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# SHORT REPORT: REQUIREMENT OF B CELLS FOR DELAYED TYPE HYPERSENSITIVITY–LIKE PATHOLOGY AFTER SECONDARY INFECTION WITH *LEISHMANIA MAJOR* IN RESISTANT C57BL/6 MICE

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*Abstract.* B cell-deficient C57Bl/6 (μMT) mice were resistant to *Leishmania major* after both primary and secondary parasite challenge. However, unlike in wild-type mice, secondary infection in μMT mice was not accompanied by a marked delayed type hypersensitivity-like response, and interferon-γ (IFN-γ) levels were approximately half of those in wild-type mice. These results suggest that B cells are involved in IFN-γ production and the pathology of secondary infection.

*Leishmania major* is an obligate intracellular parasite that induces cutaneous lesions in humans and animals. Most laboratory mice are resistant to primary infection with *L. major* because they develop protective T helper type 1 (Th1) immune responses. Unlike the essential role for T cells in this resistance, the role of B cells in primary resistance to *L. major* appears to be minimal.[1–5] Although one study has shown that depletion of B cells in normally *L. major*-resistant mice results in a lack of resistance,[6] three other studies found the opposite result: resistance to *L. major* infection developed normally in C57Bl/6-*Igh-6*$^{tm1Cgn}$ (μMT) mice, which lack mature B cells,[7] and in mice depleted of either B cells or B-1 cells using anti-μ antibody or radiation treatment, respectively.[8,9] In addition to these studies, the finding that primary resistance does not require antibodies to *L. major* supports the minimal role conclusion for B cells.[2,10,11]

Recent studies have shown that the importance of B cells in primary and secondary responses to intracellular pathogens can differ. In both wild-type and μMT mice, primary infection with the intracellular bacterial parasite *Chlamydia tracomatis* leads to immunologic resistance.[12,13] However, in contrast to wild-type mice, resistance to a secondary challenge is reduced or absent in μMT mice.[12,13] Interestingly, both interferon-γ (IFN-γ) production and delayed type hypersensitivity (DTH) responses are also reduced following secondary challenge in μMT mice.[13] Reduction of IFN-γ production in B cell-deficient mice, relative to wild-type mice, has also been reported following infection with *Listeria monocytogenes* or *Neospora caninum*.[14,15] Both DTH responses and IFN-γ production are closely associated with resistance to *L. major*, and the development of Th1 responses correlates with the ability to develop DTH responses to *Leishmania* antigens.[3,4,9,16,17] Although it is evident that the absence of B cells does not alter resistance to *L. major* following primary infection,[7–9] the question addressed here was how do B cells influence the recall response to *L. major*.

Wild-type C57Bl/6 and μMT mice were originally obtained from Jackson Laboratories (Bar Harbor, ME) and bred at the Laboratory Animal Resources facility at Colorado State University as previously described.[18] The maintenance and care of all experimental animals complied with National Institutes of Health guidelines for the humane use of laboratory animals. Female mice (6–8 weeks of age, five per group) were infected with $10^6$ *L. major* promastigotes (LV39, RHO/SU/59/P, Neal, or P strain) in one rear foot pad.[19] Lesion size was monitored over time with vernier calipers (lesion size = infected foot - contralateral uninfected foot).

As shown in Figure 1A, we confirmed that both wild-type and μMT mice were resistant to *L. major* after primary challenge. However, using an enzyme-linked immunosorbent assay technique[20] to analyze 48-hour supernatants from cultured, *L. major*-restimulated lesion-draining lymph node cells taken 20 days post-infection, we observed that cells from μMT mice produced significantly less IFN-γ (approximately half as much; $P = 0.01$) than did cells from wild-type mice (Table 1). These data are similar to those of others who showed reduced IFN-γ production after pathogen challenge in μMT mice.[13–15] In contrast, we found that production of interleukin-2 (IL-2), IL-4, and IL-10 was not different between the two mouse strains. Interestingly, Brown and Reiner[7] found no difference in IFN-γ mRNA levels between wild-type and μMT mice infected with *L. major*, but they analyzed $CD4^+$ lesion-draining lymph node cells only, rather than production by all lymph node cells as described here. A study by Harris and others[21] has shown that some B cells can produce IFN-γ. Therefore, it is possible that the reduced IFN-γ production observed here for μMT mice was due to the lack of B cell-produced IFN-γ. To examine if a similar phenomenon was occurring in the lesion itself, mRNA levels for IFN-γ were measured in lesions from wild-type and μMT mice 21 days post-infection using a reverse transcription–polymerase chain reaction technique as described previously.[19] The data from three individual animals per group are shown in Table 1 (presented as the relative ratio of mRNA for IFN-γ/β-actin) and indicate that IFN-γ mRNA levels were lower by 42% in μMT mice relative to wild-type mice. This reduction in lesion IFN-γ mRNA was approximately the same magnitude as the reduction in IFN-γ protein produced by lymph node cells from μMT mice.

