Interleukin-4 (IL-4) and IL-13 Signaling Pathways Do Not Regulate Borrelia burgdorferi-Induced Arthritis in Mice: IgG1 Is Not Required for Host Control of Tissue Spirochetes

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Lyme disease is a tick-transmitted infection caused by the spirochete Borrelia burgdorferi (17, 25, 42). Illness in humans can be successfully treated with antibiotics; however, in the absence of treatment, arthritis, carditis, neurological complications, and skin abnormalities may develop (42). Arthritis is a late-stage symptom in approximately 60% of individuals who are not treated with antibiotics at the time of infection. The arthritis is associated with the presence of spirochetes in the joint tissue and is characterized by tendonitis, synovial hyperplasia, and infiltration of neutrophils (7). There is a spectrum of disease with different mouse strains having differing susceptibilities to B. burgdorferi-induced pathology. Infected C3H mice develop severe arthritis with large numbers of bacteria infiltrating joint tissue (8, 12, 52). C57BL/6 mice predominantly develop mild arthritis and yet harbor large numbers of spirochetes that equal those in C3H mice (8, 10, 30). BALB/c mice show a spectrum of disease severity. At low inoculum doses, BALB/c mice develop mild arthritis which becomes more severe as the inoculum dose is increased (30). It appears that there may be more than one mechanism of resistance, since resistance in BALB/c mice correlates with low levels of spirochetes in the joints, while in C57BL/6 mice arthritis is mild even in the presence of large numbers of spirochetes (30).

A mapping project has been carried out in this laboratory using the F2 intercross generation of C3H/HeNCR and C57BL/6NCR mice (51). Four chromosomal regions were linked to arthritis development by quantitative trait loci (QTL) analysis, confirming that arthritis development is a complex, multigenic trait with multiple mechanisms. Interestingly, ankle swelling and histopathological severity were linked to distinct chromosomal regions in this study. An additional suggestive quantitative trait locus for histopathology was identified in a region of chromosome 11 that contains a cluster of immune and inflammatory regulatory cytokines, including interleukin-3 (IL-3), IL-4, IL-5, and IL-13. This cluster of genes is associated with the regulation of Th cell responses, regulating T-cell and inflammatory responses to infection (22).

Previous studies provide conflicting evidence regarding the involvement of IL-4 in Lyme arthritis. Antibody (Ab)-mediated depletion of IL-4 in BALB/c and C3H mice suggested a protective effect for IL-4 (29, 33), as did experiments in which treatment of C3H/HeN mice with recombinant IL-4 reduced arthritis severity (28). Others have shown that the kinetics of IL-4 production differs in severe and mildly arthritic mice (27). However, in BALB/c mice, interruption of the B7/CD28 costimulatory pathway using an Ab to CD86 prevented T-cell production of IL-4, yet it had no effect on arthritis development (48). Most recently, arthritis-resistant DBA mice that were genetically deficient in IL-4 did not show increased arthritis severity when infected with B. burgdorferi (14). These
conflicting results suggest that the regulation, production, and action of IL-4 during *B. burgdorferi* infection may be more complex than could be examined in previous experiments.

In response to conflicting results in the literature and the suggestive linkage from our mapping study, we have chosen to look at the effects of IL-4 production in Lyme disease by using mice that are genetically altered to prevent the expression of IL-4, the α chain of the IL-4 receptor, or both. These mice enabled us to examine the involvement of IL-4 in the development of Lyme arthritis, regardless of the cell type producing it, including T cells and macrophages. We also chose to use the mice in this study, as a knockout of the IL-4 receptor, or responsive cell type. In other pathogen systems, it has been found that IL-13 signaling through the IL-4Rα chain can compensate for the absence of IL-4, because the two cytokines have many of the same effects (20, 24, 39, 49). Therefore, the IL-4Rα knockout with disruption of both IL-4 and IL-13 signaling allowed us to determine whether IL-13 plays a role in the severity of Lyme arthritis and/or is able to compensate for a loss of IL-4 during infection.

