An Immunohistochemical Analysis of Naturally Occurring Chancroid

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Haemophilus ducreyi is a major cause of genital ulcer disease in many developing countries and is associated with augmented transmission of human immunodeficiency virus (HIV). However, the mechanisms through which H. ducreyi produces ulceration are poorly understood. The characteristics of the host response to H. ducreyi and the pathobiology of its potential contribution to increased HIV susceptibility are not known. Chancroid ulcer biopsies from 8 patients were analyzed histologically and immunohistochemically. All biopsies had perivascular and interstitial mononuclear cell infiltrates that extended deep into the dermis. The infiltrate, which contained macrophages and CD4 and CD8 lymphocytes, was consistent with a delayed hypersensitivity type cell-mediated immune response. The recruitment of CD4 T lymphocytes and macrophages may in part explain the facilitation of HIV transmission in patients with chancroid.

Haemophilus ducreyi, the causative organism of chancroid, is a major cause of genital ulcer disease in developing countries [1]. Moreover, its incidence of infection in the United States rose during the 1980s [2]. It is presumed that H. ducreyi enters the skin through a break in the epithelium after sexual exposure. A painful circumscribed ulcer with ragged edges ensues [1]. Histologically, the ulcer has been described as having a characteristic trilaminar zonal pattern consisting of a narrow superficial zone of necrotic tissue and neutrophils, a broad midzone of edema and prominent vascularity, and a deep zone of predominantly plasma cells and fewer lymphocytes [3, 4].

Others have reported increased susceptibility to human immunodeficiency virus (HIV) infection in patients with chancroid [1, 5]. Such a predisposition to infection could relate to the presence of lesions, which provide a portal of entry for the virus. An additional explanation might derive from the facilitation of HIV transmission in patients with chancroid.

Clinical and laboratory data. Eight men with penile ulcers clinically characteristic of chancroid infection were assessed during 1994 at the Nairobi City Special Council Treatment Center. All presented with one or two sharply circumscribed, tender, purulent, 10- to 20-mm penile ulcers. Ulceration had been present 1–4 weeks; none showed evidence of reepithelialization. Four patients had significant inguinal lymphadenopathy including 2 who had bubo formation. Seven patients were circumcised and 1 patient had visible old scar tissue, presumably from previous infections.

Swabs from ulcers for the isolation of H. ducreyi were directly inoculated onto CCA plates containing chocolate agar plus 0.2% activated charcoal (Sigma, St. Louis), 1% IsoVitalex supplement (BBL, Baltimore), and 3 mg/L vancomycin. Plates were incubated at 33°C with 5% CO2 for 72 h. Rapid plasma reagin card (Becton Dickinson Microbiology Systems, Cockeysville, MD) and Serat-E Tek microhemagglutination–Treponema pallidum (MHA-TP; Miles, Elkhart, IN) tests for syphilis were done. We made darkfield examinations of material aspirated from the ulcer surfaces. Serum was screened for HIV infection using Detect HIV1/2 (IAF Biochem, Montreal). Patients were treated orally with erythromycin base (500 mg) four times a day for 7 days.

Tissue samples. Four-millimeter punch biopsies of the ulcers were obtained under local anesthetic and divided into three equal portions. One piece was fixed in 10% formalin, a second was fixed in 2.5% glutaraldehyde, and the third was snap frozen and stored in OCT (optimum cutting temperature) compound at −70°C.

Histochemical and immunohistochemical studies. Sections from formalin-fixed paraffin-embedded tissue were stained with hematoxylin-eosin, Giemsa, and Gram's stain. Immunohistochemical analysis was done on consecutively cut 5-μm sections. Antibodies that bound to B lymphocytes (CD20), helper-inducer/memory T lymphocytes (CD45RO), Langerhans cells (S100), and macrophages (LN5; Biogenex, San Ramon, CA) were used on paraffin sections. Antibodies to CD4 and CD8 expression on T lymphocytes (CD4 and CD8), macrophages (CD68), and B cells (CD19) were used on frozen tissue sections. Unless indicated otherwise, all antibodies were purchased from Dako (Carpinteria, CA). An avidin–biotin peroxidase complex method was used to detect bound antibody as previously described [6]. Controls included species-matched irrelevant first antibody.

Quantitation and analysis of tissue biopsies. Tissue sections were analyzed by 2 of us (R.K., J.G.) in a blinded study and described according to the following criteria: presence or absence
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 1A). 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 1C), 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 1B). 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 1F, F) and expressed CD45RO, indicating memory phenotype (figure 1D). 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,
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.

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**References**