

Passive Antibody to *Bartonella henselae* Protects against Clinical Disease following Homologous Challenge but Does Not Prevent Bacteremia in Cats

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We challenged cats transfused with anti-*Bartonella* serum and kittens born to antibody-positive queens with *Bartonella henselae* to determine the contribution of antibodies to the control of *B. henselae* in cats. In both experiments, antibody-positive cats were protected from clinical disease but passive antibody to the homologous strain of *B. henselae* did not prevent bacteremia.

Bartonella henselae is the causative agent of human cat scratch disease, bacillary angiomatosis, encephalopathy, and other clinical syndromes, the most serious of which occur in immunocompromised individuals (reviewed in reference 7). Cats are the natural host and become bacteremic following infection (3, 10).

The immune mechanisms important in the control and prevention of *B. henselae* infections have not been determined, and the relative contribution of antibodies in both the human and feline hosts is unclear. Human immunodeficiency virus (HIV)-infected individuals lack a functional cellular immune system and do not mount a significant antibody response to *Bartonella* infections (12), in contrast to the strong humoral response of human (12, 18) and feline (4, 8, 15, 16, 19) hosts that ultimately control the infection. Bacteremia in experimentally infected cats decreases significantly as the level of antibody increases (1, 7, 15) but both naturally and experimentally infected cats can develop a recurrent bacteremia in the presence of high levels of antibody (1, 3, 4), suggesting that antibodies may be important in controlling only the initial bacteremia. There are at least three genotypes of *B. henselae*, and these types do not cross-protect; that is, cats infected with type 1 are protected from bacteremia following challenge with type 1 but not other types (19). The effector mechanism responsible for this protection has also not been determined.

The long generation time of *B. henselae* and the chronic nature of the infection make it difficult to determine the relative contributions of cell-mediated and antibody-mediated effector mechanisms in the control of bacteremia. The purpose of this study was to determine the role of antibody in controlling bacteremia in the absence of a cellular immune response. In this study, we used *B. henselae* LSU16, a strain that causes reproducible disease in intradermally (i.d.) inoculated cats (15). Following inoculation with this strain, naive cats develop suppurative skin lesions, fever, lethargy, anorexia, and lymph-

adenopathy, clinical signs similar to those of moderate to severe human cat scratch disease, in addition to the bacteremia characteristic of the feline infection. We were therefore able to examine the effect of antibody on clinical signs as well.

All cats were purchased from either Harlan-Sprague-Dawley (Indianapolis, Ind.) or Liberty Research, Inc. (Waverly, N.Y.). Six 10-month-old cats, culture negative for *B. henselae* and seronegative by enzyme-linked immunosorbent assay (ELISA) and Western blot analysis, were used as recipients; three of these cats were transfused with sera from antibody-positive cats and three were transfused with sera from antibody-negative cats. Six 2- to 5-year-old cats were used as serum donors; three were inoculated 11 months previously with *B. henselae* and were abacteremic at the time of donation, and three were never exposed and were seronegative.

Blood was collected from donor cats for four consecutive weeks, and sera were frozen at -20°C . Prior to transfusion, the sera were thawed, filtered through a $0.45\text{-}\mu\text{m}$ -pore-size filter, and cultured to verify the absence of *B. henselae*. Recipient cats received 40 ml of pooled sera from either *B. henselae*-positive donors ($n = 3$) or negative donors ($n = 3$). Sera were transfused intravenously (i.v.) in five of the six cats; due to transfusion difficulties, one anti-*Bartonella*-positive cat received serum subcutaneously in several sites. All six transfusion recipients were challenged with 3.6×10^7 CFU of *B. henselae* i.d. on the lateral thorax 30 min following transfusion. Blood was collected for culture and antibody analysis immediately before and after transfusion and weekly until the end of the study. Bacterial cultures, western blot analysis and ELISA were performed as previously described (8, 15).

