Background Acne inversa (hidradenitis suppurativa) is a chronic inflammatory and cicatricial disorder that affects skin areas rich in apocrine glands and terminal hairs, such as perineum and axillae. The exact pathogenesis of the disease is not well understood and the mechanisms by which bacterial superinfection contributes to the disease progression are not clear. Toll-like receptors (TLRs) expressed by inflammatory cells play a crucial role in the innate immune response to bacteria.

Objectives We sought to investigate the role of TLR2 in the pathogenesis of acne inversa.

Methods We investigated the expression of TLR2 using real-time polymerase chain reaction analysis and immunohistochemical stainings of tissue samples from patients with acne inversa. Furthermore, we phenotypically characterized the infiltrating cells and their expression of TLR2.

Results Compared with normal skin, a highly increased in situ expression of TLR2 in acne inversa skin lesions was found at both the mRNA and the protein level. The most abundant cells in the dermal infiltrate of acne inversa were CD68+ macrophages, CD209+ dendritic cells (DCs) and CD3+ T cells. CD19+ B cells and CD56+ natural killer cells were found only in small numbers. Double staining with fluorescence-labelled antibodies showed that TLR2 was expressed by infiltrating macrophages (CD68+) and DCs (CD209+). Flow cytometric analysis of isolated infiltrating cells further confirmed surface expression of TLR2 by macrophages and DCs.

Conclusions These data indicate that the enhanced expression of TLR2 by infiltrating macrophages and DCs may contribute to the pathogenesis of inflammatory lesions of acne inversa.

Acne inversa (hidradenitis suppurativa) is a chronic relapsing inflammatory skin disease predominantly affecting skin folds that carry terminal hair follicles and apocrine glands. The disease is characterized by recurrent abscesses and draining sinuses located mainly in the axillae, groins and perineum. Healing occurs with substantial scarring. The prevalence of the disease has been estimated to be 1 : 100 to 1 : 600.1 Although acne inversa is rarely a life-threatening disease, it can have a significant effect on patients’ well-being.2 The pathogenesis of acne inversa is still not well understood. Many different factors including microbiological, hormonal, immunological1 and genetic factors3 have been postulated to play a role in disease progression.

One important pathogenic factor in acne inversa is bacterial colonization. Various bacteria can be isolated from the abscesses and draining sinuses. The most frequently found are staphylococci and streptococci.4 The mechanism by which these bacteria activate the immune system in the lesions of acne inversa is poorly understood. The recently identified toll-like receptors (TLRs)5 are an important example of pattern recognition receptors (PRRs) of the innate immune system that play a crucial role in inducing immune responses and the production of various proinflammatory cytokines. TLRs mediate immune response to microbial ligands, and both staphylococcal and streptococcal components have been shown to bind to TLR2. Furthermore, there is evidence that the upregulation of TLR2 may be an important triggering factor in chronic inflammatory skin diseases.6

In the present study, we sought to elucidate the mechanisms by which the chronic bacterial colonization/infection in patients with acne inversa contributes to the chronic inflammation of the skin. Therefore, the phenotype of infiltrating...
cells as well as the expression and distribution of TLR2 were investigated in chronic inflamed lesions of acne inversa. Our results revealed a high expression of TLR2 in acne inversa lesions by macrophages and dendritic cells (DCs), indicating that the inflammation detected in acne inversa could be a result of stimulation of TLR2 by microbial ligands.

**Materials and methods**

**Samples from patients**

The study was approved by the Medical Ethics Committee of the Canton of Berne, Switzerland. Nine patients (six women and three men; median age 44 years, range 32–70) with acne inversa were included in the study. Diagnosis was based on clinical, histopathological and bacteriological criteria. Patients had a long-standing history (at least 6 months) of chronic acne inversa. Normal skin samples from seven healthy subjects undergoing abdominal surgery and mammary reconstruction served as a control. Written informed consent was obtained from all subjects enrolled in this study. Following surgical excision, tissue samples were immediately embedded in optimal cutting temperature (OCT) compound, snap frozen and stored at −70 °C until sectioning.

