ImmunoTools IT-Box-139 Award 2012

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Immunomodulating aptamers

Nowadays, anti-tumor vaccines are the most used strategy within active immunotherapy. Anti-tumor vaccines have achieved encouraging results in numerous clinical trials and thus offer great expectations. However, further research is needed to improve them, since due to the low antigenicity of tumor-induced immune responses they are too weak to have a significant clinical benefit in cancer patients. In this regard, one way of active research is trying to enhance the activation of the immune system to compensate for the low antigenicity of the tumor.T lymphocytes play a crucial role as effector cells of the immune response. The degree of activation and clonal expansion of $CD4^+$ and $CD8^+$ tumor-antigen specific T lymphocytes at the beginning of the immune response determines the efficacy of immunotherapy. From the point of view of cancer immunotherapy, CD28 and CD40 are attractive targets for enhancing the immune response against the tumor through agonistic molecules. In addition, finding molecules capable of inhibiting these receptors would be a useful tool for suppressing unwanted immune responses, as in the case of transplants or autoimmune diseases.

Here we propose to generate a specific aptamer for CD28 and CD40 via SELEX technique and to evaluate their effects as agonists and as inhibitors. We also propose to select an aptamer for mesothelin through cell surface (CS)-SELEX technique and to assess its targeting effect in mesothelin-overexpressing tumor cells. Finally, the ultimate goal is to generate a bispecific aptamer for mesothelin-CD28 and CD40-mesothelin.

From the antibody spectrum contained in the IT-Box-139, four of them are intended to be used whithin this project:

- FITC: Conjugated anti-human CD80, conjugated anti-human CD86, conjugated anti-human CD4 and conjugated anti-human CD19
- PE: Conjugated anti-human control-IgG1, conjugated anti-human CD3, conjugated anti-human CD14 and conjugated anti-human CD20
- APC: Conjugated anti-human CD40, conjugated anti-human CD8 and conjugated anti-human IL-6

I am approaching the second year of my PhD project and at the time of writing this application, aptamers for CD28 and CD40 have been selected. Whithin the next year, an aptamer for mesothelin is intended to be selected and the selected aptamers for CD28 and CD40 will to further characterization. Moreover, to find a putative blocking aptamer fo CD28 and CD40 competition asssays will be performed, and for that end the PE-conjugated anti-human control IgG1 will be used. Futhermore, FITC-conjugated anti-human CD80 and CD86 will be used to determine antigen presenting cells (APCs) activation.

It is of great interest to determine whether the aptamers are cross-reactive with their respective human proteins. Competition and proliferation assays will be performed to find blocking aptamers for human CD28 and CD40. The T cytotoxic response against tumors will be evaluated. Moreover, the type of T helper (T_{h1}, T_{h2} or T_{h17}) response which is of interest in transplants or autoimmune diseases will be assessed as well, and for that the APC-conjugated anti-human IL-6 is needed. Finally, in order to phenotype the selected B and T lymphocytes and the generated dendritic cells which are to use in the future experiments, the FITC-conjugated anti-human CD4 and CD19, the PE-conjugated anti-human CD3, CD14 and CD20 and the APC-conjugated anti-human CD40 and CD8 antibodies from the IT-Box 139 are requiered.