**MATERIALS AND METHODS**

**Mice.** Male and female C3H/HeJ mice were obtained from the National Cancer Institute. Male and female C3H/HeJ, BALB/cJ, BALB/cJ-IL-4tm2Nnt, C57BL/6J, and C57BL/6J-Il-4tm2Nnt mice were obtained from the Jackson Laboratories (Bar Harbor, Maine). BALB/cJ-IL-4tm2Nnt mice were generated as previously described (40). BALB/cJ-IL-4tm2Nnt mice were obtained by an intercross between BALB/cJ-IL-4tm2Nnt and BALB/cJ-IL-4tm2Nnt mice. The designation C3H is used when data have been pooled, including for experiments performed with both C3H/HeJ and C3H/HeJ mice. Mice were housed in the Animal Resource Center at the University of Utah Medical Center, according to the National Institutes of Health guidelines for care and use of laboratory animals.

**Bacteria and infection.** Mice between 4 and 6 weeks of age were infected by intradermal injection in the shaved back with 2 × 10^5^ bacteria of the N40 isolate of *B. burgdorferi* (provided by S. Barthold, University of California at Davis, at passage 3 from an infected mouse) (8), a mode of infection reported to require the lowest spirochetes and to most closely mimic tick transmission (6, 43). In one experiment, mice from 10 to 13 weeks of age were used, with results similar to those for mice 4 to 6 weeks of age. Passage 4 spirochetes were grown in BSK-H medium containing 6% rabbit serum (Sigma, St. Louis, Mo.) for 3 to 5 days prior to injection, enumerated using a Petroff-Hauser chamber, and diluted with sterile medium. Mock-infected animals received intradermal injection of sterile BSK-H medium in the shaved back and tested negative for *B. burgdorferi* infection by both PCR and serology.

**Measurement of ankle joints.** Rear ankle joints of mice anesthetized with methoxyflurane (Pitman-Moore) were measured with a metric caliper (Mitutoyo, Tokyo, Japan) during each week of infection. Measurements were taken in the anterior-to-posterior position, with the ankle extended, through the thickest portion of the ankle. Numerous factors can influence normal joint measurement, including age, sex, and strain of mouse; therefore, findings are reported as the change in joint measurement relative to that of mock-infected matched animals.

**Histopathology of ankle joints.** The rear ankle joint displaying the greatest swelling at the time of sacrifice was taken from each mouse for histological analysis. Samples were fixed in 10% formalin, decalcified, and embedded in paraffin, and sections were stained with hematoxylin and eosin. Sections were reviewed in a blind fashion and given a score for severity ranging from 0 to 4+, as previously described (30).

**Preparation of DNA from infected tissues.** Mice were sacrificed at 4 weeks postinfection, and rear ankle joint and heart tissues were prepared as previously described (47). Briefly, tissue specimens were incubated in 0.1% collagenase A (Boehringer Mannheim) solution at 37°C overnight and then mixed with an equal volume of protease K solution (Boehringer Mannheim) and incubated at 55°C overnight. DNA was then recovered by extraction with an equal volume of phenol-chloroform and precipitated with ethanol. After digestion with 1 mg of DNase-free RNase (Sigma) per ml the samples were extracted again, and DNA was recovered by precipitation. The precipitated DNA was resuspended in 1 to 1.5 ml of water, and the DNA content was determined by measuring the absorbance at 260 nm.

**Quantification of DNA from infected tissues.** The levels of *B. burgdorferi* DNA in joints and hearts were determined by continuous-monitoring PCR, using the LightCycler (Idaho Technologies, Idaho Falls, Idaho) as previously described (36). Briefly, 200 ng of sample DNA was amplified in a final volume of 10 μl containing 10 μl of LightCycler duplex solution (Roche Molecular Biochemicals, Indianapolis, Ind.) 4 μM MgCl₂, 4.5 μg of bovine serum albumin, 200 μM deoxynucleoside triphosphates, a 1:30,000 dilution of SYBER Green I (Molecular Probes, Eugene, Oreg.), 0.5 μM each primer, 0.5 U of Taq polymerase (GIBCO BRL, Gaithersburg, Md.), and 110 ng of TaqStart Ab (Clontech, Palo Alto, Calif.). Forty cycles of amplification were performed, with each cycle consisting of three steps: heating at 20°C to 95°C with a 1-s hold, cooling at 20°C to 60°C with a 1-s hold, and heating at 1°C to 84°C. This technique monitors the cycle-by-cycle accumulation of fluorescently labeled product. The cycle at which the product is first detected was an indicator of starting copy number and was calculated by using the LightCycler analysis software. *B. burgdorferi* was quantified by using the chromosomally encoded recA gene, and values were normalized to 10^4 copies of a single-copy mouse gene, nidogen. The oligonucleotide primers used to detect mouse nidogen were nidoF (5'-CCA GGC ACA GAA TAC CAT CC-3') and nidoR (5'-GGA CAT ACT CTG CTA CCA TC-3'). The oligonucleotide primers used to detect *B. burgdorferi* recA were nTM 17F (5'-GTT GAT CTA TGG TAT TAG ATG CTC GCG-3') and nTM17R (5'-GCC AAA GTT CCA CAA CTA CAC AAG-3').