**RNA isolation and cDNA synthesis**

OCT-embedded tissue specimens were cut into 10–20-μm sections. In total, 0.5–1 mm tissue sections were collected in a frozen tube. RNA was extracted using RNeasy Lipid Tissue Mini Kit (Qiagen, Valencia, CA, U.S.A.). Contaminating DNA was removed with an RNase-free DNase step (Qiagen) according to the manufacturer’s indications. RNA (250 ng) was used to synthesize cDNA using BD Reverse Transcriptase Kit (BD Biosciences, Franklin Lakes, NJ, U.S.A.) and random hexamers according to the manufacturer’s indications.

**Quantitative real-time polymerase chain reaction analysis**

Polymerase chain reaction (PCR) primers and probes were purchased from Applied Biosystems (Foster City, CA, U.S.A.) as pre-made assays on demand spanning exon–exon borders for TLR2 and four different control genes (18S, HPRT1, β2-microglobulin, GAPDH). The real-time quantitative PCR was performed with ABI-Prism 7300 Sequence Detector System (Applied Biosystems). For cDNA amplification a 10-min incubation at 95 °C was done to activate AmpliTaqGold; this was followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. For each sample all four different control genes were amplified. Using GenNorm software, the most stably expressed control gene was determined (HPRT1) and used for further analysis. The results were expressed as relative units (RU; fold difference).

RU were calculated by the $2^{-\Delta\Delta CT}$ method. The threshold cycle ($C_T$) for the target amplicon and the $C_T$ for the endogenous control (HPRT1) were determined for each sample. Differences in the $C_T$ for the target and the $C_T$ for the internal control, called $\Delta C_T$, were calculated to normalize for the difference in the amount of total nucleic acid added to each reaction. The $\Delta C_T$ of normal skin (calibrator) was subtracted from the $\Delta C_T$ of each sample and termed as $\Delta\Delta C_T$. The amount of target normalized to the endogenous control and relative to the calibrator was then calculated by the formula $2^{-\Delta\Delta CT}$.

**Immunohistochemical staining for quantitative analysis**

The following monoclonal mouse antihuman antibodies were used as first-stage reagents: anti-TLR2 (clone TL2.3; Alexis Corp., San Diego, CA, U.S.A.), anti-CD3 (clone PC3/188A; DakoCytomation, Glostrup, Denmark), anti-CD19 (clone HD37; DakoCytomation), anti-CD56 (clone MOC-1; DakoCytomation), anti-CD68 (clone EBM11; DakoCytomation), anti-CD11c (clone KB90; DakoCytomation), anti-CD1a (clone O10; DakoCytomation), anti-CD207 (clone DCGM4; Immunotech, Marseilles, France), anti-CD209 (clone DCN46; BD Biosciences Pharmingen, San Diego, CA, U.S.A.) and anti-CD206 (clone 19.2; BD Biosciences Pharmingen). As a negative control, the same tissue was stained with an isotype-matched antibody of irrelevant specificity. Immunostaining was performed using the avidin–biotin complex/alkaline phosphatase (ABC/AP) method as previously described. Briefly, cryostat tissue sections were air-dried, fixed for 10 min in 4% ice cold acetone and rehydrated in Tris–buffered saline with 0.1% saponin. The sections were incubated with the primary mouse antibody for 1 h at room temperature, followed by a biotinylated rabbit antimouse IgG (E0413; DakoCytomation) and thereafter with ABC/AP (K0376; DakoCytomation). Finally, all sections were developed in new fuchsin–naphthol (K0624; DakoCytomation) and counterstained with haematoxylin. In each section, at least 10 randomly selected fields were analysed at × 400 magnification with a light microscope by two independent investigators. The positive cells were counted using a 0.063 mm$^2$ grid and the mean ± SEM number of positive cells mm$^{-2}$ was calculated.

