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A novel strategy for the identification of genetic adjuvants for Adenovirus vectored vaccines against infectious diseases and cancer

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ABSTRACT

Genetic vaccines, based on the gene encoding the antigen and delivered in the form of nucleic acid into a vector, have been implemented showing a number of advantages over conventional vaccine platforms such as the ability to induce both humoral and cellular immunity and a rapid and reproducible process for large scale production. This notwithstanding, genetic vaccine technologies are still amenable to improvement of their potency and durability of the induced immunity.
Most of the known adjuvants (Alum, MF59, ASO4 etc.) co-administered with "ready to immunize" antigens (i.e., inactivated pathogens or subunit vaccines) boost adaptive immune responses by activating the innate arm of the immune system. However, in the case of genetic vaccines, these adjuvants may inhibit the *in vivo* expression of the encoded antigen thus hampering vaccination efficacy.

Therefore, new adjuvant strategies are needed to achieve more effective genetic vaccines. The aim of the current project is to validate the approach of encoding immunomodulators in Adenovirus vectors to be used as genetic vaccine adjuvants, and to identify novel Adenovirus-encoded molecules capable of improving the extent and the durability of the immune responses induced by Adenovirus vectored vaccines.
From a preliminary experiment, which demonstrated the adjuvant effect of an Adenovirus vector encoding an immunomodulatory antibody (anti-CTLA4) on anti-infectious and anti-cancer Adenovirus-based vaccines, we extended the genetic adjuvant approach to encode immunomodulatory molecules such cytokines, chemokines and other protein factors (IMs) other than antibodies. As a validation of this approach we showed that a rationally identified ligand, when encoded into an Adenovirus vector and co-administered with another Adenovirus vector encoding a vaccine antigen improved the adaptive immune response to the vaccine.
We therefore generated tools and set up protocols for the generation of a library of Adenovirus encoded immunomodulators (Ad-IMs). Firstly, we generated a list of candidate immunomodulators by *in silico* selection methods. We then generated ten Ad-IMs from the top ranking ones in our list, and tested five of them for their adjuvant activity. The results of this work showed that the approach is feasible and can lead to the identification of novel Adenovirus vectored immunomodulators with significant adjuvant activity.
The low systemic exposure to the Adenovirus encoded immunomodulators together with the fact that replication incompetent Adenovirus vectors have been shown to be immunogenic, safe and efficacious in humans and can be produced at a commercial scale and with highly competitive costs, support the clinical

development of this novel class of adjuvants for Adenovirus vectored genetic vaccines.