Infection and re-infection of domestic cats with various *Bartonella* species or types: *B. henselae* type I is protective against heterologous challenge with *B. henselae* type II

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Abstract

Four *Bartonella* species have been isolated from domestic cats, of which two serotypes/genotypes of *Bartonella henselae* and possibly *B. clarridgeiae* are human pathogens, causing cat scratch disease (CSD).

Our objectives were to evaluate infection and potential cross-protection during re-infection in domestic cats with various *Bartonella* species or types.

Thirty-six cats were primarily inoculated with *B. henselae* type I (\(n = 16\)), *B. henselae* type II (\(n = 10\)), *B. clarridgeiae* (\(n = 6\)) or *B. koehlerae* (\(n = 4\)). They were challenged with *B. henselae* type I (\(n = 15\)), *B. henselae* type II (\(n = 13\)) or *B. clarridgeiae* (\(n = 8\)).

All 36 cats became bacteremic (\(1.25 \times 10^2–1.44 \times 10^6\) CFU/ml) and bacteremia lasted from 37 to 582 days. Duration of bacteremia for cats inoculated with *B. henselae* type I was shorter than for cats inoculated with either *B. henselae* type II (\(P = 0.025\)) or *B. clarridgeiae* (\(P = 0.011\)).

After challenge, 26 cats became bacteremic. Among the nine cats primarily inoculated with *B. henselae* type I and challenged with *B. henselae* type II, six cats stayed abacteremic. The three
bacteremic cats had a transient low-level bacteremia. No bacteremia was observed in three cats primarily inoculated with \textit{B. henselae} type I and challenged with another strain of \textit{B. henselae} type I. Bacteremia levels in the 26 cats were significantly lower than for primary inoculation ($P = 0.022$) and its duration was shorter ($P = 0.012$). Among the eight cats challenged with \textit{B. claridgeiae}, duration of bacteremia in the four cats primarily inoculated with \textit{B. henselae} type I was shorter than in the four cats primarily inoculated with \textit{B. henselae} type II ($P = 0.01$). \textit{Bartonella claridgeiae} inoculated cats were more likely to have relapses for both primary and secondary infections.

This is the first demonstration of cross-protection, evidenced by absence of bacteremia, in cats primarily infected with \textit{B. henselae} type I and challenged with \textit{B. henselae} type II, whereas no cross-protection was previously shown for cats primarily infected with \textit{B. henselae} type II and challenged with \textit{B. henselae} type I. Such results are of major importance for future feline \textit{Bartonella} vaccine development.

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1. Introduction

The genus \textit{Bartonella} is currently composed of at least 20 species/subspecies, including several human pathogens (Breitschwerdt and Kordick, 2000; Dehio, 2001; Dehio et al., 2001; Bermond et al., 2002). Four \textit{Bartonella} species (\textit{Bartonella henselae}, \textit{Bartonella claridgeiae}, \textit{Bartonella koehlerae}, and \textit{Bartonella bovis}) have been isolated from domestic cats (Breitschwerdt and Kordick, 2000; Bermond et al., 2002). Cats infected with \textit{B. henselae}, \textit{B. claridgeiae} and \textit{B. koehlerae}, are bacteremic for a few months to several years (Kordick and Breitschwerdt, 1997; Kordick et al., 1997a; Abbott et al., 1997; Kordick et al., 1999; Kordick et al., 1999; Yamamoto et al., 2002b).

Domestic cats are the reservoir of \textit{B. henselae}, the main causative agent of cat scratch disease (CSD) (Regnery et al., 1992a; Childs et al., 1994; Koehler et al., 1994; Chomel et al., 1995; Yamamoto et al., 1998; Breitschwerdt and Kordick, 2000). This agent is also associated with various diseases, including endocarditis, osteomyelitis, neuroretinitis in immunocompetent persons, and bacillary angiomatosis, bacillary peliosis or endocarditis in immunocompromised individuals (Wong et al., 1995; Anderson and Neuman, 1997; Maurin et al., 1997; Reed et al., 1998; Breitschwerdt and Kordick, 2000; Chomel, 2000; Koehler, 2000). Cat fleas (\textit{Ctenocephalides felis}) are the main vectors of transmission among cats (Chomel et al., 1996; Higgins et al., 1996; Foil et al., 1998). Based on 16S ribosomal RNA gene (rDNA) sequencing, \textit{B. henselae} has been classified into two predominant serotypes/genotypes, types I and II (Houston I and BA-TF/Marseille) (Drancourt et al., 1996; Bergmans et al., 1996, 1997; Sander et al., 1997, 1998).

