Abstract

Objectives: Pertussis remains a significant public health problem in spite of newer vaccination and antibiotic treatment protocols. However, deaths and hospitalization rates due to whooping cough have continued to rise recently. One factor contributing to this is the emergence of pertussis vaccine escape strains, which is a major public health concern. Therefore, research into therapeutic treatments for pertussis is vital.

Methods: Two previously described PTs-binding monoclonal antibodies, B17 and 11E6, were chosen for this study. B17 neutralized pertussis at all titers tested. 11E6 displayed greater potency than each individual antibody in the 1:1 combination and was more potent at neutralizing PTAs than the PT-V IgG preparation. When used in combination, huB17 and hu11E6 displayed greater potency than each individual antibody. The 1:1 combination was approximately 3.5-fold more potent than huB17 alone and twice as potent as hu11E6 alone.

Results: The biological activity of the humanized antibodies was similar to that of their murine counterparts and was more potent at neutralizing PTAs than the PT-V IgG preparation. When used in combination, huB17 and hu11E6 displayed greater potency than each individual antibody. The 1:1 combination was approximately 3.5-fold more potent than huB17 alone and twice as potent as hu11E6 alone.

Two previously described PTs-binding monoclonal antibodies, B17 and 11E6 [1], were chosen for this study. Biological activity was assessed via the CHO cell clumping assay [2]. Antibody was serially diluted from 50 nM to 1 μg/mL in the presence of 5 μg/mL PTs in a 96 well plate, and 210 CHO cells well were added. Plates were incubated at 37°C for a total of 24 hours and scored for lysis.

The D040 B. pertussis strain had been isolated from a critically ill infant. 210/268/11E6/11E6 strains were inoculated into 24 well plates containing CHO cell lines and were treated with B. pertussis. Leukocytes, body weight, and bacterial colonization of the lungs were evaluated 10 days later.

Methods

HuB17 and Hu11E6 Function Synergistically to Neutralize PTx

HuB17 and Hu11E6 Function Synergistically to Neutralize PTx

Two previously described PTs-binding monoclonal antibodies, B17 and 11E6 [1], were chosen for this study. Biological activity was assessed via the CHO cell clumping assay [2]. Antibody was serially diluted from 50 nM to 1 μg/mL in the presence of 5 μg/mL PTs in a 96 well plate, and 210 CHO cells well were added. Plates were incubated at 37°C for 24 hours and scored for lysis.

The D040 B. pertussis strain had been isolated from a critically ill infant. 210/268/11E6/11E6 strains were inoculated into 24 well plates containing CHO cell lines and were treated with B. pertussis. Leukocytes, body weight, and bacterial colonization of the lungs were evaluated 10 days later.

Methods

HU17 and Hu11E6 Protected Mice from Pertussis Infection

Mice were treated with the antibodies (20 g total dose) via IP injection 2 hours prior to infection with B. pertussis. Leukocytes, body weight, and bacterial colonization of the lungs were evaluated 10 days later.

Results

The Antibody Combination Effectively Treated Pertussis in Baboons

Seven baboons were inoculated with B. pertussis (10^9). Three days later, when the WBC counts had begun to rise, four animals received IV injections of a cocktail of huB17 and 11E6 (20 mg each of IgG). Leukocytosis, nasal B. pertussis bacterial loads, and coughing were reduced. The treated animals were strung into heavily and lightly colonized groups. Please refer to Poster EV102. The combination treatment with 1 and 3 mg humanized antibodies was as effective as the combination treatment without huB17 of 20 mg huB17 to 1 mg 11E6 against another Bordetella species, whereas the heavily colonized animals (huB17 and 11E6) were viable for B. pertussis.

In all treated animals, infusion of the antibody cocktail rapidly reversed the rise in white blood cell count and reduced bacterial load of infection in the lungs. The treatment was continued to a point were complete resolution of the infection was observed, as evidenced by the decreasing white blood cell counts to near normal levels. The treatment was as effective as the combination treatment without huB17 of 20 mg huB17 to 1 mg 11E6 against another Bordetella species, whereas the heavily colonized animals (huB17 and 11E6) were viable for B. pertussis.

