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Direct Assessment of Cellular Immune Responses to Ovalbumin with a Secreted Luciferase Transgenic Reporter Mouse Strain IFNγ-Lucia

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Abstract : Objectives: Assessing antigen-specific T cell responses is of utmost importance for the pre-clinical testing of prototype vaccines against intracellular pathogens and tumor antigens. Mainly two types of in vitro assays are used for this purpose 1) enzyme-linked immunospot (ELISpot) and 2) intracellular cytokine staining (ICS). Both are time-consuming, relatively expensive, and require manual dexterity. Here, we assess if a straightforward detection of luciferase activity in blood samples of transgenic reporter mice expressing a secreted Lucia luciferase under the transcriptional control of IFN-γ promoter parallels the sensitivity of IFNγ ELISpot assay. Methods: IFN-γ-LUCIA mouse strain carrying multiple copies of Lucia luciferase transgene under the transcriptional control of IFNγ minimal promoter were generated by pronuclear injection of linear DNA. The specificity of transgene expression and mobilization was assessed in vitro using transgenic splenocytes exposed to various mitogens. The IFN-γ-LUCIA mice were immunized with 50mg of ovalbumin (OVA) emulsified in incomplete Freund's adjuvant three times every two weeks by subcutaneous injections. Blood samples were collected before and five days after each immunization. Luciferase activity was assessed in blood serum. Peripheral blood mononuclear cells were separated and assessed for frequencies of OVA-specific IFNγ-secreting T cells. Results: We show that in vitro cultured splenocytes of IFN-γ-LUCIA mice respond by 2 and 3 fold increase in secreted luciferase activity to T cell mitogens concanavalin A and phorbol myristate acetate, respectively but fail to respond to B cell-stimulating E.coli lipopolysaccharide. Immunization of IFN-γ-LUCIA mice with OVA leads to over 4 fold increase in luciferase activity in blood serum five days post-immunization with a barely detectable increase in OVA-specific, IFNγ-secreting T cells by ELISpot. Second and third immunizations, further increase the luciferase activity and coincidently also increase the frequencies of OVA-specific T cells by ELISpot. Conclusions: We conclude that minimally invasive monitoring of luciferase secretions in blood serum of IFN-γ-LUCIA mice constitutes a sensitive method for evaluating primary and memory Th1 responses to protein antigens. As such, this method may complement existing methods for rapid immunogenicity assessment of prototype vaccines.

Keywords : ELISpot, immunogenicity, interferon-gamma, reporter mice, vaccines
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