Cats also are the reservoir of \textit{B. claridgeiae}, a potential zoonotic bacterium involved in CSD (Kordick et al., 1997b; Margileth and Baehren, 1998; Breitschwerdt and Kordick, 2000; Chomel, 2000; Sander et al., 2000). Furthermore, cats can be simultaneously co-infected with \textit{B. henselae} and \textit{B. claridgeiae} or by the two variants of \textit{B. henselae} (Bergmans et al., 1996; Gurfield et al., 1997; Chomel et al., 1999).
Bartonella koehlerae has only been isolated from the blood of two cats from the same household in California (Droz et al., 1999). Experimental infection demonstrated that the domestic cat can act as a reservoir for the organism (Yamamoto et al., 2002b).

Finally, a fourth Bartonella species, initially designated as B. weissii, was isolated from cats from Utah and Illinois (Regnery et al., 2000). This species is genetically identical to B. bovis isolated from domestic ruminants (Chang et al., 2000; Bermond et al., 2002) and therefore should now be called B. bovis (Bermond et al., 2002).

Relapsing bacteremia has been reported in cats infected with B. henselae and B. clarridgeiae (Kordick and Breitschwerdt, 1997; Yamamoto et al., 1998; Mikolajczyk and O’Reilly, 2000; Yamamoto et al., 2002a), suggesting possible antigenic variation (Kordick and Breitschwerdt, 1998; Maruyama et al., 2001). However, experimental infections of cats with B. henselae (Houston I) or B. koehlerae have never led to relapsing bacteremia (Yamamoto et al., 2002a,b).

Only a few studies have been conducted so far to determine the level of cross-protection between Bartonella species or between different strains of B. henselae (Greene et al., 1996; Regnery et al., 1996; Yamamoto et al., 1998). These experimental studies demonstrated that cats primarily infected with B. henselae could be protected from homologous re-infection (Greene et al., 1996; Regnery et al., 1996; Yamamoto et al., 1998). However, cats challenged with a different species or different B. henselae types (type II followed by type I) showed bacteremia after the secondary infection (Yamamoto et al., 1998).

Therefore, the objectives of this study were to: (1) further investigate if cats, experimentally inoculated with either B. henselae type I or II, B. clarridgeiae or B. koehlerae could be protected against a heterologous challenge with either B. henselae type II or I or B. clarridgeiae; and (2) to compare the characteristics of primary and secondary infections, such as duration of bacteremia, presence of bacteremia relapses, correlation between level of bacteremia and immune response by Immunofluorescent antibody (IFA) in these cats.

2. Materials and methods

2.1. Animals

Thirty-six 3-months to 2-year-old SPF cats (20 males, 16 females), including 7 SPF cats from a previous study (Yamamoto et al., 1998), were enrolled. Before experimental infection, all 36 cats were confirmed to be Bartonella spp. serologically and bacteriologically negative. All cats were examined clinically every day for the first 2 weeks and at least weekly thereafter. The cats were housed in groups of four to six (all infected with the same Bartonella species or type) in a controlled flea-free environment.

