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Progressive and destructive hair follicle infections in a murine cutaneous anthrax model

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Abstract

Hair follicles may allow pathogen entry because they represent potential barrier defects and because there is immunological privilege within actively growing follicles. Experimental cutaneous *Bacillus anthracis* infections in mice have previously shown prominent organism invasion and proliferation within hair follicles. For the present study, C57BL/6 mice were inoculated with *B. anthracis* (Sterne) spores onto abraded skin with either anagen (actively growing) or telogen (inactive) hair follicles; skin samples were evaluated by histologic methods and electron microscopy. The infections were found to progress similarly in either anagen or telogen hair follicles, with bacilli occasionally invading deeper sites in anagen hair follicles. The infections progressed from the surface inward, rather than growing outward from within the follicles. Infecting bacilli destroyed the hair follicle keratinocytes and were initially not contacted by inflammatory cells within the follicles. However, at 3–4 days after inoculation, inflammatory cells did contact and disperse the massed follicle bacilli and led to apparent resolution of the follicle infections. Therefore, in this model system *B. anthracis* initially attacks superficial sites in active or inactive hair follicles and then progresses inward, producing destructive infections of the hair follicles; these infections clear when the massed bacilli are eventually contacted and dispersed by inflammatory cells.

Keywords: Cutaneous anthrax; *Bacillus anthracis*; Hair follicles; Keratinocytes; Anagen; Telogen

1. Introduction

Anthrax in humans usually develops through contact with infected animals or products made from them, and in developed countries the disease is approximately 95% cutaneous and 5% respiratory or gastrointestinal [1,2]. The United States mail system cases in the fall of 2001 were approximately half inhalational and half cutaneous in form [3]. Despite the high percentage of cutaneous infections in humans, very little is known regarding the pathogenesis and early pathology of these lesions, except that cutaneous anthrax is thought to begin with inoculation of spores into a cut or abrasion in the skin [1,2].

We have used experimental epicutaneous inoculation of *Bacillus anthracis* (Stern) onto intact or abraded skin of mice to investigate the interactions between the organisms and the epidermis, and these studies have demonstrated that the keratinocytes of hair follicles in inoculated abraded skin were a major site of proliferation for *B. antrhacis* bacilli [4]. Hair follicles represent a potential break in the stratum corneum barrier, which is an important mechanism of defense for the skin [5–7]. Furthermore, hair follicles in their actively growing state appear to be a site of immune privilege [8,9] that may also contribute to the susceptibility of these structures to cutaneous infections. Hair follicles are dynamic, with their states of growth termed anagen (actively growing), catagen (degenerative), and telogen (resting); anagen follicles extend more deeply into the skin, but when growth stops the deeper parts of the follicles degenerate to leave behind the shallower telogen follicles [5]. In mice, as opposed to humans, anagen and telogen follicles are grouped together

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in separate skin areas [10]. It has been found in studies of experimental dermatophytosis in mice that only the anagen skin areas are susceptible to infection [11]. Also, work with experimental cutaneous candidiasis in mice has shown that some deep foci of infection could occur in the deeper parts of the anagen hair follicles [10].

The following studies were undertaken to investigate the initiation and progression of the hair follicle infections in experimental cutaneous anthrax in mice, particularly to answer the following questions: (a) are anagen hair follicles more susceptible to *B. anthracis* than are telogen ones? (b) Do hair follicle infections begin deep in the follicle and grow outward, or do they start at the skin surface and grow inward? (c) What happens to the deep hair follicle foci when the infections begin to clear?

