Immunopathogenesis of Folliculitis Decalvans

Clues in Early Lesions

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Abstract

Folliculitis decalvans (FD) is a rare variant of primary cicatricial alopecia, for which the etiopathogenesis remains unclear. Our purpose was to evaluate whether certain immunologic mechanisms might have a significant role in the pathogenesis of FD.

Lesional scalp biopsy specimens from 7 patients with FD, 7 with lichen planopilaris, and 4 with alopecia areata were studied immunohistochemically by using monoclonal antibodies to CD1a, CD3, CD4, CD8, CD20, CD25, HLA-DR, interleukin (IL)-1β, IL-4, IL-8, interferon γ, tumor necrosis factor α, basic fibroblast growth factor (b-FGF), transforming growth factor (TGF)-β, endothelial leukocyte adhesion molecule 1, intercellular adhesion molecule (ICAM)-1, and vascular cell adhesion molecule.

We showed that early FD lesions are characterized by an infiltration of activated T-helper cells, featuring mixed T\textsubscript{H}1/T\textsubscript{H}2 polarization. IL-8 and ICAM-1 may contribute to the infiltration of neutrophils, whereas b-FGF and TGF-β may represent important mediators of the fibrosis that characterizes late-phase FD.

Folliculitis decalvans (FD), a rare variant of primary cicatricial alopecia, is characterized clinically by papules and pustules that lead to scleroatrophic plaques with a chronic, progressive course. The early histopathologic features include acneiform infundibular dilatation and an intrafollicular/perifollicular infiltrate, composed mainly of neutrophil granulocytes.1 With disease progression, the infiltrate becomes mixed, with neutrophils, lymphocytes, plasma cells, and foreign-body giant cells, and extends into the adventitial dermis. Late-stage disease is characterized by follicular and adventitial dermal fibrosis.1

The etiology and pathogenesis of FD are unclear. It has been hypothesized that cytotoxic proteins secreted by Staphylococcus aureus, frequently detected in active lesions, may act as superantigens, ie, stimulate T cells directly without the need of being processed by antigen-presenting cells.2 A congenital abnormality of follicular orifices may predispose to easy access of microorganisms.3 The only immunohistochemical study performed to date on FD did not demonstrate any specific malfunction in the local immune response, ie, the CD4/CD8 and T-cell/B-cell ratios were similar to those observed in other nonspecific, chronic dermatoses.2

The aim of the present study was to evaluate certain immunopathologic mechanisms that may have a significant role in the pathogenesis of FD. In particular, we attempted to characterize the lymphocyte immunophenotype, certain cytokines related to T-helper (T\textsubscript{H}) polarization, others known to have a fibrogenic or chemotactic function, and certain adhesion molecules. Skin specimens from prototypic forms of cicatricial alopecia, ie, lichen planopilaris (LPP), and non-cicatricial alopecia, ie, alopecia areata (AA), were used as control samples.
Materials and Methods

After written informed consent was obtained, biopsy specimens were obtained from early papulopustular lesions of 7 patients (4 men and 3 women; mean age, 41.7 years; range, 19-66 years) referred during the last 6 years to our department for newly diagnosed and untreated FD of the scalp. Routine histopathologic examination showed a dense, mixed infiltrate composed mainly of neutrophils and localized in the perivascular and perifollicular dermis, as well as in the follicular epithelium, that was mainly characterized by spongiosis. Swabs taken from lesional skin grew S. aureus in 5 cases. Lesional scalp biopsy specimens from 7 patients affected by LPP (1 man and 6 women; mean age, 60.7 years; range, 49-77 years), 4 affected by AA (1 man and 3 women; mean age, 33.2 years; range, 28-46 years), and the normal-appearing scalp of 3 healthy subjects served as control samples. LPP was diagnosed according to clinical (perifollicular erythema and scaling, patchy or diffuse hair loss, and, at the end stage, atrophic cicatricial alopecia with loss of follicular ostia) and histopathologic criteria (lichenoid inflammatory infiltrate of lymphocytes at the infundibuloisthmic region with variable perifollicular fibrosis and absence of thickened basement membrane or follicular elements as confirmed by a periodic acid–Schiff with diastase stain). In the diagnosis of AA, we considered clinical (round, smooth, bald patches featuring hair loss) and histologic features (peribulbar lymphocytic inflammation).