**Immunofluorescence double staining**

Cryostat sections (4–5 μm) were air-dried, fixed for 10 min in 4% ice cold acetone and rehydrated in Tris–buffered saline with 0.1% saponin. Double immunofluorescence was performed by serially incubating sections with mouse antihuman TLR2 for 1 h followed by incubation with fluorescein isothiocyanate (FITC)-labelled goat antimouse IgG (DakoCytomation). Irrelevant IgG subclass-matched antibodies were used for negative controls. Sections were then incubated with one of the following antibodies for 1 h: (i) anti-CD68 (clone EBM11; DakoCytomation); (ii) anti-CD209. Sections were then treated with isotype-specific phycoerythrin (PE)–conjugated goat antimouse IgG2a (Caltag Laboratories, Burlingame, CA, U.S.A.).

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Flow cytometric analysis of freshly isolated inflammatory cells

To isolate sufficient numbers of macrophages and DCs without destroying the structural and functional integrity of the extracellular receptors, freshly obtained surgical tissue specimens of acne inversa were cut into small pieces (1–2 mm) and resuspended in 2.5 mg mL$^{-1}$ collagenase F (Sigma Chemicals, St Louis, MO, U.S.A.) in sterile RPMI supplemented with 5% fetal calf serum (FCS) as previously described. The tissue pieces were then shaken for 60 min at room temperature and mechanically disrupted by short vigorous vortexing. The cell suspension was then passed through a 70-μm cell strainer (BD Biosciences) and washed with cold RPMI containing 5% FCS. Aliquots containing 5–10 × 10⁴ cells were stained with FITC-labelled anti-TLR2 (clone TL2.1; eBioscience, San Diego, CA, U.S.A.), PE-Cy5-labelled anti-CD206 and PE-labelled anti-CD209 antibodies (both from BD Biosciences) in RPMI containing 5% FCS and 0.1% NaN₃ for 30 min on ice. After two washes with RPMI containing 5% FCS, 500 mL of 1% formaldehyde in phosphate-buffered saline was added. Subsequently, the samples were investigated on a FACScan flow cytometer (BD Biosciences); 10 000–40 000 events were acquired and analysed on a Macintosh computer using CELLQUEST software (BD Biosciences). Isotype-matched irrelevant antibodies were used as negative controls.

Statistics

The mean ± SEM number of stained cells mm$^{-2}$ within each of the epidermal and dermal compartments was calculated. Statistical significance was assessed by Student’s t-test. P < 0.05 was considered significant.

Results

Expression of toll-like receptor 2 mRNA in chronic inflamed acne inversa lesions

In a first step we performed quantitative PCR for TLR2 mRNA in chronic inflamed lesions of acne inversa and in normal skin from healthy subjects serving as a control. As shown in Figure 1, a fourfold increase in the relative amount of mRNA was found in acne inversa lesions in comparison with control skin.

Tissue distribution of toll-like receptor 2

To analyse the tissue distribution of TLR2 we performed immunohistochemical analysis on tissue sections of lesional skin and normal skin serving as a control. Significant differences were observed at the level of TLR2 immunoreactivity. As shown in Figure 2, the number of TLR2+ cells was significantly increased in the dermis of acne inversa lesions as compared with normal skin controls. Furthermore, a slight increase of TLR2+ cells was detected in the epidermis of acne inversa lesions as compared with normal skin controls. Most TLR2+ cells were located in the dermis of acne inversa (Figs 2, 3a, b).

Phenotypic analysis of infiltrating mononuclear cells

To assess the different phenotypes of the mononuclear infiltrating cells in acne inversa lesions, we performed immunohistochemical analysis on serial tissue sections of chronic inflamed acne inversa lesions and on normal human skin serving as control.