**Detection of *B. burgdorferi*-specific immunoglobulin (Ig) levels.** Serum obtained by retro-orbital bleeding of experimental animals at sacrifice was analyzed for *B. burgdorferi*-specific Ab using an Ab capture ELISA. Eleven columns per 9-well plate were coated with *B. burgdorferi* sonicate at a concentration of 5 μg of sonicate/ml. The 12th column was coated with polyclonal rabbit anti-mouse immunoglobulin G (IgG) plus IgA plus IgM (Zymed, South San Francisco, Calif.) at a concentration of 5 μg/ml. Serum samples were added to *B. burgdorferi* sonicate-coated wells and analyzed for isotype-specific Ab content at the following dilutions: IgG1 at a dilution of 1:100, IgG2a at 1:50, IgG2b at 1:500, and IgG3 at 1:1,000. Known concentrations of IgG1, IgG2a, IgG2b, and IgG3 were added to the rabbit anti-mouse Ig wells to ensure that results were within the linear range. Following a 2-h incubation with serum samples, unbound sample was removed by washing, and the anti-rabbit IgG Ab was added. To this were added of alkaline phosphatase-conjugated rabbit anti-mouse IgG1, IgG2a, IgG2b, or IgG3 (Zymed), respectively, for 1 h. Plates were washed and developed with incubation with 1 mg of p-nitrophenyl phosphate per ml in Tris buffer (Sigma) for 30 min, stopped with 3 N NaOH, and read at 405 nm on a 96-well microtiter plate spectrophotometer (Molecular Devices). Values are expressed as the optical density at 405 nm (OD405).

**Statistical analysis.** The degrees of statistical significance of the quantitative differences between sample groups were determined by application of Student's t test.
greater variability in arthritis severity than the C57BL/6N substrain, it is consistently less severe than arthritis in C3H mice (16). Mice were infected with 2,000 spirochetes, and the course of infection was monitored for 4 weeks. C3H/HeN mice showed moderate to severe joint swelling throughout the 4-week course of infection, while the wild-type C57BL/6J mice had mild to moderate swelling (Fig 2.). The IL-4-deficient C57BL/6J mice showed mild to moderate joint swelling, nearly identical to that of the wild-type mice.

Joint swelling and histopathological assessments of arthritis severity are regulated by distinct genetic regions; therefore, we also assessed histopathology (51). One joint from each animal was taken at sacrifice for histological analysis and examined for arthritis severity. C3H mice had severe arthritis scores (21 to 41), while the wild-type BALB/cJ mice showed a range of mild to severe scores (11 to 41), with an overall severity slightly less than that seen in C3H mice (Table 1). The IL-4- and IL-4Rα-deficient mice showed a range of severity that was not significantly different from that of wild-type BALB/cJ mice. A dosage effect was not seen in the BALB/cJ IL-4Rα−/− mice, because in these mice arthritis severity did not differ significantly from that of wild-type mice (Fig 1; Table 1). Arthritis severity was mild to moderate in wild-type C57BL/6J mice, and mice deficient in IL-4 showed the same range of severity as the wild-type animals. These data suggest that IL-4 plays neither a protective nor an antagonistic role in the development of arthritis during B. burgdorferi infection in the BALB/cJ and C57BL/6J strains. Further, IL-13 signaling through the IL-4Rα chain does not appear to be important for the regulation of arthritis severity or to compensate for a lack of IL-4 signaling on the BALB/cJ background.

