Development of a Novel Carrier Protein for Monoclonal and Polyclonal Antibody Production

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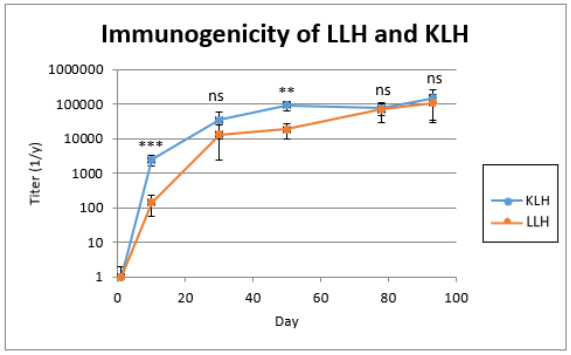
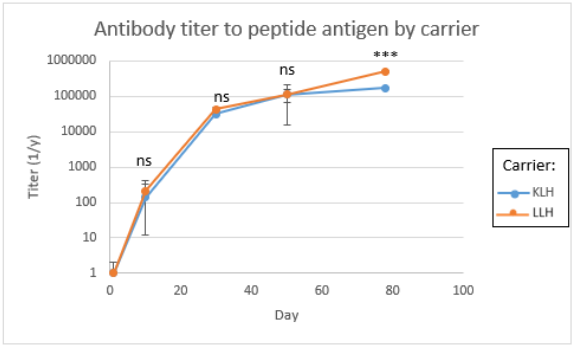
Statement of Purpose: Carrier proteins are critical in the antibody production process as they confer immunogenicity to poorly immunogenic compounds such as small molecules or peptides. Hemocyanins are copper containing proteins used for oxygen transport in arthropods and mollusks and are routinely utilized as carrier molecules due to their size, potent immunogenicity, and phylogenic distance from mammalian hosts/antigens. The most commonly used carrier protein is keyhole limpet hemocyanin (KLH). However, current immunization protocols often require alternating carrier molecules to prevent immunodominance of the carrier to the detriment of the antibody response to the hapten. Thus, there is a need for an alternative highly immunogenic carrier protein. Lobster hemocyanin (LLH), is readily available as a byproduct from the food industry. Here, we evaluate the utility of lobster hemocyanin as a carrier protein of hapten antigens in comparison with KLH.

Methods: Hemocyanin was purified from American lobster (*Homarus americanus*) serum. Peptide, protein and hormone antigens were covalently conjugated to LLH and KLH. New Zealand white rabbits and/or BALB/c mice were immunized with either the KLH-antigen conjugate or the LLH-antigen conjugate. Serum was collected at designated time points following immunizations and antigen specific antibody titers were measured by indirect ELISA. Antibody responses directed against the carrier molecule (LLH or KLH), as well as cross-reactivity of anti-LLH and anti-KLH antibodies, were also measured via indirect ELISA.

Results: Pre-existing antibodies against LLH were undetectable by ELISA in sera isolated from naïve rabbits. Immunization with both LLH and KLH conjugated antigens resulted in the production of antigen specific antibodies. There was no significant difference in the antibody titers generated against the peptide or protein antigens conjugated to LLH or KLH.

Conclusions: LLH is an immunogenic and effective carrier protein that promotes the generation of polyclonal antibodies to conjugated haptens as effectively as KLH. Anti-LLH antibodies do not cross-react with KLH, signifying that the LLH epitopes are unique from those on KLH. Thus, LLH is novel carrier protein that can be used as an alternative to or in conjunction with KLH.





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