ImmunoTools *special* Award 2014

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A combined strategy to enhance the efficacy and the delivery of anti-HER2 vaccine

As mortality due to cancer continues to rise despite progresses in cancer therapies, cancer nanotechnology is emerging as a new field of interdisciplinary research because of the great potential applications, such as a system to deliver drugs, but also cancer vaccines. Cancer vaccines offer distinct advantages over standard therapies: higher specificity, lower toxicity and long-term effects due to immunologic memory.

In order to be effective in vivo the vaccine needs to reach the antigen presenting cells (APCs), to correctly expand the immune response against tumors and overcome immunosuppressive mechanism.

The immune system contains three types of APCs, but only dendritic cells (DCs) are defined as “professional” APCs. When DCs capture and process an antigen they mature and migrate to the secondary lymphoid organs. Here, they present the antigen to T cells, initiating adaptive immunity.

Studies on the biological characteristics of new materials suited for biomedical applications are important, particularly those related to the immune system. Therefore, the interactions of nanoparticles (NPs) with the immune system and their potential effects and implications are key questions that must be answered to take full advantage of such approaches

For these reason, particular attention will be paid to the potential clinical usefulness of NPs to deliver antigens to DCs. Since NPs can be easily engineered to efficiently target DCs, they represent an effective vaccine vehicle for immunotherapy of infectious diseases and cancer.

In mice, NPs can be addressed selectively to DCs via the surface receptor CD11c. In order to maturate and migrate to the secondary lymphoid organs where lymphocytes recognize processed antigen, DCs need to receive “danger” signals or maturation stimuli. Consistent with this notion, a fusion protein consisting of antigen fused with antibodies (Ab) to DCs receptor chemically conjugated with antigen have been

shown to target DCs in vivo, inducing T-cell activation when coadministered with inflammatory stimulators such as anti-CD40 Ab.

In this context, we plan to design NPs with anti-CD11c Ab, to target DCs, together with rm sCD40L, to give the danger signal. The new conceived NPs will be loaded with DNA plasmid coding for human HER2, an oncoantigen overexpressed in many kind of tumor, and used to elicit a specific antitumor response in vitro and in vivo in mice model.

For in vitro experiment, DCs will be generated from bone marrow precursors cultured with rm GM-CSF and rm IL4. The rate of activation of NPs-pulsed DCs will be evaluated by flowcytometry (expression of activation marker CD80 and CD86) and by Boyden chamber migration assay. Medium containing rm RANTES/CCL5 or rm CCL19 will be placed in the basolateral chamber as chemoattractant. Immature DCs and DCs matured with rm IFNgamma, rm TNFalpha and rm IL-1beta will be used as negative and positive control, respectively. Efficiency of transfection will be assessed by flowcytometry using anti-HER2 PE Ab.

In order to evaluate the specificity of NPs, total splenocytes will be pulsed with NPs and after 6 and 12 hours stained with anti-HER2 PE Ab together with anti-CD11c, CD4, CD8a, CD11b, Gr1, CD19, CD34, Gr-1, NK-cells, a/b TCR, g/d TCR conjugated Ab.

NPs-pulsed splenocytes will be cultured for 7 days in presence of rm IL-2 and rm IL-7. At day 7 elicited immune response will be characterize in term of specific IFNgamma production by CD8 and CD4 T cells and killing activity using cell line negative or positive for HER2 expression.

For in vivo experiment, mice will be injected with HER2-positive cells. When tumors reach a diameter of 2 mm, mice will receive 2 transcutaneous injection of NPs at day 0 and day 14. Tumor will be monitored weekly. A tumor with 10 mm of diameter will be considered as ethical point to conclude the experiment. Elicited immune response will be characterize in term of cellular (CD8 and CD4 T cells activation) and humoral (anti-HER2 Ab production) response.