EV1029

# Antibody Cocktail Effectively Treats Pertussis in a Baboon Disease Model

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## Abstract

**Objectives:** Pertussis remains a significant public health problem in spite of near-universal vaccination and continues to kill up to 300,000 children annually. Antibiotic therapy is often ineffective, presumably due to toxins produced by the *Bordetella pertussis* bacterium. Pertussis toxin (PTx) is recognized as a major virulence factor due to its abilities to impede the innate immune response and induce severe leukocytosis. Here we developed a cocktail of humanized monoclonal antibodies designed to potently neutralize PTx. Study objectives were to characterize the antibodies *in vitro* and to demonstrate efficacy in both small and large animal models. The antibody cocktail is being developed as a therapy for critically ill infants with pertussis as well as a prophylaxis for high-risk newborns.

**Methods:** Two previously described PTx-binding monoclonal antibodies, 11E6 and 1B7 [1], were humanized. 11E6 inhibits the toxin's interaction with its receptor while 1B7 blocks the toxin's enzymatic activity by interfering with intracellular trafficking. Thus, these antibodies inactivate PTx via complementary mechanisms. Characterization of the humanized antibodies included competition ELISA, surface plasmon resonance analysis, and CHO cell-based functional assays compared to their murine precursors. Prophylactic and therapeutic efficacy of the antibody cocktail was evaluated in murine and baboon models, respectively.

**Results:** Extensive *in vitro* analyses demonstrated no changes to binding affinity and PTx neutralization compared to the parental murine antibodies. Functional analyses utilizing the CHO cell assay verified that the two antibodies behaved synergistically. *B. pertussis*-infected mice treated with the hu11E6 and hu1B7 antibodies, individually and in combination, displayed continued weight gain and diminished lung bacteria levels. Antibody treatment completely eliminated the rise in white blood cell counts characteristic of disease. Notably, these effects were more pronounced than those conferred by a high-titer polyclonal antibody preparation, P-IVIG, previously tested in humans [3-5]. Seven weanling baboons were infected with 8 x$10^9$ CFU of *B. pertussis* and four were treated on day 3 with both hu11E6 and hu1B7 (20 mg/kg of each IV). The untreated animals displayed severe leukocytosis and one became moribund. In contrast, antibody treatment reversed the rise in white blood cell counts and hastened the clearance of *B. pertussis* bacteria from the nasopharynx. The antibody half-life was 11+/-4 days.

**Conclusions:** A binary antibody cocktail functioned synergistically to neutralize PTx, was protective in mice, and demonstrated therapeutic efficacy in a newly developed, clinically relevant pertussis baboon model. This antibody cocktail is expected to improve outcomes in critically ill newborns. Finally, administration at birth to high-risk newborns in the developing world is anticipated to provide prophylaxis through the first few months, when the likelihood of pertussis-mediated fatality is greatest.

## Methods

Two previously described PTx-binding monoclonal antibodies, 1B7 and 11E6 [1], were humanized. Biological activity was assessed via the CHO cell clustering assay [2]. Antibody was serially diluted from 50 nM to 1.5 pM in the presence of 5 pM PTx in a 96 well plate and 2x$10^4$ CHO cells/well were added. Plates were incubated at 37ºC for 20 hrs and clustering was scored.

The D420 *B. pertussis* strain had been isolated from a critically ill infant. C57BL/6 mice were injected IP with 20 ug of hu1B7, hu11E6, a combination of hu1B7 and hu11E6 (10 ug of each antibody), or P-IVIG two hrs prior to inoculation with 5x$10^6$ cfu of D420 via the external nares. CD45+ WBC, body weight, and D420 lung colonization were quantified 10 days later.

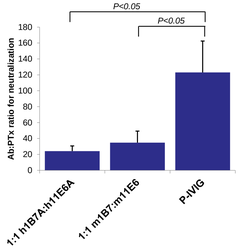
Weanling baboons were exposed to 4x$10^9$ cfu of *B. pertussis* D420 intra-tracheally followed by infusion of 2x$10^9$ cfu into each nostril. On day 3 after infection, a cocktail of hu1B7 and hu11E6 was infused intravenously at a dose of 20 mg/kg of each (40 mg/kg total). Leukocytosis, nasopharyngeal *B. pertussis* loads, and coughing were followed. Serum samples were assayed for anti-PTx antibody titers to assess pharmacokinetics.

## Results

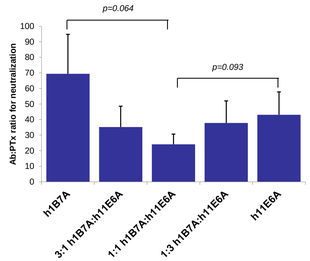
### Hu1B7 and Hu11E6 Function Synergistically to Neutralize PTx

Hu1B7 and hu11E6 were evaluated for PTx neutralization via the CHO cell assay. Each was compared to its murine counterpart and to a polyclonal pertussis immunoglobulin preparation (P-IVIG) that was used previously as a therapeutic for pertussis treatment in US clinical trials [3-5].