2. Results

2.1. Susceptibility of anagen and telogen skin areas to infection

Foci of *B. anthracis* bacilli were found below the infundibular outlets of hair follicles in inoculated abraded skin of telogen as well as anagen areas (Fig. 1a and b). Quantitative assessment of bacilli invading into interfollicular skin as assessed by either the number of fields infected or the depth of bacilli invasion was not found to be different between anagen and telogen skin (Table 1). Infections of the hair follicles themselves were also not different for anagen versus telogen, with the numbers of

Table 1
Effect of hair follicle (HF) growth stage[a] on infection after epicutaneous inoculation of *B. anthracis* spores onto abraded skin of C57BL/6 mice

	Anagen HFs	Telogen HFs
(A) Interfollicular skin		
Fields infected (%)	58.0±36.3	53.8±21.3
Bacilli depth (μm)	20.4±8.1	18.3±2.5
(B) HF infections		
Infected (%)	58.7±34.1	60.2±28.4
# Deep foci/section[b]	12.5±13.5	9.3±11.1
Foci depth (μm)	199.2±59.2	173.4±57.0

The studies were done on tissues taken at 24 h after inoculation of spores onto abraded skin, and represent results (expressed as mean±S.D.) from 10 mice tested in five experiments for anagen and 8 mice tested in four experiments for telogen. Note that the cutaneous infections of either interfollicular skin or HFs developed approximately equally after inoculations onto skin with either anagen or telogen HFs.

[a]Hair follicle growth stage = inoculations made onto skin with hair follicles in anagen (actively growing) or telogen (inactive) stages.

[b]Deep foci = bacilli observed in hair follicles at >100 μm below the skin surface.

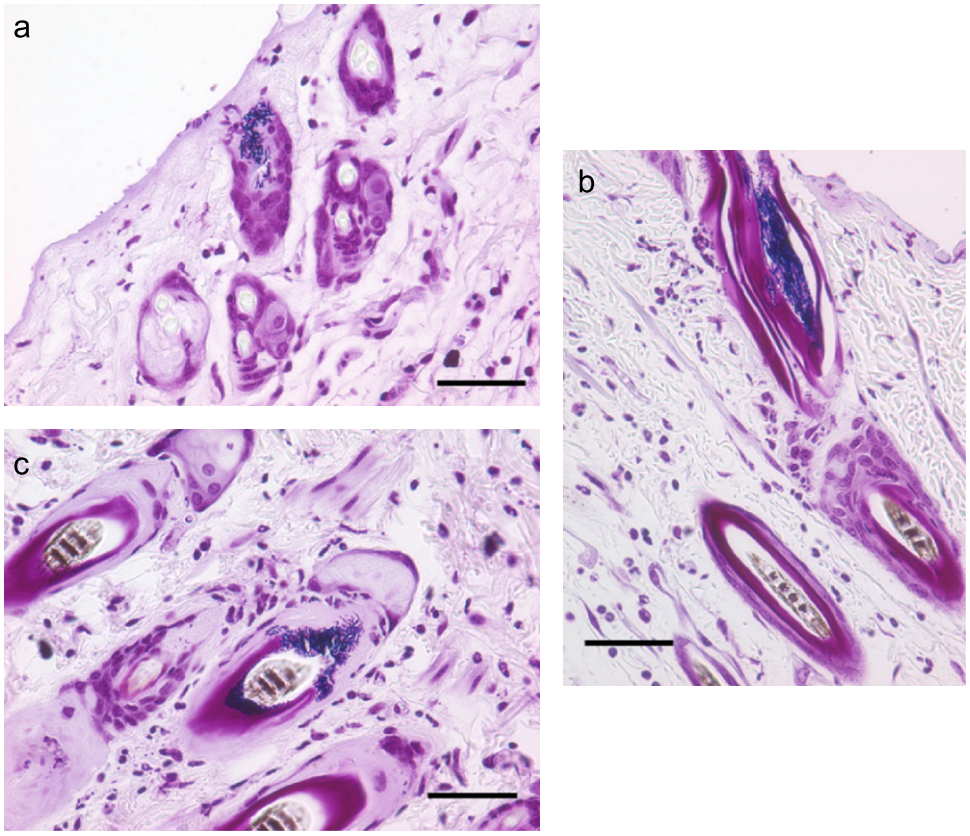

Fig. 1. *B. anthracis* bacilli infecting hair follicles in C57BL/6 mice 24 h after epicutaneous inoculation onto abraded skin, showing: (a) infection in a telogen hair follicle; (b) infection in an anagen hair follicle; (c) infection at a very deep site (below 400 μm) in an anagen hair follicle (tissue gram stain with photomicrographs taken at an original magnification of 400×; bar = 50 μm).