2.2. Experimental inoculation

The Bartonella strains used for inoculation were, respectively, B. henselae type I (either Houston I [ATCC 49882]: H1 or feline type I: F1), B. henselae type II, B. koehlerae (ATCC
B. clarridgeiae (ATCC 51734). The B. henselae F1 and B. henselae type II strains were isolated from naturally infected cats and confirmed to be B. henselae by 16S rDNA sequencing (Gurfield et al., 1997; Heller et al., 1997). These strains were grown either on blood agar plates (B. henselae and B. clarridgeiae) or chocolate agar (B. koehlerae), and incubated at 35 °C in 5% CO₂ (Yamamoto et al., 1998; Droz et al., 1999; Yamamoto et al., 2002b). The harvested colonies were suspended into sterile saline and 0.5 ml was inoculated intradermally in three to five different sites, as previously described (Abbott et al., 1997).

Of the 36 SPF cats, 16 cats were primarily infected with B. henselae type I with an inoculum of 6.64 × 10⁶–2.26 × 10⁸ CFU/ml (H1: 8 cats, F1: 8 cats), 10 cats were injected with B. henselae type II at a dose of 1.10 × 10⁶–9.60 × 10⁷ CFU/ml, 6 cats received a B. clarridgeiae inoculum of 1.00 × 10⁷ CFU/ml, and four cats were infected with B. koehlerae (inoculum dose: 8.60 × 10⁷ CFU/ml for two cats and 3.84 × 10⁸ CFU/ml for the two other cats). After the end of the initial bacteremic phase, when no colony counts could be observed on the agar plates, and after at least 2 months of successive negative cultures, these 36 cats were challenged with a heterologous strain as follows: 15 cats received B. henselae type I, (H1: 14 cats, F1: 1 cat) with an inoculum dose ranging from 3.04 × 10⁷ to 2.68 × 10⁸ CFU/ml, 13 cats were inoculated with B. henselae type II (inoculum doses: 1.00 × 10⁷–7.40 × 10⁸ CFU/ml) and 8 cats were re-infected with B. clarridgeiae (inoculum doses: 7.30 × 10⁶–1.08 × 10⁹ CFU/ml). The inoculum was confirmed to be B. henselae, B. clarridgeiae or B. koehlerae by polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) analysis (Regnery et al., 1992b; Gurfield et al., 1997; Droz et al., 1999).

2.3. Blood culture

Blood was drawn from the jugular vein of each cat weekly for the first month and every other week for the following months, up to 20 months after inoculation. Two milliliter of blood were placed into ethylenediamine tetraacetate (EDTA) tubes (Becton Dickinson, Franklin Lakes, NJ, USA) for culture and 1 ml into serum separation tubes for serological tests. The EDTA tubes were frozen at −70 °C and plated a few days later onto 5% rabbit blood agar plates for B. henselae and B. clarridgeiae and chocolate agar for B. koehlerae, as previously described (Abbott et al., 1997; Droz et al., 1999). The plates were incubated at 35 °C with 5% CO₂ for 4 weeks. Plates were examined two to three times a week for any bacterial growth. Isolated strains were confirmed to be Bartonella species by PCR/RFLP of the citrate synthase gene using HhaI and TaqI endonucleases. The number of colonies was counted and calculated as CFU per milliliter (Gurfield et al., 1997; Droz et al., 1999). A bacteremia relapse was defined as any positive culture occurring after two successive negative cultures (Yamamoto et al., 2002b).

2.4. Immunofluorescent antibody test

IgG antibody responses were detected by IFA, using antigens from the same strains used for inoculation (Childs et al., 1994; Chomel et al., 1995). Specific IgM antibody response was not investigated in the present study.
2.5. Statistical tests

For duration of bacteremia, peak level, time to reach the maximum level of bacteremia, data were analyzed by one-way analysis of variance (ANOVA). For comparison of peak levels of IgG antibodies, non-parametric Mann–Whitney rank sum test was performed with MINITAB™ statistical software Release 13.1 (MINITAB Inc., State College, PA, USA). For peak level of bacteremia, equal variances t-test was performed with MINITAB™. For univariate analysis, non-parametric test (Fisher’s exact test) was used for association between Bartonella species/types and bacteremia relapses (Epi-info version 6.04b, CDC Atlanta, GA). A P value of <0.05 was considered as significant. Correlation between level of bacteremia and IFA titer was analyzed, using transformed linear regression analysis with MINITAB™.

