BACTERIAL SUPERANTIGEN VACCINES

This application is a divisional application of U.S. patent application Ser. No. 10/767,687, filed Jan. 29, 2004, now U.S. Pat. No. 7,378,257, issued May 27, 2008, which is a divisional application of U.S. patent application Ser. No. 08/882,431, filed Jun. 25, 1997, now U.S. Pat. No. 6,713,284, issued Mar. 30, 2004.

Staphylococcal enterotoxins (SEs) A through E are the most common cause of food poisoning [Bergdoll, M. S. (1983) In Easom C S F, Aslam C., eds. *Staphylococci and staphylococcal infections*. London: Academic Press, pp 559-598] and are associated with several serious diseases [Schlievert, P. M. (1993) *J. Infect. Dis.* 167: 997-1002; Ulrich et al. (1995) *Trends Microbiol.* 3: 463-468], such as bacterial arthritis [Schwab et al. (1993) *J. Immunol.* 150; 4151-4159; Goldenberg et al. (1985) *N. Engl. J. Med.* 312: 764-771], other autoimmune disorders [Psnett, D. N. (1993) *Semin. Immunol.* 5: 65-72], and toxic shock syndrome [Schlieverst, P. M. (1986) *Lancet* 1: 1149-1150; Bohach et al. (1990) *Crit. Rev. Microbiol.* 17: 251-272]. The nonenterotoxic staphylococcal superantigen toxic shock syndrome toxin-1 (TSST-1) was first identified as a causative agent of menstrual-associated toxic-shock syndrome [Schlievert et al. (1981) *J. Infect. Dis.* 143: 509-516]. Superantigen-producing *Staphylococcus aureus* strains are also linked to Kawasaki syndrome, an inflammatory disease of children [Leung et al. (1993) *Lancet* 342: 1385-1388].

The staphylococcal enterotoxins A-E, toxic shock syndrome toxin-1 (TSST-1), and streptococcal pyrogenic exotoxins A-C are soluble 23-29-kD proteins commonly referred to as bacterial superantigens (SAgs). Bacterial superantigens are ligands for both major histocompatibility complex (MHC) class II molecules, expressed on antigen-presenting cells, and the variable portion of the T cell antigen receptor β chain (TCR Vβ) [Choi et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:8941-8945; Fraser, J. D. (1989) *Nature* 339:221-223; Marrack et al. (1990) *Science* 248: 705-711; Herman et al. (1991) *Annu. Rev. Immunol.* 9: 745-772; Mollick et al. (1989) *Science* 244:817-820].

Each bacterial superantigen has a distinct affinity to a set of TCR Vβ, and coligation of the MHC class II molecule polyclonally stimulates T cells [White et al. (1989) *Cell* 56: 27-35; Kappler et al. (1989) *Science* 244: 811-813; Takimoto et al. (1990) *Eur J. Immunol.* 140: 617-621]. Pathologically elevated levels of cytokines that are produced by activated T cells are the probable cause of toxic shock symptoms [Calson et al. (1985) *Cell. Immunol.* 96: 175-183; Stiles et al. (1993) *Infect. Immun.* 61: 5333-5338]. In addition, susceptibility to lethal gram-negative endotoxin shock is enhanced by several bacterial superantigens [Stiles, et al., supra]. Although antibodies reactive with superantigens are present at low levels in human sera [Takei et al. (1993) *J. Clin. Invest.* 91: 602-607], boosting antibody titers by specific immunization may be efficacious for patients at risk for toxic shock syndrome and the other disorders of common etiology.

A vaccine approach to controlling bacterial superantigen-associated diseases presents a unique set of challenges. Acute exposure to bacterial superantigens produces T cell anergy, a state of specific non-responsiveness [Kawabe et al. (1991) *Nature* 349: 245-248], yet T cell help is presumably a requirement for mounting an antibody response.

Presently, the only superantigen vaccines available are chemically inactivated toxoids from different bacteria which have several disadvantages. The chemical inactivation process can be variable for each production lot making the product difficult to characterize. The chemical used for inactivation, (e.g. formaldehyde), is often toxic and does not negate the possibility of reversion of the inactivated superantigen to an active form. In addition, the yields of wild-type toxin from bacterial strains used for making toxoids are often low.

SUMMARY OF THE INVENTION

The present invention relates to a vaccine which overcomes the disadvantages of the chemically inactivated toxoids described above. The superantigen vaccine(s) of the present invention is/are designed to protect individuals against the pathologies resulting from exposure to one or several related staphylococcal and streptococcal toxins. The superantigen vaccine is comprised of a purified protein product that is genetically attenuated by DNA methodologies such that superantigen attributes are absent, however the superantigen is effectively recognized by the immune system and an appropriate antibody response is produced.

Specifically, the vaccine of the present invention is a product of site-directed mutagenesis of the DNA coding sequences of superantigen toxins resulting in a disruption of binding to both the MHC class II receptor and to the T-cell antigen receptor. A comprehensive study of the relationships of the superantigen structures of TSST-1, streptococcal pyrogenic exotoxin-A (SPEa), staphylococcal enterotoxin B (SEB), and staphylococcal enterotoxin A, to receptor binding were undertaken to provide insight into the design of the vaccine. From these studies, critical amino acid residues of the toxin responsible for binding the superantigen to the human MHC receptor were defined. Site-directed mutagenesis of the gene encoding the toxin and expression of the new protein product resulted in a superantigen toxin with disrupted binding to the MHC receptors.

Therefore, it is an object of the present invention to provide a superantigen toxin DNA fragment which has been genetically altered such that binding of the encoded altered toxin to the MHC class II or T-cell antigen receptor is disrupted. Such a DNA fragment is useful in the production of a vaccine against superantigen toxin infections.

It is another object of the present invention to provide a superantigen toxin amino acid sequence which has been altered such that the binding of the encoded altered toxin to the MHC class II or T-cell antigen receptor is disrupted. Such a sequence is useful for the production of a superantigen toxin vaccine.

It is another object of the invention to provide a recombinant vector comprising a vector and the DNA fragment described above.

It is a further object of the present invention to provide host cells transformed with the above-described recombinant DNA constructs. Host cells include cells of other prokaryotic species or eukaryotic plant or animal species, including yeasts, fungi, plant culture, mammalian and nonmammalian cell lines, insect cells and transgenic plants or animals.

It is another object of the present invention to provide a method for producing altered superantigen toxin with disrupted MHC class II and T-cell antigen receptor binding which comprises culturing a host cell under conditions such that a recombinant vector comprising a vector and the DNA fragment described above is expressed and altered superantigen toxin is thereby produced, and isolating superantigen toxin for use as a vaccine against superantigen toxin-associated bacterial infections and as a diagnostic reagent.

It is still another object of the invention to provide a purified altered superantigen toxin useful as a vaccine and as a diagnostic agent.