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The adjuvant activity of alphavirus replicons is enhanced by incorporation of flagellin

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Abstract

Alphaviruses and flagellin stimulate pattern recognition receptors and can both independently act as adjuvants for antigen-specific antibody responses. Alphaviruses stimulate endosomal Toll-like receptors (TLRs) 3, 7 and 8 as well as cytoplasmic melanoma differentiation-associated gene 5 (MDA-5) and Protein Kinase RNA-activated (PKR), resulting in induction of a strong type I interferon (IFN) response. Bacterial flagellin stimulates TLR5 on cell surfaces and NLRC4 in the cytoplasm, leading to secretion of proinflammatory cytokines. Ligands of different pattern recognition receptors might act in synergy to induce greatly increased responses. We therefore hypothesized that the adjuvant activity of alphavirus replicons would be enhanced by incorporating flagellin into the replicon. To address this, we cloned flagellin into the genome of an alphavirus replicon and assessed its potential as an adjuvant on the antibody response against co-immunized protein antigen in mice. We used a flagellin variant that contains a deletion in the hypervariable region to avoid anti-flagellin immunity without compromising innate immune signaling. Using the ELISA assay, we measured total antigen-specific IgG as well as IgG1 and IgG2a/c isotype responses as indicators of Th2 and Th1 type responses, respectively. Since alphaviruses induce strong type I IFN responses and flagellin signals through TLR5, we used genetic knock-out mice to assess the

involvement of these signaling pathways on adjuvant-induced antibody responses. Antigen-directed antibody responses were potentiated by flagellin-encoding alphavirus replicons to a greater extent than by alphavirus replicons not encoding flagellin or by soluble flagellin. Both IgG1 and IgG2a/c responses were increased, indicating an enhancement of both Th2 and Th1 type antibodies. The adjuvant activity was diminished but not abolished in the absence of TLR5 or type I IFN signaling, demonstrating that several innate pathways are involved. In conclusion, we have demonstrated that incorporating flagellin into alphavirus replicons greatly potentiates antibody responses against an otherwise non-immunogenic protein antigen. Responses were characterized by both Th1 and Th2 type antibodies, and both TLR5 and type I IFN signaling were stimulated. Thus, where a balanced Th1/Th2 response is of importance, the use of VREP encoding flagellin may be an attractive choice of adjuvant. This work was supported by the Swedish Research Council and the Swedish International Development Cooperation Agency. Maria L. Knudsen is a recipient of Karolinska Institutet Faculty Funding for Doctoral Students.