

HUMAN MONOCLONAL ANTIBODIES PROTECTIVE AGAINST BUBONIC PLAGUE

This application claims the benefit of priority from an earlier filed provisional application Ser. No. 61/212,166 filed on Apr. 8, 2009, still pending.

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INTRODUCTION

Yersinia pestis (*Y. pestis*) is the causative agent of plague that has killed over an estimated 200 million people in previous pandemics (Perry and Fetherston, 1997, Clin. Microbiol. Rev. 10, 35). The current incident of plague is low but the animal reservoirs for *Y. pestis* exist worldwide. Sporadic cases have been reported recently with an average case number of 2,500 worldwide (World Health Organization, 2003, Wkly Epidemiol. Rec. 78, 130-135). *Y. pestis* can be rendered airborne and its potential use as a bioweapon is recognized as a category A agent on the NIAID list of biodefense-related pathogens (Zilinska, R. A., 2006, Crit. Rev. Microbiol. 32, 47-64). Current treatment for plague consists of antibiotics, while a live attenuated vaccine against plague is used in the former Soviet Union for prevention (Titball and Williamson, 2004, Expert. Opin. Biol. Ther. 4, 965-973). Nevertheless, live attenuated whole-cell vaccines or killed whole-cell vaccines have adverse effects to varying degrees (Titball and Williamson, 2004, supra). Though both types of treatment are efficacious, there is a need for an alternative treatment for plague (Casadevall, A., 2002, Emer. Infect. Dis. 8, 833-841). A multiple-antibiotic-resistant isolate of *Y. pestis* has been isolated, and drug resistance was shown to be mediated by a self-transferable plasmid (Galimand, M. et al., 1997, New Engl. J. Med. 337, 677-680; Welch T. J. et al., 2007, PLoS ONE 2, e309). A subunit vaccine, which consists of two virulent factors, the F1 protein and V-antigen, is currently in human clinical trials (Heath, D. G. et al., 1998, Vaccine 16, 1131-1137; Williamson, E. D. et al., 1995, Fems Immunol. Med. Microbiol. 12, 223-230; Williamson, E. D. et al., 2005, Infect. Immun. 73, 3598-3608). Studies involving the vaccine antigens in various formats have provided the proof-of-concept data that humoral response can be efficient in protection against *Y. pestis* (Anderson, G. W. et al., 1998, Am. J. Trop. Med. Hyg. 58, 793-799; Williamson, E. D. et al., 1999, Clin. Exper. Immunol. 116, 107-114). There are multiple reports that mouse anti-plague monoclonal antibodies (mAbs) against a *Y. pestis* challenge can passively protect a mouse against plague (Anderson, G. W. et al., 1997, Am. J. Trop. Med. Hyg. 56, 471-473; Hill, J. et al., 1997, Infect. Immun. 65, 4476-4482; Hill, J. et al., 2003, Infect. Immun. 71, 2234-2238). Therefore, mAb therapy is an attractive alternative to the existing treatments for plague. Despite the promising possibilities, there remains a major hurdle for the use of mouse mAbs for treatment against plague and that is the possible immune response of humans to the mouse mAbs that are currently available. One possibility to ameliorate the immune response against the mouse mAb is to humanize the mAb for use in humans. More preferable, and to avoid anti-mouse immune reactions altogether, is to develop new and fully human anti-plague monoclonal antibodies for clinical use (Park and Smolen, 2001, Monoclonal Antibody Therapy, In: Scolick M. eds. *Drug Discovery and Designs*. San Diego, Calif., Academic Press, pp 360-420).

We describe here the isolation of three mAbs antibodies from a large naive human phage-displayed Fab phage library. One is against the F1-antigen and the other two are against the V-antigen. When used alone, the human anti-F1 mAb displayed good protective effects, whereas the human anti-V mAbs did not. However, a clear synergistic effect was found when they were used together. Maximum protection by F1 alone could be achieved by altering the antibody administration schedule. This is the first report describing the isolation of fully human anti-plague mAbs that show efficacy in a mouse model of plague. These antibodies represent a significant breakthrough toward adjunctive therapeutic treatment of *Y. pestis* infection in humans.

SUMMARY OF THE INVENTION

This application describes human monoclonal antibodies against F1 and V antigen of *Y. pestis*. Fully human antibodies are antibody molecules in which the entire sequence of both the light chain and the heavy chain, including the CDRs, arise from human genes. Individual human monoclonal antibodies specific for F1 antigen, m252, and V antigen, m253 and m254, were produced from a human Fab phage library. The human monoclonal antibodies were protective against *Y. pestis* bubonic plague challenge when administered prophylactically or therapeutically. (By "prophylactic", it is meant administered before challenge, and by "therapeutic", it is meant administered after challenge.) The anti-F1 antibody protected BALB/c mice from a lethal challenge with *Y. pestis* CO92 when purified mAb was administered 24 hours before challenge. None of the anti-V mAbs were protective when administered alone. However, surprisingly, protection from *Y. pestis* challenge was increased when all three human monoclonal antibodies were administered in combination. In fact, the combination of antibodies was effective even when administered up to 2 days after challenge.

The invention provides human monoclonal antibodies recognizing the F1 and V antigen of *Y. pestis*. Exemplary human antibodies of the invention include, for example, the m252 anti-F1 human monoclonal antibody, the m253 anti-V human monoclonal antibody, and the m254 anti-V human monoclonal antibody. These antibodies have distinct specificities.

For example, human m252 mAb produces a weak-moderate binding signal with peptide 1 (SEQ ID NO:1) and peptide 2 (SEQ ID NO:2) out of 27 peptides which cover the length of the F1-antigen were tested. Both peptides 1 and 2 are located at the N-terminus of the F1-antigen. This suggests that m252 may also recognize a conformational region that involves peptides 1 and 2. The m252 mAb has a heavy chain variable region having the amino acid sequence set forth in SEQ ID NOS:3 and the nucleotide sequence set forth in SEQ ID NO:4. The light chain variable region of m252 has the amino acid sequence set forth in SEQ ID NO:5 and the nucleotide sequence set forth in SEQ ID NO:6.

The binding of human anti-V m253 mAb results in a weak signal with V-antigen peptide 1 (SEQ ID NO:7) and a strong signal with V-antigen peptide 2 (SEQ ID NO:8), near the amino-terminal of the V antigen. m253 mAb has a heavy chain variable region having the amino acid sequence of SEQ ID NOS:9, and the nucleotide sequence set forth in SEQ ID NO:10. m253 light chain variable region has the amino acid sequence set forth in SEQ ID NO:11 and the nucleotide sequence set forth in SEQ ID NO:12.

The binding of human anti-V m254 mAb did not give a specific signal with any of the 53 V-antigen peptides tested, suggesting that the m254 mAb recognizes a conformational region on the V-antigen. Even though m253 and m254 recog-