Tissue specimens were stored immediately at −80°C and cut into 5-µm-thick sections that were stained immunohistochemically using the alkaline phosphatase/anti–alkaline phosphatase method, as described previously. The monoclonal antibodies used included those to CD1a (dilution 1:20; DAKO, Copenhagen, Denmark), CD3 (dilution 1:20; DAKO), CD4 (dilution 1:20; DAKO), CD8 (dilution 1:20; DAKO), CD20 (dilution 1:20; DAKO), CD25 (dilution 1:10; DAKO), HLA-DR (dilution 1:100; DAKO), interleukin (IL)-1β (dilution 1:20; R & D Systems, Minneapolis, MN), IL-4 (dilution 1:20; R & D Systems), IL-8 (dilution 1:20; R & D Systems), interferon (IFN)-γ (dilution 1:75; R & D Systems, tumor necrosis factor (TNF)-α (dilution 1:20; R & D Systems), basic fibroblast growth factor (b-FGF) (dilution 1:1,000; Sigma, St. Louis, MO), transforming growth factor (TGF)-β (dilution 1:1,000; Anagen, Atlanta, GA), endothelial leukocyte adhesion molecule (ELAM)-1 (dilution 1:20; R & D Systems), intercellular adhesion molecule (ICAM)-1 (dilution 1:20; R & D Systems), and vascular cell adhesion molecule (VCAM) (dilution 1:20; R & D Systems). Negative control sections were incubated with nonimmune mouse serum samples.

For membrane receptor analysis, the stained cells were counted in 3 consecutive microscopic fields (×250), and the mean was calculated. Expression of the studied molecules was scored semiquantitatively as follows: 0, no expression or expression in negative control sections; 1, weak expression or mean cell count per field (MCC) between 1 and 10; 2, moderate expression or MCC between 10 and 20; or 3, strong expression or MCC greater than 20.

The procedures followed were in accordance with the Helsinki Declaration of 1975.

Results

CD1a+ Langerhans cells were strongly present in the epidermis of FD, LPP, and AA cases (Table 1, Image 1A).

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Folliculitis Decalvans (n = 7)</th>
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<th>Alopecia Areata (n = 4)</th>
<th>Control Cases (n = 3)</th>
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b-FGF, basic fibroblast growth factor; ELAM, endothelial leukocyte adhesion molecule; FE, follicular epithelium; ICAM, intercellular adhesion molecule; IFD, interfollicular dermis; IFN, interferon; IL, interleukin; ND, not done; PFD, perifollicular dermis; TGF, transforming growth factor; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule.

* Expression of the studied molecules was scored semiquantitatively as follows: 0, no expression or expression in negative control sections; 1, weak expression or mean cell count per field (MCC) between 1 and 10; 2, moderate expression or MCC between 10 and 20; or 3, strong expression or MCC greater than 20.
Immunohistochemical analysis of cases of folliculitis decalvans (FD), lichen planopilaris (LPP), and alopecia areata (AA) and healthy skin control samples (HS). A and B, FD (A) and LPP (B) CD1a+ cells in the follicular epithelium and perivascular dermis. C, HS CD1a+ cells in the epidermis with few elements in the dermis. D, FD with many CD4+ cells infiltrating the junctional dermis and the follicular epithelium. CD8+ cells in the LPP dermis was higher than in FD and AA dermis. Few CD20+ cells were found (scattered) in the FD and control specimens. There was strong expression of CD25 and HLA-DR by many cells infiltrating FD and LPP specimens, but less expression in AA and healthy control skin.

IFN-γ and IL-4 were distributed diffusely in the dermis and follicular epithelium of FD, LPP, and AA specimens, but almost not at all in healthy control specimens. The intensity of IFN-γ staining was weak in FD lesions and moderate in diseased control specimens, whereas the expression of IL-4 was weak to moderate in FD, LPP, and AA lesional compartments. IL-1β and TNF-α were
E, LPP with CD4+ cells localized around and within the hair follicle. F, AA with many CD4+ cells infiltrating the perivascular and perifollicular dermis. G, FD showing perivascular CD8+ cells. H, LPP with many perifollicular and intrafollicular CD8+ cells. I, AA with CD8+ cells scattered throughout the dermis (A-I, ×100).
weakly to moderately expressed in FD and diseased control specimens and were negative in healthy control specimens. In particular, there was greater expression of both cytokines in AA than in FD perifollicular dermis. b-FGF Image 2A, Image 2B, and Image 2C and TGF-β Image 2D, Image 2E, and Image 2F were strongly expressed in FD and LPP dermis and only weakly in AA and healthy control specimens. Immunostaining for b-FGF was distributed mainly in dermal cells, whereas that for TGF-β was diffuse in all dermal compartments. IL-8 was expressed by many keratinocytes and was strongly present in the perifollicular dermis of FD lesions, but it was weakly expressed in control specimens Image 2G, Image 2H, and Image 2I.

There was strong ICAM-1 Image 2J, Image 2K, and Image 2L expression in the perifollicular dermis and basal layer of the follicular epithelium of FD lesions, but immunostaining for VCAM and ELAM was weak in the same sites. The expression of ICAM-1 was weak in AA epidermis and negative in AA dermis. VCAM and ELAM were negative in the follicular epithelium and the perifollicular dermis of AA and healthy skin specimens. All adhesion molecules studied were expressed by the endothelial cells in the interfollicular dermis of all specimens.

