An Immunohistochemical Analysis of Naturally Occurring Chancroid


Materials and Methods

Clinical and laboratory data. Eight men with penile ulcers clinically characteristic of chancroid infection were assessed dur-
of epithelium, changes present in the epithelium, presence of ulceration, nature and extent of inflammatory infiltrate, type of inflammatory cells present, and vascular changes.

The degree of infiltrate of the various inflammatory cells was graded semiquantitatively as follows: +, minimal infiltrate/scattered cells only; + +, patchy infiltrate involving several areas; and + ++, diffuse infiltrate.

Results

Culture and serology. Seven patients had H. ducreyi-positive cultures; 1 was culture-negative. One patient, whose ulcer was positive for H. ducreyi, was also positive for syphilis by the rapid plasma reagin and macrohemagglutinin—Treponema pallidum serologic tests. Darkfield examinations were negative in all cases. All patients were HIV-negative.

Histology. Similar histologic findings were noted in all biopsies. Epithelium was present in all 8 biopsies. Epithelial changes included hyperplasia, spongiosis, and lymphocytic and neutrophilic infiltration of the epidermis with early pustule formation.

A dense inflammatory infiltrate was present in all biopsies and extended from the reticular to deep dermis (figure IA). The infiltrate was both interstitial and perivascular and in 1 case showed a predominantly perivascular distribution. It was apparent on the tissue sections that the infiltrate became predominantly perivascular in the deep dermis and in the direction away from the ulcer bed.

Four patients had focal aggregates of epithelioid macrophages surrounded by a collar of mononuclear leukocytes, predominantly lymphocytes, characteristic of a granulomatous inflammatory reaction (figure IC), which was subsequently confirmed by immunohistochemical staining with macrophage marker CD68 (data not shown). Vascular changes were prominent in all cases with marked endothelial swelling and proliferation, but no definitive evidence of vasculitis was observed.

By light microscopy the inflammatory infiltrate in all cases consisted predominantly of mononuclear leukocytes: The vast majority were lymphocytes and histiocytes (figure IB). Neutrophils intermixed with necrotic slough were diffusely present in large numbers at the ulcer base. Plasma cells were not prominently represented in any case; however, 3 cases had individual and scattered clusters of cells. In 1 case, plasma cells were perivascularly distributed.

Immunohistochemistry (table 1). CD4 and CD8 lymphocytes were present in nearly equal amounts (figure IF, F) and expressed CD45RO, indicating memory phenotype (figure ID). Small clusters of subepidermal B cells (CD19, CD20) were present in 3 cases, whereas 2 cases demonstrated isolated scattered B cells, and 1 case had no evidence of a B cell infiltrate. All cases showed diffuse infiltrates of macrophages (CD68) corresponding with the light microscopic findings. Langerhans cells were strongly positive for S100, and a very few isolated dermal macrophages stained positive for this marker.

Discussion

All patients studied presented with genital ulceration clinically typical for chancroid, and cultures for H. ducreyi were positive in 7 patients. A similar inflammatory response to H. ducreyi was observed in all 8 patients. All biopsies demonstrated a dense perivascular and interstitial inflammatory infiltrate. The cellular infiltrate consisted predominantly of T lymphocytes (both CD4 and CD8 subsets) and macrophages with areas of granulomatous change. Vascular endothelial changes consisting of endothelial swelling, endothelial cell proliferation, and erythrocyte extravasation were noted in all cases. Histologically these changes were consistent with a cell-mediated immune response.

Previous descriptions of chancroid have emphasized an inflammatory process divided into 3 distinct zones: a superficial zone of necrotic tissue containing fibrinous exudate and neutrophils, a broad midzone of edematous tissue containing numerous dilated vessels, and a deep zone consisting of an inflammatory infiltrate containing mainly plasma cells and fewer lymphocytes [3, 4]. Our biopsies were similar to prior descriptions with respect to the presence of a superficial zone of necrotic slough and neutrophils and tissue edema. However, significant differences were noted.

The characteristic trilaminar zonal pattern was absent, as was a plasma cell-rich infiltrate. In addition, the presence of a cell-mediated response consisting predominantly of T lymphocytes and macrophages, to our knowledge, has not been described in chancroid. This may be a consequence of the fact that previous studies did not include immunohistochemical analysis, nor were the biopsies proven to be H. ducreyi-positive by culture [4].

Another explanation for these apparent differences could be the site from which the biopsies were obtained. While all of our specimens were taken from the edge of the ulcers to include adjacent epithelium, previous histologic studies appear to describe biopsies taken from the ulcer bed itself. In support of this, it appeared that in a direction away from the ulcer site, the inflammatory response localized in a perivascular fashion. Another possible explanation for the discrepancies could be the time in the disease process at which the biopsies were taken. However, this seems to be a less likely explanation as our patients had well formed ulcers that had persisted 1–4 weeks, yet all displayed a similar response pattern, suggesting the pattern was unrelated to disease duration.

In vitro responses to H. ducreyi provide support for the presence of a Th1 response to whole organism or to sonicated preparations, as antigen-specific induction of interleukin (IL)-2 synthesis was observed [7]. Consistent with these findings was the observation that patients with chancroid had increased levels of soluble IL-2 receptors in urine and serum [8]. IL-2 is secreted by the Th1 subset of CD4 lymphocytes, which are predominantly associated with the genesis of cell-mediated immunity and delayed-type hypersensitivity reactions. Such
responses can activate monocytes and macrophages, which would be consistent with our biopsy findings.

Furthermore, experimental infection with *H. ducreyi* in normal healthy volunteers [9] and in a swine model of *H. ducreyi* infection [10] resulted in recruitment of T cells and macrophages to the inoculation site. In contrast, previous studies based on mouse and rabbit models have elicited a purulent inflammatory response with abscess formation [11, 12]. A comparison of the response patterns in experimental systems to those in our clinical specimens suggests that the experimental human and swine models of infection most closely resemble the human response in naturally occurring chancroid.

The mechanisms by which *H. ducreyi* increases the host susceptibility to HIV infection are unknown. However, the induction of a cell-mediated immune response may be important in the facilitation of the transmission of HIV. High-affinity binding of HIV envelope gp120 to cell surface CD4 on T lymphocytes is the major mechanism for viral entry into lymphocytes and is followed by integration into the host genome [13, 14]. In addition, cells of the monocyte-macrophage lineage are particularly susceptible to HIV infection both in vivo and in vitro [14, 15], leading to a chronic, productive, and noncytotoxic cellular infection. Macrophages can live for months while carrying a significant virus burden. Therefore,

![Image of histology and immunohistochemistry of human chancroid](image-url)

### Table 1. Semiquantitative analysis of immunohistochemical data in 8 patients with chancroid.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>CD45 T cells</th>
<th>CD20 B cells</th>
<th>CD68 macrophages</th>
<th>CD4 T cells</th>
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**NOTE.** - , scattered positive cells; + , patchy positive infiltrate involving several areas; ++ , diffusely positive infiltrate; -, negative.
macrophages may serve as primary targets for HIV infection and dissemination and play an important role in the pathogenesis of the disease. The host response in *H. ducreyi* genital ulcers provides an ideal environment for the facilitation and transmission of HIV infection to either of these cell types.

In summary, the immune response seen in naturally occurring *H. ducreyi*–induced chancroid genital ulcers displayed a histologic pattern consistent with that of a delayed-type hypersensitivity reaction with the recruitment of both CD4 and CD8 T lymphocytes and macrophages. Such recruitment may play a role in the facilitation of HIV transmission to chancroid-infected patients.

Acknowledgments

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References