3. Results

No obvious clinical signs were observed in the 28 cats inoculated with either *B. henselae* type I (H1), *B. henselae* type II, *B. clarridgeiae* or *B. koehlerae*. However, six of the eight cats inoculated with *B. henselae* F1 developed fever (>39.2 °C) within 2–12 days (mean: 5.8 days).

3.1. Primary inoculation

All 36 cats became bacteremic within 1–2 weeks after the primary inoculation, regardless of the Bartonella species or type inoculated (Table 1). Overall, bacteremia peaked between 14 and 52 days (median: 28 days) after primary inoculation (Table 2). No significant differences in time to reach the bacteremia peak were detected among cats

<table>
<thead>
<tr>
<th>Primary infection strain</th>
<th>No. of bacteremic cats/no. of inoculated cats</th>
<th>Challenge strain</th>
<th>No. of bacteremic cats/no. of inoculated cats</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. henselae</em> type Ia</td>
<td>3/3</td>
<td><em>B. henselae</em> type Ib</td>
<td>0/3</td>
</tr>
<tr>
<td><em>B. henselae</em> type Ib</td>
<td>9/9</td>
<td><em>B. henselae</em> type IIa</td>
<td>3/9</td>
</tr>
<tr>
<td><em>B. henselae</em> type I</td>
<td>6/6</td>
<td><em>B. henselae</em> type Ib</td>
<td>6/6</td>
</tr>
<tr>
<td><em>B. henselae</em> type II</td>
<td>4/4</td>
<td><em>B. clarridgeiae</em></td>
<td>4/4</td>
</tr>
<tr>
<td><em>B. henselae</em> type II</td>
<td>4/4</td>
<td><em>B. clarridgeiae</em></td>
<td>4/4</td>
</tr>
<tr>
<td><em>B. clarridgeiae</em></td>
<td>4/4</td>
<td><em>B. henselae</em> type I</td>
<td>3/4</td>
</tr>
<tr>
<td><em>B. clarridgeiae</em></td>
<td>2/2</td>
<td><em>B. henselae</em> type I</td>
<td>2/2</td>
</tr>
<tr>
<td><em>B. koehlerae</em></td>
<td>2/2</td>
<td><em>B. henselae</em> type I</td>
<td>2/2</td>
</tr>
<tr>
<td><em>B. koehlerae</em></td>
<td>2/2</td>
<td><em>B. henselae</em> type I</td>
<td>2/2</td>
</tr>
<tr>
<td>Overall</td>
<td>36/36</td>
<td></td>
<td>26/36</td>
</tr>
</tbody>
</table>

* B. henselae* feline type I.

b B. henselae* Houston I.

c Small number of colonies. Mean: 8.5 CFU/ml (range: 2.6–17.0 CFU/ml).
primarily infected with either *B. henselae* type I, *B. henselae* type II, *B. clarridgeiae* or *B. koehlerae* (*P* > 0.05). The maximum level of bacteremia ranged from 1.25 × 10^2 to 1.44 × 10^6 CFU/ml (median: 9.27 × 10^4 CFU/ml) (Table 2) and no statistically significant differences between *Bartonella* species or types were detected (*P* > 0.05). Duration of bacteremia in the 36 cats ranged from 37 to 582 days (median: 98 days) (Table 2). Duration of bacteremia in the 16 cats inoculated with *B. henselae* type I was significantly shorter than for the 10 cats inoculated with either *B. henselae* type II (*P* = 0.025) or the 6 cats inoculated with *B. clarridgeiae* (*P* = 0.011). However, among the 16 cats infected with *B. henselae* type I, cats inoculated with *B. henselae* F1 had a longer duration of bacteremia than cats inoculated with either *B. henselae* H1 (*P* < 0.001) or *B. koehlerae* (*P* = 0.004). Similarly, cats inoculated with *B. clarridgeiae* showed a significantly longer duration of bacteremia than cats inoculated with *B. koehlerae* (*P* = 0.01).

