EV1030

Spontaneous Identification of *Bordetella bronchiseptica* in a Baboon Colony: Potential Ramifications for *Bordetella pertussis* Modeling

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Abstract

Objectives: While developing pertussis therapies, we performed efficacy studies in a recently described baboon infection model. Surprisingly, despite intratracheal infusion of *B. pertussis*, some animals failed to develop the leukocytosis and/or the high levels of pertussis bacteria in the nasopharynx that are characteristic of the model. We utilized early time points from these efficacy studies to determine if infection with another *Bordetella* species could have modified the disease course.

Methods: Sixteen weanling baboons were infected with 6 x 10^9 CFU of *B. pertussis* via intratracheal and intranasal infusions. Pre-infection serum samples were assayed by ELISA for antibodies against filamentous hemaglutinin (FHA), which is expressed on multiple *Bordetella* species. Titers were graded (-) to (+++). On days 2/3 post-infection, prior to any experimental intervention, WBC counts and *B. pertussis* from nasal washes were quantified. In seven animals, anti-FHA levels were followed for 3 weeks to determine if the titer changes were characteristic of primary or secondary immune responses.

Results: Eight baboons had undetectable anti-FHA antibodies prior to infection. At the 2/3 day time point, all eight had high *B. pertussis* levels (>10^7 CFU/ml) in the nasal washes, and seven had elevated WBC counts. Two baboons, #115 (+) and #113 (++), had elevated WBC counts, but the *B. pertussis* levels remained low. In the other six baboons, with anti-FHA titers of (++) to (+++), the WBC counts remained normal and the *B. pertussis* levels remained low. Three of these animals were followed for 3 weeks, and they never had an elevated WBC count. Nasal washes from #63, #67, #99, and #113 were overgrown with a second bacterium definitively identified as *B. bronchiseptica* in animal #113. Two of these animals, #63 and #67, had no prior exposure to *B. bronchiseptica* (based on their negative FHA serology), and became unusually debilitated for this model. FHA antibody levels were followed in five animals that were FHA(-) prior to infection, and #113 and #115. The FHA(-) animals did not develop FHA titers until 3 weeks, whereas the FHA(+) animals displayed a rapid titer increase indicating prior *Bordetella* exposure.

Conclusion: These data indicate that *B. bronchiseptica* can spread in baboon colonies and can alter the course of infection in baboons used for pertussis modeling. Prior exposure to *B. bronchiseptica*, as suggested by high FHA titers, provided protection from infection by *B. pertussis*, whereas coincident initial exposure to both *Bordetella* species appeared to exacerbate the clinical course (#63 and #67). Thus, when using this baboon model it is important to be cognizant of *B. bronchiseptica* in the colony. Finally, since recent data from Dr. Merkel's lab [1,3-5] indicate that *B. pertussis* can spread between baboons, it may wise to investigate *B. pertussis* exposure in colonies used for pertussis modeling.

Methods

Baboon serum was screened by ELISA for the presence of anti-filamentous hemaglutinin (FHA) antibodies. Titers were scored as (-) to (+++).

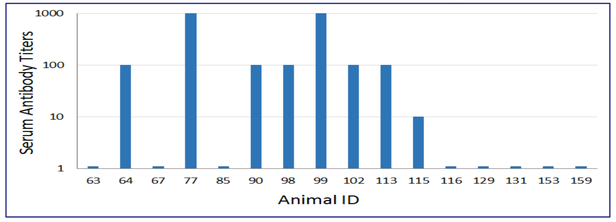
To determine the effect of pre-existing anti-FHA antibodies on subsequent B. pertussis infection, selected animals were exposed to 4x10^9 cfu *B. pertussis* D420 intracheally followed by infusion of 2x10^9 cfu into each nare. Blood was drawn under anesthesia to assess white blood cell (WBC) counts and antibody levels. Bacterial nasal carriage was evaluated from nasopharyngeal washes followed by plating on Regan-Lowe agar.

Results

Pre-Treatment Screening for anti-FHA Antibodies

Serum collected from 16 weanling baboons prior to *B. pertussis* infection was screened by ELISA for anti-FHA antibodies. The presence of anti-FHA antibodies indicates prior exposure to a *Bordetella* species.

Pre-Infection Serum Anti-FHA Antibody Titers



Eight animals had undetectable anti-FHA titers. The other eight displayed titers that ranged from (+) to (+++) indicating previous exposure to a Bordetella species.

Elevated Anti-FHA Titers Preclude Infection with *B. pertussis*

Selected animals were infected with *B. pertussis*. After two or three days, prior to any therapeutic intervention, WBC counts and nasopharyngeal pertussis levels were determined. Unexpectedly, 4 of 16 nasal wash plates were overgrown with a second bacterium, confirmed to be *B. bronchiseptica* in animal 113.

