The Capsule of *Bacillus anthracis* Behaves as a Thymus-Independent Type 2 Antigen

Taia T. Wang and Alexander H. Lucas*

Children’s Hospital Oakland Research Institute, Oakland, California

Received 22 April 2004/Returned for modification 1 June 2004/Accepted 14 June 2004

*Bacillus anthracis* elaborates a homopolymeric capsule composed of γ-D-glutamic acid residues. Mice were immunized with formalin-fixed encapsulated *B. anthracis* bacilli, and the serum antibody response to a γ-D-glutamyl capsular epitope was measured. Antiglutamyl antibodies were elicited in athymic BALB/c Nu/Nu, BALB/c Nu/+, and CBA/J mice but not in CBA/N xid mice. These response patterns define the capsule of *B. anthracis* as a thymus-independent type 2 antigen.

*Bacillus anthracis* is an encapsulated, endospore-forming, gram-positive rod and is the causative agent of anthrax (1, 8). Pathogenicity of *B. anthracis* depends upon elaboration of both toxins and capsule. The capsule, a homopolymer of D-glutamic acid residues linked via the γ-carboxyl (2), is thought to function as a virulence factor by inhibiting phagocytosis. The *B. anthracis* capsule consists of a repeating epitope structure, is of high molecular weight, and is likely resistant to degradation. These properties are characteristic of antigens classified as thymus-independent type 2 (TI-2) (9). TI-2 antigens are able to directly activate B lymphocytes in the absence of T cells, presumably by their ability to extensively cross-link membrane immunoglobulin. Activation of B lymphocytes by TI-2 antigens requires functional expression of Bruton’s tyrosine kinase (Btk), a molecule involved in the surface immunoglobulin receptor-mediated signaling cascade. CBA/N xid mice have an X-linked mutation that results in the failure to express functional Btk, and consequently, they do not respond to TI-2 antigens. Although polysaccharides are the prototypic example of TI-2 antigens, proteins containing repeating antigenic determinants, viruses, and synthetic amino acid polymers also may function as TI-2 antigens (9, 12, 13).

In this study we sought to determine whether the capsule of *B. anthracis* behaved as a TI-2 antigen. Mice received two intraperitoneal injections of 2 × 10^8 formalin-fixed *B. anthracis* bacilli (Ames strain). Animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee. The culture and preparation of the bacilli have been described in a previous report (15). Sera were taken before immunization and 10 days after each injection. Serum anti-γ-D-glutamyl antibody levels were measured in pre- and postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in pre- and postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in pre- and postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in pre- and postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immunosorbent assay (ELISA), where glutamyl antibody levels were measured in postvaccination sera by enzyme-linked immuno...
Mice received two intraperitoneal injections of conjugate spaced 4 weeks apart. A single dose consisted of 20 μg of conjugate in 1.0 mg of aluminum hydroxide. As might be expected, (γ-D-Glu)₉-KLH behaved as a thymus-dependent (TD) antigen as shown by the lack of antiglutamyl antibody response in Nu/Nu mice (Fig. 1B). There was no difference between the magnitude of the antibody responses of CBA/N xid and CBA/J mice (P > 0.99), a result showing that glutamyl-specific B cells are present in xid mice but that their activation requires presentation of the epitope in a TD form. As seen with B. anthracis, immunization with (γ-D-Glu)₉-KLH induced significantly greater responses in CBA mice than in BALB/c Nu/+ mice (P < 0.001). Thus, the difference in responsiveness between these two strains is apparent with both TI and TD forms of the glutamyl epitope.

Isotype analysis of the antiglutamyl antibodies showed that B. anthracis immunization elicited an IgM response (Fig. 2A), whereas (γ-D-glu)₉-KLH elicited an IgG response (Fig. 2B). These isotype patterns are consistent with the conclusion that the capsule of B. anthracis functions as a TI-2 antigen and is unable to promote isotype switching. In contrast, when presented as a hapten-like molecule coupled to KLH, the glutamyl epitope functions as a TD antigen and is able to stimulate extensive switching to a variety of IgG subclasses. IgM and IgG3 typically predominate in murine responses to TI-2 polysaccharide antigens, although there are exceptions (9). In humans, TI-2 antigens, such as the pneumococcal polysaccharides, can elicit IgG responses, but this probably results from activation of preexistent class-switched memory B cells and not from the ability of the polysaccharide to directly induce isotype switching (6).

Intact B. anthracis bacilli, as opposed to purified capsule, were used as an immunogen in this study in order to simulate the antigenic stimulus encountered by the host during natural infection. High-dose intraperitoneal injection of bacilli, as used in this study, and the high concentration of bacteria present in the bloodstream during the later stage of inhalational anthrax infection are conditions that favor activation of B1 and marginal-zone B cells. Both populations have been implicated in responses to TI-2 antigens (7). Encapsulated bacteria are complex immunogens comprised not only of surface antigens but also of other molecules having adjuvant and immunomodulatory properties that activate both the adaptive and innate arms of the immune system (5, 9, 14). While the capsule of B. anthracis behaves as a TI-2 antigen in accordance with the classical criteria, the response to B. anthracis bacilli likely involves multiple immunoregulatory pathways. T cells, NK cells, complement, and cytokines are known to play a role in responses to TI antigens (9, 14), and antibody responses to TI antigens have TD components as well (4, 5).

Based upon previous studies of humoral responses to encapsulated bacterial pathogens (14), we think that the host response to B. anthracis infection will include a substantive TI-2 component. Antcapsular marginal-zone B lymphocytes would be rapidly mobilized by the septicemia which develops during the fulminant stage of inhalational anthrax infection (7). Although this TI-2 response may provide a rapid and early defense mechanism, it may not be sufficiently robust to clear an infection where bacterial levels in the blood can reach 10⁸ organisms per ml. We and others have suggested that conversion of the capsular antigen into a TD form by coupling it to immunogenic carrier proteins may be a worthwhile approach towards the development of an anthrax vaccine component that elicits an immune response directed against encapsulated vegetative cells (5a, 6, 10, 11). The benefit of having a TD capsular component in an anthrax vaccine would be the generation of an expanded, isotype-switched and possibly affinity-maturated memory-B-cell population capable of secreting IgG.
antibodies that would opsonize and promote clearance of vegetative cells during systemic anthrax infection.

We thank Patricia F. Fellows, Southern Research Institute, Frederick, Md., for provision of B. anthracis.

This work was supported by Public Health Service grant AI25008 from the National Institute of Allergy and Infectious Diseases and by a grant from Children’s Hospital Oakland Research Institute.

REFERENCES