### 3.2. Challenge

Twenty-six of the 36 cats became bacteremic within 1–2 weeks after the challenge (Table 1). Overall, the bacteremia peaked between 14 and 48 days (median: 27 days) after re-infection (Table 2). However, 3 of these 26 cats that were primarily inoculated with *B. henselae* type I and challenged with *B. henselae* type II had a transient and low bacteremia level (Table 1). No bacteremia was detected in three cats primarily inoculated with *B. henselae* F1 and challenged with *B. henselae* H1 (Table 1). Similarly, six of the nine cats primarily inoculated with *B. henselae* type I and challenged with *B. henselae* type II did not develop any bacteremia, as shown in Fig. 1 for one of these cats. Among those nine cats, five (83%) of the six cats primarily inoculated with *B. henselae* H1 were protected from re-infection with *B. henselae* type II, whereas only one (33%) of the three cats primarily

<table>
<thead>
<tr>
<th>Bartonella species/types (no. of cats)</th>
<th>Median days to reach peak bacteremia (range)</th>
<th>Median maximum level of bacteremia (CFU/ml) (range)</th>
<th>Median duration in days of bacteremia (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Primary inoculation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. henselae</em> type I (<em>n</em> = 16)</td>
<td>25 (14–48)</td>
<td>9.33 × 10^4 (1.25 × 10^2–4.45 × 10^5)</td>
<td>80 (37–357)</td>
</tr>
<tr>
<td><em>B. henselae</em> type II (<em>n</em> = 10)</td>
<td>18 (14–52)</td>
<td>9.27 × 10^5 (2.40 × 10^4–6.08 × 10^5)</td>
<td>181 (49–582)</td>
</tr>
<tr>
<td><em>B. clarridgeiae</em> (<em>n</em> = 6)</td>
<td>36 (28–36)</td>
<td>9.49 × 10^6 (3.06 × 10^5–3.20 × 10^6)</td>
<td>284 (140–363)</td>
</tr>
<tr>
<td><em>B. koehlerae</em> (<em>n</em> = 4)</td>
<td>36 (14–36)</td>
<td>7.12 × 10^7 (3.36 × 10^5–1.44 × 10^6)</td>
<td>74 (70–78)</td>
</tr>
<tr>
<td>Overall (<em>n</em> = 36)</td>
<td>28 (14–52)</td>
<td>9.27 × 10^4 (1.25 × 10^2–1.44 × 10^6)</td>
<td>98 (37–582)</td>
</tr>
<tr>
<td>(B) Challenge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. henselae</em> type I (<em>n</em> = 11)</td>
<td>28 (22–48)</td>
<td>1.12 × 10^4 (8.89 × 10^3–9.36 × 10^4)</td>
<td>62 (14–77)</td>
</tr>
<tr>
<td><em>B. henselae</em> type II (<em>n</em> = 7)</td>
<td>28 (14–35)</td>
<td>3.00 × 10^5 (2.67 × 10^4–1.60 × 10^5)</td>
<td>70 (37–203)</td>
</tr>
<tr>
<td><em>B. clarridgeiae</em> (<em>n</em> = 8)</td>
<td>22 (22–36)</td>
<td>3.35 × 10^6 (1.92 × 10^5–8.69 × 10^6)</td>
<td>138 (43–405)</td>
</tr>
<tr>
<td>Overall (<em>n</em> = 26)</td>
<td>27 (14–48)</td>
<td>1.92 × 10^5 (2.67 × 10^4–8.69 × 10^5)</td>
<td>63 (14–405)</td>
</tr>
</tbody>
</table>
Fig. 1. Bacteremia and IgG antibody response in a cat infected with *B. henselae* type I and challenged with *B. henselae* type II.
inoculated with *B. henselae* F1 was protected from re-infection. However, that difference was not statistically significant (*P* = 0.222). Finally, one of the four cats primarily inoculated with *B. clarridgeiae* and challenged with *B. henselae* type I did not become bacteremic (Table 1). No significant differences in time to reach the bacteremia peak were observed among the cats secondarily infected with either *B. henselae* type I, *B. henselae* type II or *B. clarridgeiae* (*P* > 0.05).