Spirochete numbers in the joint and heart tissues of animals lacking IL-4 or the IL-4Rα chain. Although IL-4 does not appear to play a role in regulating arthritis severity, it may be involved in the control of B. burgdorferi numbers. In previous experiments, animals which had been treated with an IL-4 Ab yielded higher numbers of spirochetes following culture for several days, suggesting that IL-4 is involved in the control of spirochete numbers (29). To more directly address the numbers of B. burgdorferi in tissues, we performed continuously monitored PCR on DNA collected from tissues at 4 weeks postinfection. Previously, using a different PCR technology, we had shown that, at an inoculum dose of 2,000 B. burgdorferi cells, 5- to 10-fold fewer spirochetes persist in the joint and heart tissues of BALB/cAn than of C3H/HeJ mice (30). In this study, with a newly developed PCR method, fivefold fewer spirochetes were found in the joints of wild-type BALB/cJ mice than of C3H/HeN mice (Fig. 3A). A similar three to fivefold difference in spirochete numbers was seen between the IL-4- and IL-4Rα-deficient mice and the C3H/HeN mice. Spirochete numbers were threefold higher in the hearts of C3H/HeN mice than in those of the wild-type, IL-4-, or IL-4Rα-
deficient BALB/cJ mice (Fig. 3B). Overall, deficiency in IL-4 or IL-4Rα had no effect on spirochete numbers in tissues from BALB/cJ mice.

C57BL/6N mice are resistant to severe arthritis when infected with *B. burgdorferi*; however, the levels of spirochetes persisting in their joints are approximately equal to those found in C3H/HeN mice (30). In contrast, C57BL/6N mice have very few spirochetes in their hearts compared to the number in C3H/HeN mice (30). Continuous-monitoring PCR analysis revealed nearly equivalent numbers of spirochetes in the joints of C57BL/6J and C3H/HeN mice (Fig. 4A). The IL-4-deficient C57BL/6J mice showed a slightly larger range of spirochete numbers in the joints; however, the differences in the numbers of spirochetes present were not significant. Hearts from the C3H/HeN mice contained fivefold more spirochetes than those of either the wild-type or IL-4-deficient C57BL/6J mice. The lack of difference in spirochete numbers between any of the IL-4- or IL-4Rα-deficient mice and their wild-type controls demonstrated that IL-4 signaling and IL-13 signaling through the IL-4Rα chain are not required for control of spirochete numbers in joints and hearts.

**Immunoglobulin isotype profiles in mice lacking IL-4 and IL-4Rα.** Passive immunization of naive mice with serum from animals infected with *B. burgdorferi* prior to infection has been shown to be effective in preventing infection (9, 11). The importance of antibodies for resolution of Lyme disease has also been confirmed in studies using immune-compromised *scid* and *rag*-deficient mice, which are unable to resolve infection (13, 15, 45, 46). It has not directly been shown that any single isotype of Ab is more effective at clearing *B. burgdorferi* or influencing the development of severe arthritis. However, it has been inferred from the reported effects of IL-4 depletion that Th2-dependent isotype IgG1 could be more effective than other isotypes at clearing *B. burgdorferi* (29, 33). Because IL-4 induces class switching to IgG1, we determined by ELISA the relative levels of various IgG isotypes in mice infected with 2,000 *B. burgdorferi* cells. Table 2 shows a comparison of the relative amounts of Ab produced by wild-type C3H and BALB/cJ mice, as well as the IL-4- and IL-4Rα-deficient mice on the BALB/cJ background. It is quite interesting that the C3H mice produced greater quantities of all Ab isotypes than the wild-type BALB/cJ mice did. The IL-4- and IL-4Rα-deficient mice had barely detectable levels of IgG1, which were greatly reduced compared to levels in the wild-type BALB/cJ mice. IL-4- and IL-4Rα-deficient mice produced increased amounts of IgG2b. IgG2a and IgG3 levels showed little variation between mice of the BALB/cJ background.