**Discussion**

FD is a rare and seldom studied disease. In particular, the mechanisms by which early papulopustular lesions lead to cicatricial alopecia are almost unknown. We studied active lesions of patients with FD to try to understand better what underlies the massive infiltration of neutrophils, which characterizes early lesions, and whether certain mechanisms, known to be able to induce fibrosis, are already present in the inflammatory phases of the disease.
D and E, FD and LPP showing diffuse and strong immunostaining for transforming growth factor (TGF)- in the dermis with perifollicular enhancement. F, HS showing weak staining for TGF-. G, FD showing many interleukin (IL)-8+ follicular keratinocytes and perifollicular cells. H, AA showing few IL-8+ follicular keratinocytes and dermal cells. I, HS showing weak dermal staining for IL-8.
The number of CD1a+ Langerhans cells was similar in lesional and healthy specimens, whereas several cells were observed also in all lesional specimens in the dermis, probably resulting from the migration of such antigen-presenting cells to local lymph nodes.

The lymphocyte infiltrate was composed mainly of CD3+ (mature T) cells with few CD20+ (mature B) cells. CD4+ cells outnumbered CD8+ cells, confirming the only information from the literature. Moreover, CD3+ cells, mostly belonging to the CD4+ subset, showed a marked tendency to infiltrate the dermoepidermal junction and the follicular epithelium, allowing one to hypothesize a specific reaction toward one or more specific intrafollicular antigens. The comparison with control diseases further highlighted that FD is characterized by a high CD4/CD8 ratio that was more similar to AA, characterized by a prevalent CD4+ pattern, than to LPP, which is mediated mainly by CD8+ cells. The finding of few B cells may imply a nonsignificant role of such cells in FD. The markers of cellular activation studied, ie, the receptor of IL-2 (CD25) and II-class molecules (HLA-DR), almost colocalized with T lymphocytes.

The comparison between IFN-γ, a T H1-polarized cytokine, and IL-4, secreted mainly by T H2 cells, showed that in FD, both molecules were similarly expressed in all lesional specimens, thus configuring a mixed T H1/T H2 pattern. Although IFN-γ may be involved in the enhancement and maintenance of tissue immunoinflammation, IL-4 may also contribute to the scarring process, given its stimulating activity on fibroblasts.

Although S aureus may act as a superantigen in FD, IL-1β and TNF-α, known to be abundantly produced along this pathway, were expressed weakly to moderately in FD specimens. These cytokines, which showed expression similar to that observed in AA, may act as proinflammatory and profibrogenic mediators.
b-FGF and TGF-β, mainly produced by T lymphocytes, macrophages, and fibroblasts, are known to induce activation of fibroblasts and strong production of many components of the extracellular matrix. In our FD series, as well as in LPP, these 2 mediators were abundantly produced in the dermis. Thus, one could hypothesize that b-FGF– and TGF-β–induced secretion of collagen represents an important mechanism of late fibrosis and cicatricial alopecia in FD and LPP.

It is interesting that IL-8, a potent chemoattractant of neutrophils that is produced by several cell types, including epithelial cells, was expressed mainly in the follicular epithelium and perifollicular dermis of FD lesions. This chemokine may represent an important mechanism in the recruitment of neutrophils toward the lesional follicles.

Adhesion molecules were expressed on endothelial cells belonging to interfollicular microvessels in all lesional specimens, thus promoting the extravasation of circulating blood cells. Moreover, ICAM-1, involved in the adhesion of T lymphocytes and granulocytes, was the only molecule expressed in the perifollicular dermis and basal layer of the follicular epithelium. Thus, it can be hypothesized that ICAM-1 is a key factor in the recruitment of T lymphocytes and neutrophils that constitute the main cell population in lesional FD skin.

On the basis of previous and present data, it can be hypothesized that in genetically predisposed people, the infection of hair follicles by Staphylococcus aureus induces an intense migration of neutrophils, recruited in the perifollicular/intrafollicular dermis by innate immunity mechanisms, such as IL-8. Neutrophils damage the follicular epithelium and penetrate into the follicle where they phagocytize Staphylococcus aureus.

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Figure 1. Schematic representation of the immunopathogenesis of folliculitis decalvans on the basis of our findings. Staphylococcus aureus (SA) may be processed by antigen-presenting cells, such as Langerhans cells (LC) and, thus, activate T cells (T). Moreover, enterotoxins (ET) of Staphylococcus aureus may act as superantigens by activating T cells via the Vβ domain of the T-cell receptor (TCR). Neutrophils (N) may be recruited also by innate immunity mechanisms, such as interleukin (IL)-8. T cells may promote immunoinflammation mainly via interferon (IFN)-γ and tumor necrosis factor (TNF)-α and fibrogenesis via transforming growth factor (TGF)-β, basic fibroblast growth factor (b-FGF), IL-1β, and IL-4. Activated fibroblasts overproduce the extracellular matrix, the progressive accumulation of which leads to fibrosis.
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References