Overall, the peak levels of bacteremia in the 26 bacteremic challenged cats ranged from 2.67 × 10^5 to 8.69 × 10^5 CFU/ml (median: 1.92 × 10^4 CFU/ml) (Table 2) and these levels were significantly lower than those for primary inoculation (*P* = 0.022). However, no significant differences in peak levels of bacteremia were observed among cats secondary infected with either *B. henselae* type I, *B. henselae* type II or *B. clarridgeiae* (*P* > 0.10). In the 26 cats that were bacteremic after challenge, bacteremia lasted 14–405 days (median: 63 days) (Table 2). Overall, the duration of bacteremia after challenge was significantly shorter than after primary inoculation (median: 98 days) (*P* = 0.012). In particular, cats challenged with *B. henselae* (either type I or II) had a significantly shorter duration of bacteremia (median: 63 days, *n* = 18) than for cats primarily infected with *B. henselae* (median: 97 days, *n* = 26) (*P* = 0.013). Interestingly, only three (33%) of the nine cats primarily inoculated with *B. henselae* type I and challenged with *B. henselae* type II became bacteremic. Furthermore, these three cats had a significantly lower level (*P* < 0.01) and shorter duration of bacteremia (*P* < 0.05) when compared to any other groups of bacteremic cats after re-infection. Among the eight cats challenged with *B. clarridgeiae*, duration of bacteremia in the four cats primarily inoculated with *B. henselae* type I (median: 50 days) was significantly shorter than the duration of bacteremia in the four cats primarily inoculated with *B. henselae* type II (median: 284 days) (*P* = 0.01). However, no statistically significant differences in the level of bacteremia were observed between these two groups (*P* > 0.30).

Forty-four percent (*n* = 16/36) of the primarily inoculated cats developed relapsing bacteremia compared to 27% (*n* = 7/26) of the challenged bacteremic cats, but that difference was not statistically significant (*P* = 0.16). During primary infection, cats inoculated with *B. clarridgeiae* were more likely to have relapses (100%; *n* = 6/6) than the cats inoculated with either *B. henselae* type I (25%; *n* = 4/16, *P* = 0.002) or *B. koehlerae* (0%; *n* = 0/4; *P* = 0.03). Similarly, among challenged cats, the ones challenged with *B. clarridgeiae* were more likely to develop relapses (63%; *n* = 5/8) than cats challenged with *B. henselae* (8%; *n* = 2/25; *P* = 0.004). Cats primarily inoculated with *B. henselae* showed more relapses (38%; *n* = 10/26) than cats heterologously challenged with *B. henselae* (11%; *n* = 2/18; *P* = 0.03).

All 36 cats primarily infected with *Bartonella* sp. developed IgG antibodies, which were detected within 1–2 weeks (mean: 11 days) by IFA. Overall, IgG antibody titers peaked between 1 and 21 weeks post-infection (PI) (mean: 6 weeks PI) and ranged from 1:512 to 1:8192 (mean: 1:1024). Positive IgG antibody titers (IFA titers >1:64) persisted in all 36 cats for the duration of the study and no statistical differences were identified between each of the four *Bartonella* groups (*P* > 0.11). Within each of the four *Bartonella* groups of primarily infected cats, a positive correlation (*P* < 0.05) was observed between the level of bacteremia and the IFA titer for respectively 38% (*n* = 6/16) of the cats inoculated with *B. henselae* type I, 20% (*n* = 2/10) of the cats inoculated with *B. henselae* type II, 67%
(n = 4/6) of the cats inoculated with *B. clarridgeiae* and 25% (n = 1/4) of the cats inoculated with *B. koehlerae*.