Cats that received anti-*Bartonella* sera i.v. had measurable antibody levels to *B. henselae* 30 min following transfusion. The anti-*B. henselae* titer following transfusion (400:1) was eightfold lower than that of the pooled donor sera (3,200:1) and was roughly equivalent to the expected dilution of the sera based on the body weight of the recipient cats. The cat that received serum subcutaneously did not have measurable antibody immediately following transfusion but, 1 week postchallenge, had antibody levels indistinguishable from those in the cats receiving i.v. transfusion. By 3 weeks postchallenge, measurable anti-

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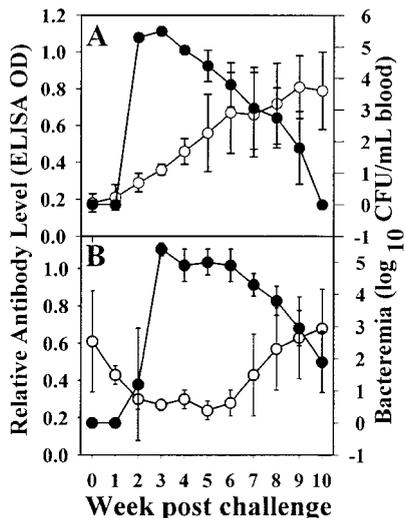


FIG. 1. Mean ELISA OD and standard deviation as a measure of anti-*B. henselae* antibody levels (open circles, *n* = 3) and mean levels of bacteremia and standard deviation (closed circles, *n* = 3) in control serum-transfused cats (A) and anti-*B. henselae* serum-transfused cats (B).

Bartonella antibodies were present in the sera of all three control cats while antibody levels decreased for 2 weeks in cats that received anti-*B. henselae* antisera and did not increase until week 7 (Fig. 1).

By week 2 postchallenge, all three control cats had high levels of circulating *B. henselae* while one anti-*Bartonella*-positive (i.v.-transfused) cat was bacteremic (7.7×10^3 CFU/ml of blood). Despite the delay, the level of bacteremia between the two groups was indistinguishable by week 3 (Fig. 1).

The cats were monitored daily for signs of clinical disease, and skin lesions were scored on a relative scale for diameter of swelling (0, no change; 1, 0.1 to 0.5 cm; 2, 0.6 to 1.0 cm; 3, 1.1 to 1.5 cm; 4, greater than 1.5 cm), color (0, no change; 1, slightly pink; 2, pink; 3, red; 4, pink/purple), and the presence of pus (0, no pus; 1, pustule; 2, pus). The total skin lesion score was the sum of the scores in the three categories. Cats that received anti-*Bartonella* antisera did not develop significant clinical disease. While all six cats developed some redness and swelling at the site of injection within 2 days of challenge, the lesions were less severe and of shorter duration in the anti-*Bartonella*-positive cats than in the control cats (Fig. 2), and, in contrast to the control cats, the anti-*Bartonella*-positive cats did not develop pustules. Control cats developed fever (39.5 to 40.4°C), which peaked between days 4 and 12 postchallenge. In contrast, one anti-*Bartonella*-positive cat (i.v. transfused) developed a fever (40.2°C) on day 18 postchallenge, a timing consistent with the loss of measurable passive antibody.

In a second experiment, we examined the role of natural passive antibody on the development of bacteremia and clinical disease. Four kittens from each of three queens were used. The first queen had been infected i.d. with 2.0×10^7 CFU of *B. henselae* at 12 weeks of age (chronically infected), the second queen was infected i.d. with 1.0×10^7 CFU of *B. henselae* at mid-gestation (acutely infected), and the final queen was maintained *B. henselae* free (control). All three queens were mated to *B. henselae*-negative toms. At 6 weeks of age, while

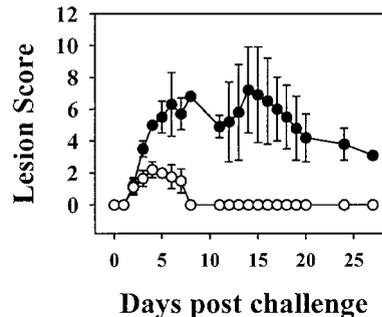


FIG. 2. Mean lesion scores and standard deviation for anti-*B. henselae* serum-transfused cats (open circles, *n* = 3) and control serum-transfused cats (closed circles, *n* = 3).

still nursing, each kitten was challenged i.d. with 10^5 to 10^6 CFU of *B. henselae*. All 12 kittens had been culture negative since birth and were *B. henselae* culture negative at the time of challenge. The kittens were bled prior to challenge and at 2-week intervals for 6 weeks or until they were bacteremic. Rectal temperatures were taken daily, and the kittens were monitored for inflammation at the injection site.

