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Pore-forming proteins (PFPs) from a sea anemone encapsulated into liposomes are able to enhance an antigen specific cytotoxic T lymphocytes response

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Sticholysins I and II (StI/II, Sts) are two PFPs produced by the sea anemone *Stichodactyla helianthus*, exhibiting a preference for sphingomyelin-containing membranes. Different strategies employing bacterial PFPs have been used to improve the antigen-specific cytotoxic T CD8⁺ lymphocytes (CTLs) response. Furthermore, liposomes have been used as adjuvants due to their ability to improve antigen uptake by antigen presenting cells. We studied the enhancement of CTLs response by liposomes co-encapsulating Sts with ovalbumin as model antigen (Lp/OVA/Sts). C67BL/6 mice were immunized twice with Lp/OVA/Sts or Lp/OVA without Sts. SIINFEKL-specific B3Z CD8⁺ T and OVA-expressing EG-7 tumor cells were used to measure the antigen cross-presentation *in vitro* and antitumor activity *in vivo*, respectively. Lp/OVA/StII induced an OVA-specific CD8⁺ T-cell expansion superior to that observed with Lp/OVA and *in vitro* significantly enhanced activation of the SIINFEKL-specific B3Z CD8⁺ T cells as a consequence of antigen cross-presentation by macrophages, but not by dendritic cells. Interestingly, Lp/OVA/StII-induced activation was inhibited by lysosomes proteases inhibitors, but not proteasome inhibitor indicating that StII induces antigen cross-presentation by vacuolar pathway. The formulations Lp/OVA/Sts enhanced the OVA-specific CTLs response *in vivo* in comparison with Lp/OVA and also conferred a higher protection to mice challenged with OVA-expressing tumor cells. Additionally, CTLs activity induced by Lp/OVA/StII was independent of CD4⁺ T-cells, while anti-tumor response was strongly affected by CD8⁺ T-cells depletion. Interestingly, free-Sts were able to induce activation of DCs and it was dependent of TLR-4 and MyD88, suggesting that the effect of these proteins on the cellular immune response could be beyond their pore-forming ability. The antigen-specific CTLs immune response enhanced by immunization of wild type mice with Lp/OVA/StII was significant reduced in TLR-4 knockout mice. Our results suggest the potentialities of Sts encapsulated into liposomes as adjuvant for enhancing effective CTLs mediated immune responses.

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