Baboons with No Pre-Infection Titer

Animal	Pre-Infection FHA titer	WBC counts (1000/ul)	B. pertussis nasal wash (CFU)	Bacterial overgrowth*
116	-	33.6	3 x 10^7	-
129	-	14.2	1 x 10^8	-
131	-	19.0	3 x 10^7	-
153	-	27.6	2 x 10^7	-
159	-	30.3	4 x 10^7	-
85	-	18.4	8 x 10^7	-
63	-	5.1	2 x 10^7	+
67	-	19.3	4 x 10^7	+

*Growth on Regan-Lowe agar, suspected to be B. *bronchiseptica*

Baboons with Pre-Infection Titer

Animal	Pre-Infection FHA titer	WBC counts (1000/ul)	B. pertussis bacterial nasal wash counts	Bacterial overgrowth*
115	+	23.0	2 x 10^4	-
113	++	28.4	2 x 10^3	+
64	++	8.0	7 x 10^4	-
77	+++	6.9	3 x 10^4	-
90	++	6.3	1 x 10^4	-
98	++	8.4	6 x 10^3	-
99	++	12.6	1 x 10^3	+
102	++	6.7	0	-

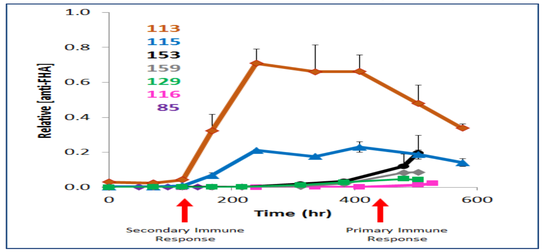
*Confirmed to be B. *bronchiseptica in Animal 113*

Animals with no pre-infection anti-FHA titers were readily infected with *B. pertussis* as 7 of 8 displayed leukocytosis and all had nasal pertussis levels >10^7 CFU/ml. In contrast, 6 of 8 with pre-infection titers failed to become infected, with no leukocytosis and nasal wash pertussis levels of 10^3-10^4 CFU/ml. Three of these animals were watched for 3 weeks and none developed leukocytosis. Interestingly, a third category with animals 113 and 115 that had (+) or (++) titers, displayed an intermediate infection profile, with elevated white counts but low nasal pertussis levels. As nasal washes from animal 113 were confirmed positive for *B. bronchiseptica*, we postulated that both 113 and 115 had been exposed to *B. bronchiseptica* shortly before *B. pertussis* infection. Accordingly, these two animals would have a primed immune response prior to B. pertussis infection, but not one that was sufficiently developed to be completely protective.

Immune Response Kinetics Confirm Prior Exposure to Bordetella

The anti-FHA antibody titers were followed in 7 animals for 3 weeks to determine if the titer changes were characteristic of primary or secondary immune responses.

Time Course of Anti-FHA Immune Response



The kinetics of the anti-FHA immune response confirmed that animals 113 and 115 had been exposed to *B. bronchiseptica* shortly before *B. pertussis* infection. Specifically, the titers in the naïve animals did not begin to rise for at least two weeks, consistent with a primary immune response. In contrast, the titers in animals 113 and 115 began to rise much earlier, consistent with a secondary immune response, indicating that these animals had a primed immune response to Bordetella infection that was partially protective. These data are consistent with the human situation where some infants are completely naïve to pertussis, whereas others are partially protected via maternal antibodies or postnatal vaccination.

Mixed Infection Can Lead to Severe Disease

The clinical status of the *B. bronchiseptica*-positive animals and their cage-mates was followed.

Cage 1

Animal	Pre-Infection FHA titer	WBC counts (1000/ul)	Bacterial overgrowth*	Clinical Appearance
63	-	5.1	+	Severe
67	-	19.3	+	Severe
85	-	18.4	-	Died

Cage 2

Animal	Pre-Infection FHA titer	WBC counts (1000/ul)	Bacterial overgrowth*	Clinical Appearance
99	++	12.6	+	Normal
102	++	6.7	-	Normal
116	-	33.6	-	Died

Concurrent infection with *B. pertussis* and *B. bronchiseptica* led to a clinical picture that was more severe than that described for this model. Accordingly, animals 63, 67, and 85 were housed together. All three had no detectable anti-FHA titers prior to the study. 63 and 67 were presumed to have an active *B. bronchiseptica* infection at the time of B. pertussis exposure. All three became extremely sick, huddled on the floor of the cage, and had poor food intake. Animal 85 died, which is unusual for this model. Similarly, animals 99, 102, and 116 were housed together. Animal 99 was presumed to be actively infected with *B. bronchiseptica* at the time of *B. pertussis* exposure. While animals 99 and 102 were protected from infection by high anti-FHA titers, animal 116 was not. Animal 116 became critically ill and was euthanized.

Conclusions

- *B. bronchiseptica* can spread in baboon colonies
- Exposure to *B. bronchiseptica* can alter the course of *B. pertussis* infection in baboons
- Prior exposure to *B. bronchiseptica* provided protection from *B. pertussis* infection
- Concurrent exposure to both Bordetella species results in a mixed infection with severe pathology that may be fatal
- Baboon facilities should consider developing procedures to minimize the spread of Bordetella infections
- Baboons should be screened for anti-FHA titers prior to inclusion in pertussis studies
- Since *B. pertussis* can also spread between naive baboons [5], procedures should be developed to pre-screen animals for prior pertussis exposure

References

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