

P. Heinzl, M.D.

Murine leishmaniasis as a model for testing immunotherapies during chronic infection. Frederick P. Heinzl, M.D. Division of Geographic Medicine, Case Western Reserve University School of Medicine, Cleveland, OH 44106

Several models of murine leishmaniasis exist, each using distinct species of this protozoan parasite and each demonstrating unique types of immunopathology. These models have provided important insights into the nature of protective or exacerbative immunity against intracellular infection. Cutaneous infection of different inbred strains of mice with *Leishmania major* results in highly polarized disease outcomes dependent on the early development of predominant Th1 or Th2-type CD4⁺ T cell responses. Deleterious Th2-type responses in susceptible BALB/c mice mediate progression of cutaneous disease and are dependent on early expression of IL-4 by a unique population of Va8/Vb4 CD4⁺ T cells that may be primed by cross-reactive antigens within the intestinal microflora. Although Th2 cell development and disease progression can be blocked by preemptive disruption of co-receptor and cytokine-dependent signals in vivo, established Th2 cell responses (> 2 weeks of disease) become unresponsive to therapeutic manipulation.

Dendritic-cell directed immunotherapies were studied as a means of reversing established Th2 responses in this model. Because dendritic cells expand to three-fold greater numbers in the regional lymph node of resistant mice during infection, we tested whether cytokine-induced increases in DC numbers would affect the course of disease. However, pre-treatment of BALB/c mice with the hematopoietic cytokine rFlt3 ligand only prevented disease progression in 40% of treated mice and did so without any associated changes in Th1/Th2 cytokine balance during the primary infection. Alternatively, the role of therapeutic CD40 activation as a means of recruiting protective DC function in established disease was tested. Injection with activating anti-CD40 antibody was extremely effective in reversing the rapid expansion of Th2-type cells after subcutaneous injection with *Schistosoma mansoni* eggs. However, anti-CD40 did not protect against *L. major* in susceptible mice unless administered for a minimum of three weeks and only if commenced at the time of infection. Delayed therapy was ineffective. These studies indicated that enhanced DC numbers and activation do not effectively reverse established Th2 responses in murine leishmaniasis.

As an alternative approach for reversing established Th2-biased responses, CD4⁺ T cells were transiently depleted using cytotoxic antibody and the recovering lymphocyte population subsequently biased towards the Th1 phenotype by cytokine-directed immune reconstitution. This method cured >80% of mice already infected for two to three weeks at a time when mice were otherwise unresponsive to cytokines or CD4 depletion alone. Treatment with anti-IL-4 antibody alone after CD4⁺ depletion also resulted in cure, showing that accessory cell functions were sufficient to support expansion of Th1 type cells despite the presence of large numbers of parasites. These findings support the central role of the Th2-differentiated CD4⁺ cell as the key mediator of BALB/c susceptibility to progressive leishmaniasis. They further suggest that the sequential depletion and immune reconstitution of CD4⁺ cells may be a useful means for abrogating pathologic T cell responses otherwise resistant to immunotherapy. Supported by funds from NIAID grants AI 35979 and AI 45602 and by the VA Medical Research Service.