect might be compatible with the hypothesis of an autoimmune predilection in hidradenitis suppurativa. Furthermore, the disease may co-exist with Crohn disease, which is an autoimmune disorder, and pyoderma gangrenosum, in which derangements of the immune function have been reported.

Therapeutic benefit with immunosuppressive and anti-TNF-\(\alpha\) therapies would be compatible with the existence of intense reactions of both the innate and adaptive immune systems upon antigenic triggering in hidradenitis suppurativa. Presented findings pointing towards defective immune responses give rise to much skepticism about the existence of an autoimmune predilection of the disease. However, these diminished immune responses may provide an adequate explanation for the high recurrence rates even in patients undergoing extensive surgical excision of the affected sites.

The presented results reveal the existence of alterations of the function of the innate immune system and to a lesser extent of the adaptive immune system in patients with hidradenitis suppurativa. These changes mainly involve a reduction in the percentage of NK cells over time and a lower monocyte response upon triggering by bacterial components. Further research is mandatory to clarify if these changes are connected to an autoimmune mechanism in the pathogenesis of hidradenitis suppurativa.

Acknowledgments

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References