Differences in Ab production were also assessed on the C57BL/6J background (Table 3). The amounts of IgG2a pro-

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**TABLE 1. Histopathological assessment of arthritis severity at 4 weeks postinfection**

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>n</th>
<th>Avg histopathology score (0–4+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3H</td>
<td>28</td>
<td>3.0 ± 0.8</td>
</tr>
<tr>
<td>BALB/cJ</td>
<td>35</td>
<td>2.7 ± 1.2</td>
</tr>
<tr>
<td>BALB/cJ-IL-4Rα--</td>
<td>39</td>
<td>2.8 ± 1.3</td>
</tr>
<tr>
<td>BALB/cJ-IL-4Rα--</td>
<td>6</td>
<td>2.2 ± 1.0</td>
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<tr>
<td>BALB/cJ-IL-4Rα--</td>
<td>31</td>
<td>2.9 ± 1.3</td>
</tr>
<tr>
<td>BALB/cJ-IL-4Rα--</td>
<td>20</td>
<td>2.5 ± 1.2</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>7</td>
<td>1.7 ± 1.1</td>
</tr>
<tr>
<td>C57BL/6J-IL-4Rα--</td>
<td>7</td>
<td>1.4 ± 1.0</td>
</tr>
</tbody>
</table>

*a* Mice were infected with 2,000 *B. burgdorferi* cells. Arthritis severity was assessed as described in Materials and Methods.

*b* Number of mice per group.

*c* Results are representative of five experiments.
duced were not determined in these mice because C57BL/6 mice do not possess the gene for IgG2a, but instead they possess a novel IgG2c gene, which is not recognized by commercial antibodies to IgG2 (26, 31, 32). Wild-type C57BL/6J mice produced only a small amount of IgG1 antibody, barely detectable above background, as did the C57BL/6 IL-4-deficient mice. As was seen previously, in the IL-4- and IL-4Rα-deficient mice on the BALB/cJ background, IL-4-deficient C57BL/6J mice produced greater amounts of IgG2b and similar levels of IgG3 Ab compared to levels in the wild-type mice. Although there were alterations in the amounts and isotypes of Abs produced in the IL-4- and IL-4Rα-deficient mice, these differences did not correlate with changes in arthritis severity or B. burgdorferi numbers during the first 4 weeks of infection.

DISCUSSION

In this study, we examined the effects of genetic ablation of IL-4 and the IL-4Rα chain in order to determine the roles that IL-4 and IL-13 play in host defense against B. burgdorferi infection. IL-4 has previously been shown to play a role in resistance to severe arthritis, and its presence correlated with decreased joint swelling during infection (28, 29, 33).

Throughout the study we saw variability in the severity of arthritis in BALB/cJ wild-type mice. By doing several experiments, we were able to conclude that the absence of IL-4 or the IL-4Rα chain did not lead to more severe arthritis or joint swelling when wild-type mice had mild arthritis or to less severe arthritis and joint swelling when wild-type mice had severe arthritis. This conclusion points out the care that must be exercised in using the dose-responsive BALB/c mice and may explain why others using BALB/c mice previously reported a greater effect for IL-4 (28, 29, 33). The results of experiments with mice on the C57BL/6J background were quite clear, showing no differences in arthritis severity or joint swelling between IL-4-deficient and wild-type mice. Our results show that IL-4 and IL-13 are not required for resistance to arthritis upon infection with B. burgdorferi. These results are consistent with the findings for another IL-4-deficient mouse on the resistant DBA background (14).

The role of IL-4 in the host response to B. burgdorferi infection has previously been addressed in the context of Th1 versus Th2 responses in the mouse. A Th1 response including gamma interferon production and isotype switching to IgG2a was suggested to be detrimental to the host, leading to the development of severe arthritis and the inability to control spirochete numbers (28, 29, 33). IL-4 production, as part of a Th2 response, and isotype switching to IgG1 were believed to be crucial for resistance to severe arthritis and control of spiro-
chete numbers (28, 29, 33). Surprisingly, our experiments show that arthritis-susceptible C3H mice make Borrelia-specific Abs of all isotypes—IgG1, IgG2a, IgG2b, and IgG3, in larger amounts than those of either of the resistant BALB/cJ or C57BL/6J strains. Although C3H mice are able to produce IgG1 in amounts equivalent to those of BALB/cJ mice, they still harbor fivefold more bacteria in their joints. IL-4- and IL-4Rα-deficient BALB/cJ mice produce almost no IgG1, yet they are able to control spirochete numbers in both joint and heart tissues, suggesting that IgG1 is not required for host control of spirochete numbers. Interestingly, all of the IL-4- and IL-4Rα-deficient mice produced more IgG2b than did their wild-type counterparts; however, this change had no effect on spirochete numbers. The C57BL/6J mice that were deficient in IL-4 retained similar bacterial numbers in joints and hearts compared to those retained by wild-type C57BL/6J mice. Overall, the absence of IL-4 and IL-4 receptor signaling had no effect on spirochete presence in tissues; therefore, these cytokines do not appear to be necessary for host control of Borrelia burgdorferi during the first 4 weeks of infection.