Expression of different cell markers in the dermis and epidermis was separately assessed. The mean ± SEM number of stained cells mm$^{-2}$ is listed in Table 1. When compared with normal skin controls, there was a substantial increase in different DC subsets, macrophages and T cells in the epidermis and dermis of chronic inflamed acne inversa skin lesions. An increased number of CD68+ cells was detected in the dermis of acne inversa lesions. Some CD68+ cells at the basal layer were found protruding and infiltrating into the epidermis. The
number of CD11c+ cells was notably increased in the dermis of acne inversa lesions. A substantially increased immunoreactivity for C-type lectin receptor markers including CD206/mannose receptor, expressed by a subset of macrophages and DCs, CD209/DC-SIGN, expressed by a subset of immature DCs, and CD207/langerin, expressed by immature Langerhans cell-type DCs, was detected in both the dermis and epidermis of acne inversa. Furthermore, an increased number of CD1a+ DCs was detected in the epidermis of acne inversa lesions. There was an increased number of CD3+ T cells in both the epidermis and dermis of acne inversa. In contrast, only few CD19+ (B cells) and CD56+ (natural killer cells) cells were detected in the dermis of acne inversa.

In addition, increased immunoreactivity for HLA-DR was detected in the epidermis and dermis. Clearly, the DCs and macrophages constituted the major component of the mononuclear cell infiltrate in acne inversa.

**Phenotype of toll-like receptor 2-expressing cells**

To assess the phenotype and distribution of cells expressing TLR2 in the lesions of acne inversa, we performed double immunofluorescence labelling. The colocalization of TLR2 with CD68 and CD209 was examined because cells expressing these markers appeared to be the most highly abundant in acne inversa lesions. CD68 and CD209 colocalized with TLR2, suggesting that TLR2 is expressed on macrophages as well as DCs in acne inversa lesions. The TLR2+ macrophages and TLR2+ DCs were mainly located in the inflammatory infiltrate in the dermis (Fig. 4).

**Flow cytometric analysis of infiltrating cells**

Further to demonstrate the expression of TLR2 by C-type lectin receptor-bearing macrophages and DCs in acne inversa skin lesions, we performed flow cytometric analysis of infiltrating cells.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Specificity</th>
<th>Acne inversa (mean ± SEM)</th>
<th>Normal skin (mean ± SEM)</th>
<th>Acne inversa (mean ± SEM)</th>
<th>Normal skin (mean ± SEM)</th>
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</thead>
<tbody>
<tr>
<td>CD3</td>
<td>T cells</td>
<td>10.3 ± 6.0</td>
<td>1.6 ± 1.1</td>
<td>20.6 ± 93.1*</td>
<td>10.0 ± 6.6</td>
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<tr>
<td>CD19</td>
<td>B cells</td>
<td>30.4 ± 12.4</td>
<td>6.4 ± 9.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD56</td>
<td>Natural killer cells</td>
<td>43.1 ± 11.4</td>
<td>55.3 ± 23.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD68</td>
<td>Macrophages</td>
<td>71.2 ± 162.0*</td>
<td>165 ± 30.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD11c</td>
<td>DCs/macrophages</td>
<td>32.2 ± 117.5*</td>
<td>56.9 ± 14.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD207</td>
<td>Immature LCs</td>
<td>15.5 ± 6.9</td>
<td>3.0 ± 2.8</td>
<td></td>
<td></td>
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<tr>
<td>CD1a</td>
<td>Immature DCs</td>
<td>63.3 ± 28.3</td>
<td>62.4 ± 15.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD209</td>
<td>Immature DCs</td>
<td>463.2 ± 72.4*</td>
<td>192.4 ± 32.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD206</td>
<td>DCs/macrophages</td>
<td>467.3 ± 141.5*</td>
<td>141.5 ± 67.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DCs, dendritic cells; LCs, Langerhans cells. Asterix (*) indicates significant statistical differences (P < 0.05) between marker expression in acne inversa lesions (n = 9) compared with normal skin (n = 7).
cells in skin lesions of acne inversa in four patients. As shown in Figure 5, TLR expression by macrophages and DCs was evident.