**CHO Cell Assay Comparing Human to Murine Antibodies and to P-IVIG**




**CHO Cell Assay Comparing Individual Antibodies to Combinations**

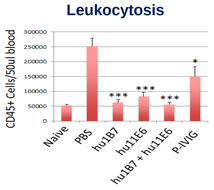



The biological activity of the humanized antibodies was similar to that of their murine counterparts and was more potent at neutralizing PTx than the polyclonal P-IVIG preparation. When used in combination, hu1B7 and hu11E6 displayed greater potency than each individual antibody. The 1:1 combination was approximately 3.5-fold more potent than hu1B7 alone and twice as potent as hu11E6 alone.

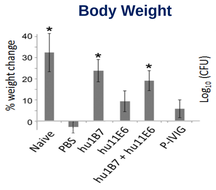
### Hu1B7 and Hu11E6 Protected Mice from Pertussis Infection

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

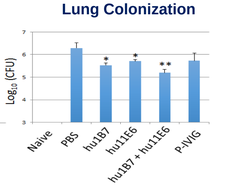
**Leukocytosis**




**Body Weight**




**Lung Colonization**



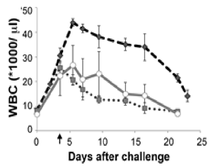

*P<0.05; **P<0.01; ***P<0.001 vs PBS

When given individually and in combination, hu1B7 and hu11E6 protected mice from *B. pertussis* infection. Most importantly, antibody treatment completely eliminated the rise in white blood cell counts characteristic of disease. Treated mice also displayed continued weight gain and diminished pulmonary bacteria levels. The monoclonal antibodies were more protective than the high-titer P-IVIG preparation previously tested in humans [3-5]. Finally, the 20 ug dose chosen for this study was too high to demonstrate antibody synergy.

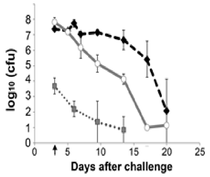
### The Antibody Combination Effectively Treated Pertussis in Baboons

Seven weanling baboons were inoculated with *B. pertussis* [6-8]. Three days later, after the WBC counts had begun to rise, four animals received IV injections of a cocktail of hu1B7 and hu11E6 (20 mg/kg of each). Leukocytosis, nasal *B. pertussis* bacterial levels, and coughing were followed.

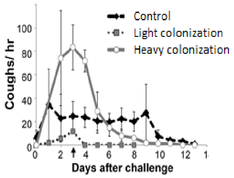
**Leukocytosis**




**Nasal *B. pertussis* Levels**




**Cough Counts**



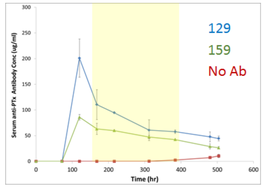

The treated animals were stratified into heavily and lightly colonized groups. Please refer to Poster EV1030. The lightly colonized animals (#113 and #115) may have been partially protected from *B. pertussis* via prior exposure to another *Bordetella* species, whereas the heavily colonized animals (#129 and #159) were naïve for *Bordetella*.

In all treated animals, infusion of the antibody cocktail rapidly reversed the rise in white blood cell counts, which then returned to normal. Antibody treatment also accelerated removal of *B. pertussis* from the nasopharynx of those animals that were heavily colonized. While all infected animals coughed, the coughing rate was variable. Antibody treatment resulted in a rapid improvement of the severe bouts of coughing (80/hr on day 3) experienced by the heavily colonized experimental group.

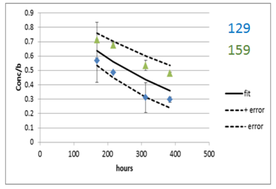
### Half-Life Analyses of the Humanized Antibodies in Baboons

To assess the half-life of the humanized antibody cocktail, anti-PTx antibodies were monitored for three weeks, by which time the endogenous immune response to infection became apparent. Data from two representative animals is shown below.

**Serum Antibody Levels**




**Beta Half-Life**




A two-phase elimination profile was observed with a beta phase half-life of 11+/-4 days. The predicted longer half-life in humans suggests that an injection of monoclonal antibody at birth could potentially protect newborns for several months, the high risk period for fatal pertussis. Since up to 300,000 newborns die annually in the developing world, this prophylactic strategy could address a significant unmet medical need.

## Conclusions

- Monoclonal antibodies 1B7 and 11E6 potently and synergistically neutralize pertussis toxin.
- Humanization of each antibody was achieved without loss of affinity or functional activity.
- Prophylactic administration in mice with hu1B7 and hu11E6, individually or in combination, completely blocked leukocytosis, enabled the mice to gain weight, and diminished pulmonary bacterial loads.
- Therapeutic administration of a cocktail of the two antibodies in baboons rapidly reversed the rise in white blood cell count and acclerated clearance of the *B. pertussis* bacteria.
- The data suggest that hu1B7 and hu11E6 have therapeutic potential to diminish morbidity, long-term sequelae, and mortality in critically ill infants with pertussis.
- The data also suggest the potential for prophylactic use at birth, particularly in the developing world, to save thousands of lives annually.

## References

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