4. Discussion

Choosing appropriate strains of viruses or bacteria is of critical importance for the development of vaccines, especially for infectious agents with a wide diversity of strains (Gupta et al., 1998). The present study is the first one to report protection of SPF cats by *B. henselae* type I (H1 or F1) from secondary infection by *B. henselae* type II. Similarly, we identified for the first time a reduction in the duration of bacteremia in SPF cats challenged with *B. clarridgeiae* after being primarily inoculated with *B. henselae* type I, whereas a similar effect was not observed for cats primarily infected with *B. henselae* type II. Previous studies on a limited number of cats have demonstrated the lack of re-infection after primary infection with a homologous strain of *B. henselae* (Greene et al., 1996; Regner et al., 1996; Abbott et al., 1997; Yamamoto et al., 1998). We had also shown protection in cats infected and re-infected with different isolates of *B. henselae* type II and identified a lack of cross-protection in cats primarily infected with *B. henselae* type II and challenged with *B. henselae* type I (H1) or primarily infected with *B. henselae* type II and challenged with *B. clarridgeiae* (Yamamoto et al., 1998).

We also confirmed that SPF cats inoculated with *B. henselae* type I and challenged with the same (H1) strain (Yamamoto et al., 1998) or a different strain (F1) of *B. henselae* type I did not become bacteremic. Despite a wide genomic diversity of *B. henselae* isolates as analyzed by pulsed-field gel electrophoresis (Sander et al., 1998; Maruyama et al., 2001), cats challenged with different strains of the same type of *B. henselae* (either type I or II) were protected from re-infection. In contrast, 17 (94%) of the 18 cats challenged with a different *Bartonella* species became bacteremic. These observations support the lack of species cross-protection for feline *Bartonella* infection and only a unidirectional cross-protection for *B. henselae* types (type I/II, but not type II/I). Our data are also supported by the fact that co-infection with *B. henselae* and *B. clarridgeiae* have been observed in naturally infected cats (Bergmans et al., 1997; Gurfield et al., 1997, 2001; Chomel et al., 1999; Maruyama et al., 2000). Interestingly, natural co-infection by *B. henselae* types I and II strains has also been reported (Gurfield et al., 1997), but it could not be documented if such cats could have been initially infected with *B. henselae* type II and later *B. henselae* type I.

We confirmed that duration of bacteremia in cats primarily inoculated with *B. clarridgeiae* was longer than in cats inoculated with either *B. henselae* type I or *B. koehlerae* (Yamamoto et al., 1998, 2002b). Furthermore, cats inoculated with *B. henselae* type II showed a longer duration of bacteremia than those inoculated with *B. henselae* type I. We also confirmed that cats inoculated with *B. henselae* type I (H1) had a significantly shorter duration of bacteremia than cats infected with *B. henselae* type I (F1) (Yamamoto et al., 2002a).

Among the 36 challenged cats, 26 developed bacteremia. All but one cat challenged with a different *Bartonella* species became bacteremic. Among the 10 cats that did not become bacteremic, 6 were from a group of 9 cats primarily infected with *B. henselae* type I and challenged with *B. henselae* type II. Furthermore, the three of these nine cats that became
bacteremic had a significantly lower bacteremia (mean: 8.5 CFU/ml) than for other infections.

Bacteremia was also significantly shorter in cats after challenge than after primary inoculation. Specifically, cats challenged with *B. henselae* (types I and II) had a shorter duration of bacteremia than cats primarily infected with *B. henselae* (types I and II). Cats challenged with *B. clarridgeiae* were more likely to have a shorter bacteremia when primarily infected with *B. henselae* type I than primarily infected with *B. henselae* type II. These observations suggest a possible cross-protection against re-infection with *B. clarridgeiae* in cats primarily infected with *B. henselae* type I.