Kittens born to infected queens had high but variable levels of antibody at birth (ELISA optical density [OD], 0.69 to 2.10), which fell steadily and were weakly positive at the time of challenge (ELISA OD, 0.06 to 0.21). Kittens born to the control queen were negative for antibodies to *B. henselae* at birth and at challenge (ELISA OD, <0.05). Following challenge, the kittens born to the acutely infected and chronically infected queens showed no measurable signs of disease, while the kittens born to the control queen developed fever ($>39.5^\circ\text{C}$) and significant skin lesions, similar to those seen in the adult cats. The clinical signs peaked in severity at 18 days postchallenge (Fig. 3). At the termination of the experiment, 7 weeks postchallenge, 11 of 12 kittens were bacteremic; only 1 kitten, born to the acutely infected queen, failed to develop measurable bacteremia.

Together, these data suggest that even during homologous challenge, *B. henselae* can escape antibody-mediated effector mechanisms and cause bacteremia. The titer of antibody in transfused cats was eightfold lower than that of the pooled sera

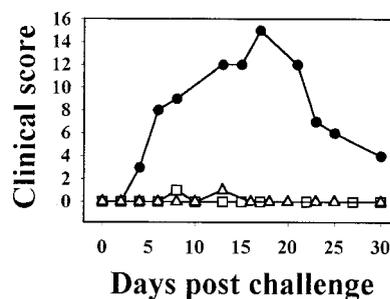


FIG. 3. Clinical scores for kittens challenged with *B. henselae* at 6 weeks of age. Each kitten could score 4 points per day (1 point for fever of $>39.5^\circ\text{C}$, 1 point for lesion swelling, 1 point for lesion redness, and 1 point for lesion pus). The results for kittens from each litter (acutely infected queen [open triangles], chronically infected queen [open squares], and control queen [closed circles]) are combined for a total of 16 possible points per litter.

used in the transfusion (400:1 versus 3,200:1), a level approximately equivalent to that seen at 4 weeks postchallenge in the control cats (Fig. 1). It is possible that the level of antibody in the cats following transfusion was not sufficient to completely prevent bacteremia and that antibody levels approaching those in the donor cats may have prevented bacteremia. Yamamoto et al. (19) demonstrated that previous *B. henselae* exposure protected cats from bacteremia following challenge with a homologous strain. Our results suggest that antibody-mediated mechanisms may not be responsible for that protection, at least at the antibody levels present in our transfused cats and maternally protected kittens.

The inability of antibody to prevent bacteremia suggests a sequestered site of replication, either in blood cells or in some tissue that could seed the blood. *Bartonella bacilliformis* invades erythrocytes (2), and this has been suggested as an evasion mechanism for *B. henselae*. Intraerythrocytic bodies have been reported in naturally infected cats (11) and in feline erythrocytes infected in vitro (13). However, using immunohistochemistry, Guptill et al. (9) demonstrated the presence of extracellular bacteria in the blood and spleens of cats infected 8 weeks previously but were unable to demonstrate intracellular *B. henselae* in any of the tissues they examined including blood, although they saw pseudoinclusions in 5 to 6% of erythrocytes. Blood-associated cells, such as endothelial cells, could act as a site of replication and seeding of the blood. Dehio et al. (5, 6) demonstrated *B. henselae* invasion of human endothelial cells in vitro, although this has not been demonstrated in feline endothelial cells. *Bartonella henselae* is sensitive to killing by human complement via the alternate pathway, and the bactericidal effects are not increased by the addition of antibody (17). We have also observed this using feline complement (data not shown). One possible explanation consistent with our observations is that antibody accelerates the sequestering process, resulting in decreased complement activation and decreased inflammation. Clearly, additional work is required to address the bacterial and host mechanisms involved in the establishment and control of *B. henselae* bacteremia in the cat.

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