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Tuberculosis Verrucosa Cutis Lesions Exhibit a Greater Microvessel Count than Lupus Vulgaris Lesions

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BACKGROUND

Tuberculosis is reported to be the second most common infectious cause of death after HIV. The causative organism of tuberculosis, Mycobacterium tuberculosis (MTB) is capable of infecting virtually every organ of the body, including the skin (1). The top three most common cutaneous forms of tuberculosis are Tuberculosis Verrucosa Cutis (TBVC), Lupus Vulgaris (LV) and Scrofuloderma. TBVC is often self limited, and may clear without antibiotic treatment, while LV is progressive without treatment. TBVC may thus represent a Th1 immune response to MTB, while LV may represent a Th2 response. This is analogous to the differentiation between tuberculoid leprosy (Th1) and lepromatous leprosy (Th2) (2).

Previously, we examined angiogenesis in differing stages of leprosy, caused by a Mycobacterium (3). We demonstrated that lepromatous leprosy had a higher microvessel count than tuberculoid leprosy. In the current study of cutaneous Mycobacterium tuberculosis infection, we found that TBVC had an elevated microvessel count compared to LV, despite LV having a clinically more aggressive course (3). This difference suggests that

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Study concept & design: JLA SSB

Acquisition of data: JLA, SSB

Analysis & interpretation of data: SSB, EV, JLA

Drafting of the manuscript: JLA, MYB and SSB

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Mycobacterium tuberculosis is controlled in the skin through a different mechanism than Mycobacterium leprae. The increased microvessel count in TBVC compared to LV suggests a possible requirement for vessel associated cytokines in the control of MTB, and conversely, that tissue hypoxia may prevent an effective immune response to MTB.

**QUESTIONS ADDRESSED**

We wanted to determine whether the two most common forms of cutaneous tuberculosis differ in terms of angiogenesis. This knowledge may be beneficial in modifying host responses to tuberculosis.

**EXPERIMENTAL DESIGN**

We studied 14 cutaneous lesions, one from each of the 14 patients with cutaneous tuberculosis. The paraffin blocks were received from Dr. Padmavathy from Annamalai University, India. The diagnosis of cutaneous tuberculosis in the patients was supported by hematoxylin-eosin-stained histology of the biopsied lesions and the clinical history. Seven patients had lupus vulgaris and seven had tuberculosis verrucosa cutis.

A total of 14 sections (5 mm) of formalin-fixed, paraffin-embedded tissue were immunostained with monoclonal antibodies against CD31 (clone JC170A,1/80, Dako Corp, Carpinteria, CA) using a polymer with the pressure cooker heat–induced antigen retrieval and an autostainer (Dako Corp). Negative controls had primary antibody replaced by buffer. Sections of myometrium (blood vessels) were used as positive control for CD31. The CD31 mean and microvessel density were quantitated microscopically by 1 observer (J.M). The number of CD31 positive blood vessels in the sections and in two hotspots at a power of 20X according to the method of Weidner et al (4). Hot spots were areas determined by the pathologist to represent the fields of greatest vascular density within a given section.

We used the nonparametric Wilcoxon matched pairs test to compare the durations of efficacy between the first and last injections, with a significance threshold of 5%. Statistical analyses were carried out with SAS 9.3 software.

**RESULTS**

The results of lesion vascularity evaluated with CD31 range from a mean of 4.8 in the Lupus Vulgaris spectrum and a mean of 10.1 in the Tuberculosis Verrucosa Cutis spectrum. The mean CD31 microvessel density in both types of lesions is shown in Figure 1. Both LV and TBVC demonstrated angiogenesis, but TBVC was significantly more angiogenic. CD31 values for TBVC were significantly higher than values for LV. There almost was no overlap in their distribution and test reported them as different with possible error of no more than 0.0106. Lupus vulgaris showed a significantly lower CD31 count when compared to Tuberculosis Verrucosa Cutis.
CONCLUSION

Cutaneous Tuberculosis is not as common as pulmonary tuberculosis, but it is still a significant infectious problem in developing countries (5). Lupus vulgaris is reported to be the most common in adults (6) in many countries and currently considered the most prevalent type (7). We decided to study angiogenesis patterns in these two forms to determine the role of angiogenesis, if any, in these two common types of cutaneous tuberculosis.

TNF increases the antimicrobial capacity of macrophages through the induction of reactive oxygen, but excess reactive oxygen can cause necroptosis of bacteria laden macrophages, leading to release and dissemination of MTB, while low concentrations of reactive oxygen favor enhanced intracellular growth (8, 9). Therefore a state of moderate elevation of reactive oxygen may be required for control of MTB. TNF is also a well-known inducer of angiogenesis and inducer of nitric oxide synthesis, which can be clinically observed in psoriasis. One possibility is that TBVC has increased blood vessels due to increased cytokines and reactive oxygen. The cytokines that have been implicated in control of MTB include TNF, superoxide and nitric oxide, all well-established mediators of angiogenesis (8, 9).

We found that lupus vulgaris has significantly lower angiogenic vessel counts than tuberculosis verrucosa cutis. Clinically, LV is more aggressive than TBVC, a possible manifestation of invasive behavior. These differences may play a role in host control of tuberculosis infection. LV has relatively fewer vessels, suggesting that LV lesions may be relatively hypoxic compared with TBVC.

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Figure 1. Distribution of Wilcoxon Scores for vessel count (CD31) in Lupus Vulgaris (LV) and Tuberculosis Verrucosa Cutis (TBVC) specimens

TBVC was significantly more angiogenic than LV.
Figure 2. Representative CD31 staining in TBVC (A.) and LV (B.)
The mean staining of CD31 in TBVC was 10.1 compared to LV, which had a mean CD31 staining of 4.8.