

Analysis of the mouse immune response to *Leishmania major* using 'LACK' antigen-deficient parasites

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Studies of *Leishmania major* susceptibility in BALB/c mice suggest a pathologic Th2 response nucleated by the response to an epitope derived from the *L.major* antigen LACK. The LACK genes of this diploid parasite are tandemly arranged as two gene copies on each allele. Gene targeting has been used to delete one allele, thus creating heterozygous LACK-deficient *L.major*. As compared to wild-type parasites, LACK heterozygotes demonstrated no loss of pathogenicity for BALB/c mice. Although single deletion of either remaining LACK gene was tolerated, LACK null parasites could not be created, indicating that LACK is essential for *L.major*. LACK1 or LACK2 single-copy organisms showed moderately reduced levels of LACK protein by Western analysis and lesion progression was severely delayed in both WT and T cell-deficient BALB/c mice. A knock-in approach has been used to replace the remaining LACK gene with mutations that destroy the crucial I-Ad epitope that drives the LACK response in BALB/c mice. □ LACK-specific tetramers have been used together with IL-4/GFP reporter mice in order to analyze expansion and IL-4 expression among LACK-specific CD4 T cells, in response to infection with these LACK-deficient parasites. □ Initial experiments suggest reduced expansion of IL-4-expressing LACK-specific T cells for inoculated parasites lacking the wildtype LACK epitope. The impact of this reduced LACK-specific response on the susceptibility of mice to *L.major* is currently being determined by monitoring lesion development following inoculation with these mutant organisms.

This approach will confirm definitively whether this single parasite antigen epitope is critical to susceptibility in BALB/c mice.