Angiogenesis in Cutaneous Lesions of Leprosy

Implications for Treatment

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Objective: To examine the potential role of angiogenesis in leprosy.

Design: Immunohistochemical analysis of leprosy lesions.

Setting: Department of Dermatology, Venereology, and Leprology, Kasturba Medical College; Division of Dermatology, University of California at Los Angeles; and Departments of Dermatology and Pathology, Emory University.

Patients: Thirty-two cutaneous lesions that represented the spectrum of leprosy were obtained from 32 patients.

Main Outcome Measure: CD31 microvessel counts.

Results: The mean CD31 microvessel count in borderline tuberculoid, midborderline, and lepromatous leprosy lesions was significantly higher than in indeterminate leprosy lesions.

Conclusions: Increased bacterial load is associated with increased angiogenesis. Angiogenesis inhibitors may be of benefit in the treatment of leprosy.

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Kasturba Medical College, and 4 sections were received from the Division of Dermatology, University of California at Los Angeles. The diagnosis of leprosy in the patients was supported by hematoxylin-eosin–stained histologic analyses of the biopsied lesions and the clinical history of the patients. We studied 4 sections of indeterminate leprosy, 5 of tuberculoid leprosy, 12 of borderline tuberculoid leprosy, 3 of midborderline leprosy, 3 of borderline lepromatous leprosy, and 5 of lepromatous leprosy.

IMMUNOHISTOCHEMICAL ANALYSIS

A total of 32 sections (5 mm) of formalin-fixed, paraffin-embedded tissue (5 µm) were immunostained with monoclonal antibodies against CD31 (1:80) (clone JC170A, 1/80; Dako Corp, Carpinteria, California) using a horseradish peroxidase–labeled polymer, a heat-induced antigen retrieval, and an autostainer (Dako). The Dako Envision system is a 2-step horseradish peroxidase–labeled polymer that is conjugated with secondary antibodies and is used in combination with the automated Dako Autostainer. Hematoxylin was used as the counterstain, and the negative control was a primary antibody replaced by buffer. Sections of myometrium (blood vessels) were used as the positive control for CD31. Diaminobenzidine was the chromogen used, and Dako automation hematoxylin was used as a counterstain for 15 minutes. The mean CD31 microvessel density was quantitated microscopically by 2 independent observers (C.C. and J.B.M.). The number of CD31-positive blood vessels in the whole biopsy specimen and in 2 hot spots at a power of ×20 was determined according to the method of Weidner et al.16 Hot spots were areas determined by the observers to represent the fields of greatest vascular density within a given section.19 The mean number of microvessels in 2 hot spots from each section was determined, and the total mean of each spectrum was compared with indeterminate leprosy.

STATISTICAL ANALYSIS

P < .05 was considered statistically significant. A t test was used for statistical analysis, which was performed with Excel (Microsoft Inc, Redmond, Washington).

RESULTS

The results of lesion vascularity evaluated with CD31 ranged from a low of 7 vessels per high-powered field in the indeterminate leprosy lesions to a high of 68.5 in lepromatous leprosy lesions. The mean±SEM CD31 microvessel density was 25.3±3.7 vessels per high-powered field in the borderline tuberculoid lesions, 36.2±5.2 in the midborderline lesions, and 44.0±9.8 in the lepromatous lesions. These numbers were significantly higher (P = .02, .004, and .03, respectively) when compared with a mean of 13.3±3.7 vessels per high-powered field in indeterminate leprosy lesions. The mean CD31 microvessel densities in the different leprosy types are shown in Figure 1A.

Figure 1. Mean CD31 microvessel density for each leprosy type. Error bars indicate SEM. *P < .05 compared with indeterminate leprosy.

For this patient, a total of 3 hot spots with 8, 6, and 7 vessels were counted, and the mean number of microvessels in 2 hot spots was 7.5. Figure 2B shows a borderline lepromatous lesion, with a mean CD31 value of 39. For this slide, a total of 5 hot spots with values of 38, 31, 26, 40, and 23 were calculated, and the mean number of microvessels in 2 hot spots was 39.
Leprosy or Hansen disease is a chronic infectious disease that primarily affects the peripheral nerves and the skin. *Mycobacterium leprae* has a unique predilection for Schwann cells and replicates inside the Schwann cells slowly throughout the years. The clinical response of the susceptible patient forms a spectrum according to the type of host immune response to *M. leprae* and forms the basis of various classifications of leprosy. Ridley and Jopling classified the clinical response into tuberculoid, borderline tuberculoid, borderline lepromatous, and lepromatous leprosy. Patients with tuberculoid leprosy with strong cell-mediated immunity are at one end of the spectrum, and patients with lepromatous leprosy and poor cell-mediated immunity are at the other end of the spectrum. Indeterminate leprosy was first described as a new clinical variant by Findlay and was included in the World Health Organization 1952 classification. Indeterminate leprosy is often the clinical beginning of the disease, and approximately 80% of susceptible patients will show spontaneous regression of lesions, and 20% will progress to a more definitive form of leprosy. The role of immunologic responses in all the different forms of leprosy has been long established, but data are lacking on the role of angiogenesis in the spectrum of leprosy. We wanted to determine the role of angiogenesis, if any, in the entire spectrum of leprosy, including indeterminate leprosy.

*Mycobacterium leprae* has been found in endothelial cells of blood vessels, and anti–factor VIII–related antigen antibody has been used to demonstrate a difference in the microvascular pattern between the 2 ends of the spectrum, with lepromatous leprosy demonstrating a tortuous mesh of microvessels among the *M. leprae*–laded macrophages; however, the microvessels in the tuberculoid lesions were restricted to the periphery of the granulomas. We studied the microvessel counts in the entire Ridley-Jopling spectrum and found an apparent overall increase in microvessel count toward lepromatous lesions compared with indeterminate leprosy lesions. The microvessel count was significantly higher in borderline tuberculoid lesions, midborderline lesions, and lepromatous lesions when compared with indeterminate lesions. We observed that the increase in microvessel count follows the same pattern of increase as *M. leprae* loads toward the lepromatous end of the spectrum. We propose that new treatments, such as angiogenesis inhibitors directed toward leprosy, could potentiate the current multidrug treatment for leprosy.

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Author Contributions: Dr Arbiser had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Bhandarkar and Arbiser. Acquisition of data: Bhandarkar, Cohen, Kuruwila, Rea, Lee, and Modlin. Analysis and interpretation of data: Bhandarkar, Cohen, MacKelfresh, and Arbiser. Drafting of the manuscript: Bhandarkar, Kuruwila, MacKelfresh, Modlin, and Arbiser. Critical revision of the manuscript for important intellectual content: Cohen, Rea, Lee, Modlin, and Arbiser. Statistical analysis: Bhandarkar. Obtained funding: Arbiser. Administrative, technical, or material support: Bhandarkar, Cohen, Kuruwila, Rea, Lee, Modlin, and Arbiser.

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REFERENCES