A recent study addressed the role of IL-4 in B. burgdorferi infection by using an IL-4-deficient mouse on the DBA background (14). The results presented here confirm and expand on those results by looking at additional mouse strains, as well as examining B. burgdorferi infection in a mouse lacking the IL-4Rα chain. IL-4 and IL-13 share the IL-4Rα chain as a common component in their receptor complexes (1, 21, 23, 35), leading to many similar downstream effects (18, 37, 53). For example, granuloma formation in Schistosoma mansoni-infected mice lacking IL-4 was found to be dependent on IL-13 and disappeared when IL-4Rα−/− mice were infected (19, 20, 24, 34, 44, 49, 50). Through the use of an IL-4Rα-deficient mouse, the present study has shown that IL-4 is not required for resistance to the development of severe arthritis or for control of bacterial numbers in the joint and heart tissues and that, in BALB/cJ mice, IL-13 is not compensating for the absence of IL-4.

Genes encoding IL-4 and IL-13 can now be eliminated as candidate genes for the region of chromosome 11 with suggestive linkage to arthritis severity (51). Experiments have now shown that the absence of IL-4 on the resistant DBA (14), C57BL/6J, and BALB/cJ backgrounds or the absence of the IL-4Rα chain on the BALB/cJ background has no effect on arthritis severity. Although IL-4 and IL-13 are not required for resistance to severe arthritis, production of the inflammatory molecules IL-6, IL-11, and IL-12 appears to regulate the host response to B. burgdorferi infection (2–5). Furthermore, the absence of the anti-inflammatory cytokine IL-10 on the C57BL/6J background leads to increased arthritis severity in a resistant mouse strain (16). These results suggest that the balance of pro- and anti-inflammatory molecules may be involved in regulating Lyme arthritis severity, but at a level other than the production of IL-4 or through the IL-4–IL-13 signaling pathway.

ACKNOWLEDGMENTS
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REFERENCES

TABLE 2. Levels of B. burgdorferi-specific IgG isotypes in sera from IL-4- and IL-4Rα-deficient BALB/cJ mice

<table>
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<th>Avg Ig OD&lt;sub&gt;405&lt;/sub&gt;</th>
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<tr>
<td></td>
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<td>IgG1</td>
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<tr>
<td>C3H</td>
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<td>BALB/c-IL-4&lt;sup&gt;−/−&lt;/sup&gt;</td>
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<td>BALB/c-IL-4&lt;sup&gt;−/−&lt;/sup&gt; IL-4R&lt;sup&gt;−/−&lt;/sup&gt;</td>
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<td>0.146 ± 0.09&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>C57BL/6J</td>
<td>21</td>
<td>0.117 ± 0.11&lt;sup&gt;c&lt;/sup&gt;</td>
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</table>

<sup>a</sup> Mice were infected with 2,000 B. burgdorferi cells. Serum collected at 4 weeks postinfection was assessed by ELISA for IgG content as described in Materials and Methods.

<sup>b</sup> Number of mice per group. Results are combined from four individual experiments.

<sup>c</sup> Significantly different from values obtained for wild-type BALB/cJ mice (P ≤ 0.001).

TABLE 3. Levels of B. burgdorferi-specific IgG isotypes in sera from IL-4-deficient C57BL/6J mice

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>n</th>
<th>Avg Ig OD&lt;sub&gt;405&lt;/sub&gt;</th>
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<td></td>
<td></td>
<td>IgG1</td>
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<tr>
<td>C57BL/6J-IL-4&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>7</td>
<td>0.048 ± 0.02</td>
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</table>

<sup>a</sup> Mice were infected with 2,000 B. burgdorferi cells. Serum collected at 4 weeks postinfection was assessed by ELISA for IgG content as described in Materials and Methods.

<sup>b</sup> Number of mice per group.

<sup>c</sup> Significantly different from values obtained for wild-type C57BL/6J mice (P ≤ 0.01).