Cats primarily inoculated or challenged with *B. clarridgeiae* showed more relapses than cats inoculated with *B. henselae*. Relapsing bacteremia has previously been observed in cats infected or re-infected with *B. henselae* and *B. clarridgeiae* (Kordick and Breitschwerdt, 1997; Yamamoto et al., 1998, 2002a; Mikolajczyk and O’Reilly, 2000). However, the presence or absence of relapsing bacteremia in cats inoculated with *B. henselae* appeared to be associated with the inoculum strain used (Kordick et al., 1995; Kordick and Breitschwerdt, 1997; Guptill et al., 1998; Yamamoto et al., 1998). For instance, cats inoculated with *B. henselae* type I (H1; a strain of human origin submitted to several passages), never presented relapsing bacteremia, whereas cats infected with feline *B. henselae* strains (either type I or II) had relapses. Additionally, cats inoculated with *B. henselae* were more likely to develop relapses after primary inoculation than after challenge, suggesting induction of a partial protection.

IgG antibodies were detected by IFA in all 36 cats within a few weeks after primary inoculation. Cats experimentally infected with *B. henselae*, *B. clarridgeiae* or *B. koehlerae* developed specific IgG antibodies, detectable by IFA within 1–3 weeks after infection (Greene et al., 1996; Regnery et al., 1996; Abbott et al., 1997; Kordick et al., 1997b; O’Reilly et al., 1999; Yamamoto et al., 1998; Yamamoto et al., 2002a,b). These antibodies can persist for several months, despite the absence of bacteremia (Abbott et al., 1997; Yamamoto et al., 1998; Yamamoto et al., 2002a,b). During primary infection with *B. clarridgeiae*, a positive correlation between bacteremia and IFA titers was observed in 67% of the cats, suggesting a limited role of the IgG antibody response in the organism defense mechanisms against *Bartonella* infection. Epidemiological and experimental studies of cats infected with *Bartonella* showed that long-term bacteremia was often concomitant with the presence of positive IFA titers (Chomel et al., 1995, 1999, 2002). Similarly, most of the cats challenged with a heterologous strain of *Bartonella* developed bacteremia despite the presence of circulating antibodies. Therefore, the humoral immune response may not be sufficient to prevent *Bartonella* infection in cats (Abbott et al., 1997; Kordick and Breitschwerdt, 1997). A booster effect after challenge was not clearly identified for some cats, which could be explained by strain variations of the antigens used for IFA testing (Sander et al., 2001; Yamamoto et al., 2002a).

In contrast to the humoral response, a predominant role of the cellular immune response has been established for many intracellular pathogens (Kaufmann, 1995; Mollenkopf et al., 1998; Pedersen et al., 1998; Pappalardo et al., 2001). *B. henselae* is an intracellular bacterium, located inside the host’s erythrocytes (Kordick and Breitschwerdt, 1995; Rolain et al., 2001), and also has a tropism for endothelial cells (Dehio, 2001). For cellular immunity, the major histocompatibility complex (MHC) class I pathway plays an
important role for antigen presentation to CD8+ T cells (Kaufmann, 1995; Pedersen et al., 1998), but is not expressed on the surface of mammalian erythrocytes. Therefore, despite the presence of Bartonella organisms inside the erythrocytes, the cellular immune response, especially the CD8+ pathway, may not be triggered, leading to chronic Bartonella infection (Dehio, 2001).

Immunopathological investigation of Bartonella infection in animal models is essential for the development of vaccines. For feline infections, Pedersen et al. (1998) demonstrated the essential role of CD4+ effector cells (Th1 and Th2) for activation of the immune system against intracellular bacteria. The importance of CD4+ Th1 T-cell dependent immune response leading to cell-mediated immune response was also demonstrated in a Bartonella murine infection model (Arvand et al., 2001). Similarly, dogs experimentally infected with B. vinsonii subsp. berkhoffii developed cell-mediated immune response through CD4+ cells (Pappalardo et al., 2001). Immunocompromised individuals infected with Bartonella, especially AIDS patients with very low CD4+ cell counts (<50 cells/ml), present more severe clinical disease, such as bacillary angiomatosis (Koehler, 2000). These observations clearly support the role of CD4+ cell-mediated immune pathway in Bartonella infections. However, pathological mechanisms of feline Bartonella infection and re-infection are not clearly understood. Particularly, some major components of the Bartonella organisms not yet identified may be recognized at the species or type levels, triggering a partial to complete protection against re-infection.

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