The combination treatment resulted in a rapid improvement of the severe bouts of coughing (30th on day 3) experienced by the heavily colonized group.

Half-Life Analyses of the Humanized Antibodies in Baboons

To assess the half-life of the humanized antibody cocktail, anti-PTx antibodies were monitored for 14 days. Three weeks after antibody treatment, immunoglobulin was assayed in serum, and 24 hours following antibody treatment, immunoglobulin was assayed in serum.

In all treated animals, infusion of the antibody cocktail rapidly reversed the rise in white blood cell count and reduced bacterial load of infection in the lungs. The treatment was continued to a point where complete resolution of the infection was observed, as evidenced by the decreasing white blood cell counts to near normal levels. The treatment was as effective as the combination treatment without huB17 of 20 mg huB17 to 1 mg 11E6 against another Bordetella species, whereas the heavily colonized animals (huB17 and 11E6) were viable for B. pertussis.

The combination treatment resulted in a rapid improvement of the severe bouts of coughing (30th on day 3) experienced by the heavily colonized group.

The treated animals were strung into heavily and lightly colonized groups. Please refer to Poster EV102. The combination treatment with 1 and 3 mg humanized antibodies was as effective as the combination treatment without huB17 of 20 mg huB17 to 1 mg 11E6 against another Bordetella species, whereas the heavily colonized animals (huB17 and 11E6) were viable for B. pertussis.

In all treated animals, infusion of the antibody cocktail rapidly reversed the rise in white blood cell count and reduced bacterial load of infection in the lungs. The treatment was continued to a point where complete resolution of the infection was observed, as evidenced by the decreasing white blood cell counts to near normal levels. The treatment was as effective as the combination treatment without huB17 of 20 mg huB17 to 1 mg 11E6 against another Bordetella species, whereas the heavily colonized animals (huB17 and 11E6) were viable for B. pertussis.

The combination treatment resulted in a rapid improvement of the severe bouts of coughing (30th on day 3) experienced by the heavily colonized group.

The treated animals were strung into heavily and lightly colonized groups. Please refer to Poster EV102. The combination treatment with 1 and 3 mg humanized antibodies was as effective as the combination treatment without huB17 of 20 mg huB17 to 1 mg 11E6 against another Bordetella species, whereas the heavily colonized animals (huB17 and 11E6) were viable for B. pertussis.

In all treated animals, infusion of the antibody cocktail rapidly reversed the rise in white blood cell count and reduced bacterial load of infection in the lungs. The treatment was continued to a point where complete resolution of the infection was observed, as evidenced by the decreasing white blood cell counts to near normal levels. The treatment was as effective as the combination treatment without huB17 of 20 mg huB17 to 1 mg 11E6 against another Bordetella species, whereas the heavily colonized animals (huB17 and 11E6) were viable for B. pertussis.

The combination treatment resulted in a rapid improvement of the severe bouts of coughing (30th on day 3) experienced by the heavily colonized group.

The treated animals were strung into heavily and lightly colonized groups. Please refer to Poster EV102. The combination treatment with 1 and 3 mg humanized antibodies was as effective as the combination treatment without huB17 of 20 mg huB17 to 1 mg 11E6 against another Bordetella species, whereas the heavily colonized animals (huB17 and 11E6) were viable for B. pertussis.

In all treated animals, infusion of the antibody cocktail rapidly reversed the rise in white blood cell count and reduced bacterial load of infection in the lungs. The treatment was continued to a point where complete resolution of the infection was observed, as evidenced by the decreasing white blood cell counts to near normal levels. The treatment was as effective as the combination treatment without huB17 of 20 mg huB17 to 1 mg 11E6 against another Bordetella species, whereas the heavily colonized animals (huB17 and 11E6) were viable for B. pertussis.

In all treated animals, infusion of the antibody cocktail rapidly reversed the rise in white blood cell count and reduced bacterial load of infection in the lungs. The treatment was continued to a point where complete resolution of the infection was observed, as evidenced by the decreasing white blood cell counts to near normal levels. The treatment was as effective as the combination treatment without huB17 of 20 mg huB17 to 1 mg 11E6 against another Bordetella species, whereas the heavily colonized animals (huB17 and 11E6) were viable for B. pertussis.