**Discussion**

The recognition of microbial pathogens by cells of the immune systems triggers host defence mechanisms to combat infection and prevent disease. The same mechanisms may, however, result in pronounced inflammation at the site of disease, leading to subsequent tissue damage. In acne inversa, streptococci, staphylococci and *Escherichia coli* have been identified in the early stages of the disease, while anaerobic bacteria and *Proteus* species have more commonly been isolated in the chronic relapsing stages. These organisms are thought to secrete microbial products that stimulate the production of proinflammatory cytokines and chemokines, which may initiate an immune response at the site of infection. However, the exact mechanism by which these microbial products trigger the immune response and cause inflammation is poorly understood. In last years, TLRs have emerged as a key component of the innate immune system that detect different microbial ligands, e.g. from Gram-positive and Gram-negative organisms, and trigger antimicrobial host defence responses. TLRs have been shown to activate multiple steps in the inflammatory reactions that help to eliminate the invading pathogens and coordinate systemic defence. In addition, TLRs have been shown to control multiple DC functions and activate signals that are critically involved in the initiation of adaptive immune responses. For example, the increased expression of TLR2 was shown to be vital in the inflammation occurring in acne vulgaris by mediating *Propionibacterium acne*-induced cytokine production. The fact that acne inversa and acne vulgaris may share similar pathophysiological mechanisms as regards follicular occlusion and bacterial colonization prompted us to analyse the expression of TLR2 in chronic inflamed acne inversa lesions with the aim of providing further understanding of the disease pathogenesis.

In this study, we demonstrated an increased expression of TLR2 at both the mRNA and protein levels in chronic inflamed acne lesions. These findings were statistically significant when compared with normal skin controls. Using immunohistochemical staining, we further showed that TLR2+ cells are particularly located in the dermis. Furthermore, our findings demonstrated a significantly increased number of macrophages and DCs which constituted the major portion of the mononuclear cell infiltrate in acne.

![Fig 4](image_url) Phenotype of cells expressing toll-like receptor 2 (TLR2) in lesions of acne inversa. Two-colour immunofluorescence images were obtained for TLR2 (first panel of each row, red), and CD68 and CD209 (second panel of each row, green). The two images were then superimposed (third panel of each row). Double-positive cells are shown in yellow. TLR2-positive cells colocalize with CD68 (macrophages) and CD209 (dendritic cells).

![Fig 5](image_url) Representative examples of FACScan-based analysis of toll-like receptor 2 (TLR2) expression of freshly isolated inflammatory cells from lesions of acne inversa. After gating on the dendritic-monocytic cell population by side and forward light scatter, CD209 and CD206 expression was assessed (c). TLR2 expression (red line) was assessed on the CD206+ CD209− cell population (monocytes) (a) and on the CD206+ CD209+ cell population (dendritic cells) (b). The blue lines in (a) and (b) indicate IgG control staining. FITC, fluorescein isothiocyanate; PerCP, peridinin chlorophyll protein; PE, phycoerythrin.
Inversa lesions. There was a significant increase in CD68+, 
CD11c+ and C-type lectin receptor-bearing cells (CD209+,
CD206+ and CD207+ cells) in both the epidermis and der-
mis of acne inversa lesions. An increased number of CD3+ T
 cells was also detected in the epidermis and dermis of acne
inversa lesions. Furthermore, by using double immunoflu-
orescence labelling, TLR2 was found to colocalize with CD68
and CD209. Taken together, these findings indicate a signifi-
cantly increased expression of TLR2 on activated macrophages
and DC subsets in acne inversa lesions, and suggest a novel
pathomechanism by which the colonizing bacteria in acne
inversa lesions may contribute to inflammation at the site of
disease activity.

