PALADINS: PROTECTIVE APPROACHES LEVERAGING AD-APTIVE AND IN-NATE SYSTEMS

Burkholderia Pseudomallei Infection Models In Humanized Hla Transgenic Mice

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Vaccine-induced T cell responses to Burkholderia pseudomallei are critical for protective immunity because B. pseudomallei is capable of evading antibodies by invading non-phagocytic cells. Human leukocyte antigen (HLA) transgenic mouse models offer an in vivo system for assessment of T cell responses induced by human MHC restriction that cannot be replicated by native mouse MHC and make it possible to more readily translate findings in animal studies to human applications. In this study, we established a model of B. pseudomallei infection in HLA-DR3 transgenic mice. Virulence of B. pseudomallei K96243 was evaluated in an acute melioidosis model using C57BL/6 (WT) and HLA-DR3 transgenic mice. The acute infection in WT mice resulted in 100% mortality at 2500 CFU with survival at lower doses, while the HLA-DR3 mice were highly susceptible to the infection with a 100% mortality by day 6 post-infection via intranasal route. We hypothesized that poor control of the infection was due to enhanced activity of the regulatory T cells (Tregs). We then sought to characterize how the proportion of CD3+,CD4+, CD25+, FoxP3+ cells within splenocytes related to disease severity. We found that acutely infected mice self-segregated into distinct infection groups, as defined primarily by lung bacterial loads, although similar burdens were observed systemically within the liver and spleen. By flow cytometry, we observed an increased frequency of Tregs as well as increased IL-10 production as compared to uninfected animals only in those exhibiting the most severe infections. The proportion of Teff cells remained stable among all cohorts, however only those with moderate infection displayed significantly enhanced IFNy activity. This could explain that the response was due to the increased activation of Th1-skewed splenocytes, as there was likely an increase of TCR engagement given the elevated bacterial burdens without inhibition by Tregs that were upregulated in severe infection. In addition, we also sought to determine if active Treg populations could be identified in a chronic model. HLA-DR3 mice were infected with 5 CFU of K96243 and euthanized on day 28. We found that all mice had detectable bacteria within the lung, and 50% had systemic infection detectable in the liver. No mice exhibited splenic infection. We stratified mice into mild (2-fold increase) and moderate (>2x102-fold increase) infection cohorts and, phenotyped CD4+ T cells using flow cytometry. Compared to the acute infection, cytokine production was less robust in the sub-acute and chronic models. In addition, the effector functions of CD4+ T cell subsets at later time points may be diminished due to the combined effects of prolonged inhibitory signaling (e.g., PD-1, IL-10) as well as the absence of additional pro-inflammatory signals subsequent to the quelling of the primary innate immune response. Collectively, this study has demonstrated that the HLA-DR3 mouse is a suitable model for studying T cell responses in melioidosis. We have confirmed that the poor control of B. pseudomallei infection in the host is associated with enhanced Treg activity. Vaccines developed against this deadly bacterial threat agent should consider the balance of functional Th1 and Treg cell induction.

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