

1

PROPHYLACTIC AND THERAPEUTIC MONOCLONAL ANTIBODIES

This application claims the benefit of priority from an earlier filed provisional application Ser. No. 60/519,384 filed on Nov. 12, 2003.

INTRODUCTION

Bubonic plague whose causative agent is the bacterium *Yersinia pestis* is a zoonotic infection that is spread from natural reservoirs via the bite of infected fleas. The bubonic form of the disease characterized by a severe inflammation of the lymph nodes involved can develop into a highly infectious pneumonic form that is spread from person to person in airborne droplets. The pneumonic form of the disease is highly contagious and is usually fatal without treatment. Due to the high infectivity and mortality of pneumonic plague, *Y. pestis* is considered a likely biological threat agent. At present, there is no effective licensed vaccine for plague, and diagnostic assays are limited. Thus, the need exists for the development of sensitive and specific diagnostic assays to detect plague infection, and effective vaccines for people living in or traveling to areas in which plague is endemic, for laboratory workers handling specimens from plague patients, and for high-risk individuals who may be exposed to aerosolized *Y. pestis* in a bioterrorist attack.

The two main protective antigens of *Yersinia pestis* are the Virulence (V) antigen and the F1 capsular antigen. The V-antigen is a protein that is required for virulence and is also a protective antigen against lethal infection by *Y. pestis* (T. W. Burrows, 1957, Virulence of *Pasteurelia pestis*, Nature, 179: 1246-1247; T. Une et al., 1984, J. Immunol. 133 (4): 2226-2230; R. D. Perry and J. D. Fetherston, 1997, Clin. Microbiol. Rev. 10: 35-66). Active immunization with V-antigen or V-antigen containing recombinant antigens confers a high level of protection to both parenteral and aerosol-induced mortality by homologous strains of *Y. pestis* in mice (S. E. C. Leary et al., 1995, Contr. Microbiol. Immunol. 13: 216-217; S. E. Leary et al., 1995, Infect. Immun. 63: 2854-2858; G. W. Anderson et al., 1996, Infect. Immun. 64: 4580-4585). Passive transfer of antibody-containing serum from V-immunized mice to naïve mice protected the naïve mice from a subsequent lethal parenteral or aerosol challenge with *Y. pestis* (T. Une et al., 1987, Microbiol. Immunol. 9: 179-185; V. L. Motin et al., 1994, Infect. Immun. 62: 4192-4201; A. Roggenkamp et al., 1997, Infect. Immun. 65: 446-451). A monoclonal antibody (mAb) specific for a peptide on the V antigen has been reported to passively protect mice against a challenge with a low infectious dose of *Y. pestis* given subcutaneously (J. Hill et al., 1997, Infect. Immun. 65: 4476-4482). Passive protection against a lethal aerosol challenge with *Y. pestis* in mice using anti-V monoclonal antibodies has not been previously demonstrated.

In order to identify potentially important epitopes on the V-antigen, monoclonal antibodies to a recombinant V antigen (rV) were generated. Hybridoma supernatants were screened for reactivity to *Y. pestis* rV by ELISA. A panel of 84 mAbs specific for the V antigen was generated and have been assigned to 38 binding groups. Four hybridomas were chosen for further study: 74-1, 84-1, 125-1, and 141-1. Surprisingly, the mAbs produced from these hybridomas, mAb 74-1, mAb 84-1, mAb 125-1, and mAb 141-1, were found to protect mice from a lethal aerosol dose of *Y. pestis* strain C12. This is the first time anti-V mAbs were shown to protect against an aerosol challenge. Previous experimental results suggest that it is significantly more difficult to protect against the capsule-

2

negative, F1-negative C12 strain of *Y. pestis* as compared to the capsule-positive, F1-positive CO92 strain. The finding that mAbs with this specificity are protective is novel and unexpected. In general, it is usually easier to protect against a subcutaneous challenge than an aerosol one. Bubonic plague resulting from a subcutaneous infection or challenge usually involves the lymphatic system and takes more time to develop. Pneumonic plague which involves the respiratory system is harder to protect partly because of the delay in serum antibodies diffusing into the respiratory system where the bacteria resides. The second reason is that unlike the capsular antigen (F1) which is expressed on the surface of the bacteria, the V antigen is not believed to be abundantly expressed on the surface of the bacteria. The V antigen is usually released in the environment surrounding the bacteria. Therefore, one would expect that it would be easier to protect with anti-F1 antibodies which can readily bind to the surface bound F1 antigen and neutralize the pathogen.

SUMMARY OF THE INVENTION

Therefore, this application describes protective V-antigen-specific mAbs. The antibodies are classified into four binding groups. All the mAbs protect mice from a lethal aerosol dose of *Y. pestis* strain C12 when the mAbs are administered to mice prior to challenge. The mAbs of the present invention may be used as a prophylactic or therapeutic treatment for *Y. pestis* infections in other animal species, or in people. In addition, these mAbs are able to detect and capture V-antigen and can be incorporated into a specific, rapid diagnostic assay for the sensitive detection of V antigen of *Y. pestis*.

Therefore, it is an object of the present invention to provide monoclonal antibodies which protect against an aerosol challenge with *Y. pestis* and bind to epitopes on the *Y. pestis* V-antigen. Such antibodies are, for instance, produced by the hybridoma cell lines 74-1, deposit designation PTA-6289, 84-1, deposit designation PTA-6290, 125-1, deposit designation PTA-6291, and 141-1, deposit designation PTA-6292, deposited under the Budapest Treaty at American Type Culture Collection, Manassas, Va. on Nov. 9, 2004.

It is another object of the invention to provide for antibodies that are functionally equivalent to the antibodies listed above. These functionally equivalent antibodies substantially share at least one major functional property with an antibody listed above and herein described comprising: binding specificity to V antigen, protection against aerosol challenge when administered prophylactically or therapeutically, or competition for the same binding site on *Y. pestis* V antigen. The antibodies can be of any class such as IgG, IgM, or IgA or any subclass such as IgG1, IgG2a, and other subclasses known in the art. Further, the antibodies can be produced by any method, such as phage display, or produced in any organism or cell line, including bacteria, plant, insect, mammal or other type of cell or cell line which produces antibodies with desired characteristics, such as humanized antibodies. The antibodies can also be formed by combining a Fab portion and a Fc region from different species.

The monoclonal antibodies of the present invention described below recognize epitopes on *Y. pestis* V antigen. The immunogen was a recombinant V antigen obtained from Dr. P. Worsham of the Bacteriology Division of USAMRIID (Leary, S. et al., 1995, Infect. Imm. 63, 2854-2858).

The sequence of the V antigen is presented in SEQ ID NO:1. This sequence or portions thereof can be used as immunogens for the production of protective antibodies, polyclonal