Previous studies have demonstrated an increased number of
TLR2+ cells in different inflammatory diseases such as tuber-
culoid lesions of leprosy,10 acne vulgaris6 and rheumatoid
arthritis.11,12 Furthermore, the activation of TLR2 on mono-
cytes/macrophages in certain autoimmune and inflammatory
diseases was shown to induce the production of proinflamma-
tory cytokines, e.g. interleukin (IL)-12 and tumour necrosis
factor (TNF)-α, which in turn may promote a Th1-mediated
immune response.13,14 Interestingly, therapeutic agents which
target TLR2-induced cytokines, e.g. TNF-α or TLR2 ligands,
including etanercept and clindamycin-rifampacin combination
therapy, respectively, have demonstrated their efficacy in treat-
ment of acne inversa.15,16

PRRs including TLRs and C-type lectin receptors expressed
by DCs and macrophages have been shown to regulate impor-
tant cell functions and interactions including pathogen recog-
nition, phagocytosis, antigen presentation, cell adhesion, the
secretion of cytokines and chemokines and T lymphocyte acti-
vation.17 In our study, we were able to demonstrate not only
an increased expression of TLR2 in acne inversa lesions, but
also an increased expression of different C-type lectin recep-
tors including CD206/mannose receptor, CD209/DC-SIGN
and CD207/langerin. Interestingly, we found that CD209 and
TLR2 colocalize on DCs infiltrating the chronic inflamed
lesions of acne inversa. Whereas TLRs mainly act to alert DCs,
causing their maturation and inducing cytokine production,
e.g. TNF-α and IL-12, evidence now shows that C-type lectin
receptors, by recognizing pathogen-associated molecular pat-
terns, can operate as constituents of the powerful antigen cap-
ture and uptake mechanism of macrophages and DCs.18,Furthermore, evidence is emerging that C-type lectin receptors
not only play a role as phagocytic PRRs but they also might
synergize or antagonize TLR signals.19,20 For example, myco-
bacteria can simultaneously interact with TLRs and C-type lec-
tin receptors. Binding of mycobacterial lipoproteins to TLRs
on DCs triggers production of IL-12, essential to initiate
immune responses to eliminate intracellular mycobacteria.21
Interestingly, several groups recently showed that Mycobact-
rium-derived mannosylated lipoarabinomannans bind to DCs
via CD209/DC-SIGN and CD206/mannose receptor and inhi-
bbit TLR-mediated IL-12 production. These observations led
to the suggestion that simultaneous binding of Mycobacterium
components to CD209/DC-SIGN, CD206/mannose receptor and
TLRs might alter the immune system from a protective Th1
response towards a tolerogenic Th2 response, facilitating
immune escape of mycobacteria.18,22,23 Indeed, it is plausible
that acne inversa may represent another example of the
so-called cross-talk between C-type lectin receptors and TLRs
where the collaborative recognition of distinct microbial com-
ponents by different classes of innate immune receptors (TLRs
and C-type lectin receptors) is crucial for orchestrating inflam-
matory or inhibitory responses and may in turn contribute to
the chronic state of the disease.

In conclusion, our study demonstrates for the first time an
increased expression of TLR2 together with different C-type
lectin receptors in acne inversa lesions. These data suggest a
possible mechanism by which bacteria colonizing chronic
inflamed acne inversa lesions may cause tissue damage through
stimulating TLR2 expressed by infiltrating macrophages and
DCs. It is tempting to speculate that by synchronizing with
TLR2, C-type lectin receptors may play a role in the pathogen-
esis of acne inversa and in maintenance of the chronic
inflammatory state of the disease. Future studies are warranted,
further to unravel the role of TLRs in acne inversa and to eluci-
date the potential therapeutic role of anti-TLRs in the manage-
ment of this frustrating disease.

Acknowledgments

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