After lesion resolution (approximately 70 days), some mice (2–4 per group) were reinfected with $10^6$ *L. major* in the contralateral uninfected footpad. As shown in Figure 1B, an increase in lesion size indicative of a DTH response was observed in wild-type mice and was typical for secondary exposures to *L. major* antigens.[8] The peak of this response occurred on day three after rechallenge, and lesion resolution was complete by approximately day 40. In contrast, in μMT mice, little or no increase in lesion size was observed after secondary infection, and only small lesions were observed to develop in any of these mice in two separate experiments.

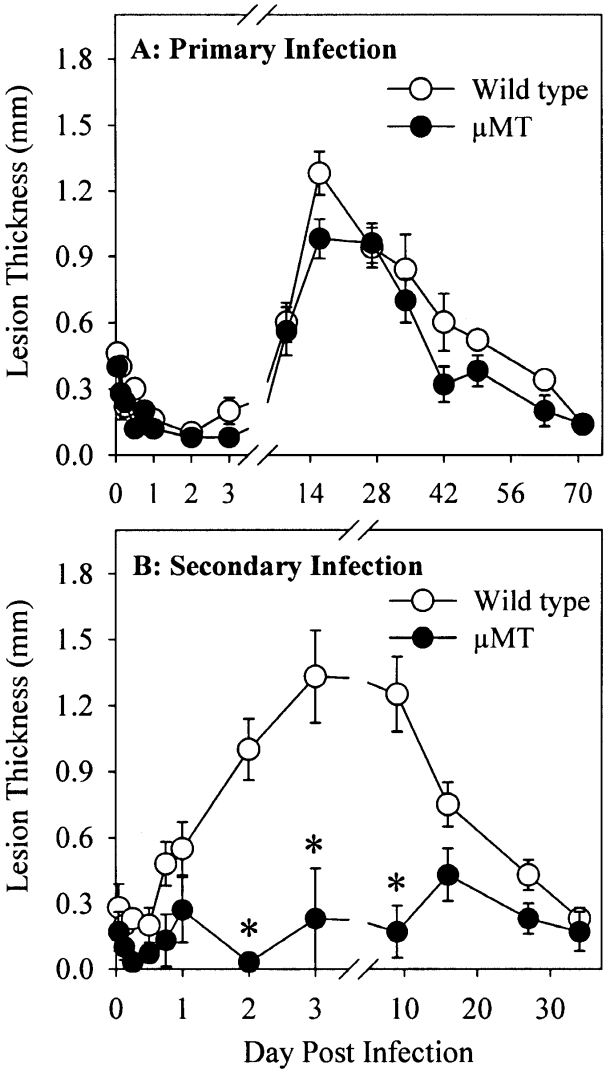


FIGURE 1. Requirement of B cells for the expression of delayed type hypersensitivity–like pathology following secondary infection with *Leishmania major*. Foot lesion size over time is shown for C57B1/6 and μMT mice after primary (**A**) and secondary (**B**) infection with $10^6$ *L. major*. Data represent the mean ± SEM for 4–5 animals per group.

Importantly, all lesions resolved in μMT mice over the same time frame as observed for wild-type mice, and no difference in parasite burdens was found. Thus, a lack of B cells did not alter resistance to *L. major* after secondary challenge. Because DTH responses are associated with IFN-γ production in *L. major*-infected animals,[3,4,9,16,17] IFN-γ mRNA levels were measured in lesions taken three days after reinfection with parasites. As shown in Table 1, IFN-γ mRNA levels were reduced by 47% on average in two experiments (the lesions from two animals were pooled per group in each experiment). Clearly, although reduced in the absence of B cells, the amount of IFN-γ was sufficient to facilitate resistance in μMT mice. It is unclear, however, if the lack of a DTH response was the result of reduced IFN-γ expression or if they are merely coexpressed phenomena. It is interesting that Babai and others[8] found that in B-1 cell-depleted mice, the DTH response to *L. major* antigen was unchanged, relative to wild-type mice, nine weeks after primary challenge with parasites. In light of their results, the results shown here suggest that classic B cells (those other than B-1 cells), may have a major role in the development of DTH responses to *L. major* in wild-type *L. major*-resistant mice. Because DTH responses do not correlate with antibody levels to *Leishmania*,[10] B cells are likely to influence DTH responses through a non-antibody-mediated pathway.

TABLE 1

Interferon-γ measurements for lesion-draining lymph node cells and foot lesions

| | Wild-type mice | μMT mice |
|---|---|---|
| Primary infection | | |
| Experiment 1* | 49.1 ± 2.7 ng/ml | 22.7 ± 4.1 ng/ml† |
| Experiment 2†‡ | 1.00 ± 0.39 | 0.58 ± 0.14 |
| Secondary infection | | |
| Experiment 1§ | 1.00 | 0.58 |
| Experiment 2§ | 1.00 | 0.48 |

* Values represent restimulated lymph node cell production (mean ± SEM) on day 20 post infection.
† Significantly different from wild-type ($P = 0.01$).
‡ Values represent relative mRNA levels in foot lesions (mean ± SEM) on day 21 post-infection.
§ Values represent relative mRNA levels in foot lesions (pooled sample) 72 hours post-secondary infection.

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