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$CD4^+$ $Foxp3^+$ Regulatory T Cells in Persistence of Leishmania donovani in the Liver of Immunocompromis

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Abstract

Visceral leishmaniasis (VL) is a chronic and fatal disease in humans and dogs caused by the intracellular protozoan parasites, Leishmania donovani and L. infantum (L. chagasi). Reactivation of parasites and relapse of VL are frequent in the immunocompromised patients, in which the number of cases has been increasing recently. The liver, spleen, bone marrow and lymph nodes are the major sites of parasite growth, disease pathology and persistent infection. However, the mechanisms underlying the parasite persistence in the immunocompromised status have not been clarified. The present study is aimed to improve the understanding of mechanisms of L. donovani persistence in the liver in an immunocompromised status using alymphoplastic aly/aly mice that carry a mutation within the gene encoding NF-κB inducing kinase (NIK). Hepatic parasite burden, granuloma formation, expression of cytokine/chemokine mRNA and induction of regulatory T cells (Tregs) for up to 7 months after the intravenous inoculation with L. donovani promastigotes were determined. While control aly/+ mice showed an early peak of hepatic parasite growth at 4 weeks post infection (WPI) and resolved the infection by 8 WPI, aly/aly mice showed long-term parasite persistence in the chronic phase of the infection in the liver, which was associated with delayed and impaired granuloma maturation. Although hepatic $CD4^+Foxp3^+$ but not $CD8^+Foxp3^+$ T cells were first detected at 4 WPI in both strains of mice, the number of $CD4^+Foxp3^+$ T cells and expression of Foxp3 mRNA were significantly increased in aly/aly mice from 8 WPI. Immunohistochemical analysis demonstrated the presence of $Foxp3^+$ T cells in L. donovani-induced hepatic granulomas and perivascular neo-lymphoid aggregates. Laser microdissection and quantitative RT-PCR assay of

mature granulomas revealed the correlation of Foxp3 and IL-10 mRNA level. Furthermore, treatment of infected aly/aly mice with anti-CD25 or anti-FR4 mAb resulted in significant reductions in both hepatic $Foxp3^+$ cells and parasite burden. Bone marrow (BM) chimeric mice, aly/aly → aly/+ and aly/+ → aly/aly mice, were produced to examine the factors underlying the improper T cell-mediated immune response in the liver of aly/aly mice. In the liver of both BM chimeric mice, parasite persistence, delayed and impaired hepatic immune response and Tregs expansion were found. These results suggest that both structural defects of secondary lymphoid tissues and the NIK gene mutation of hematopoietic cells account for the diminished hepatic immune response to L. donovani infection, but not for the induction and suppressive function of Tregs in aly/aly mice. Conclusion, this study provide the first evidence that $CD4^+Foxp3^+$ Tregs mediate L. donovani persistence in the liver of immunodeficient aly/aly and BM chimeric aly/aly → aly/+ mice. In addition, this study is the first report on the effectiveness of anti-FR4 mAb to control the systemic L. donovani infection in mice, a result that will help to establish new strategies of immunotherapy against this intracellular protozoan pathogen.