NON-VIRAL NANOVECTORS TO ENGINEER T CELLS

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Nowadays, the use of engineered immune cells is one of the most promising approaches to treat cancer [1]. In particular, adoptive T cell therapies are widely used for haematological cancers and the principal way to deliver nucleic acids into cells is based on viral vectors [2, 3]. Notwithstanding the success of this approved treatment, the use of viral vectors faces with some obstacles such as their complex, highly specialized and expensive manufacture process, as well as limits in genetic cargo capacity, biocompatibility, variability of transduction efficiency between patients, and the risk of semirandom gene integration with potential oncogenic transformation/clonal expansion [4, 5]. In this context, we focused on the development of nanovector-based approach to genetically engineer T cells, which proved being challenging since primary T cells are refractory to transfection [6]. To achieve this aim, it is important to consider not only the physico-chemical properties of nanocarrier but also the phenotype and the status of T cells. Indeed, memory T with stem cell-like (T_{SCM}) phenotype creates a link between naïve and central memory cells entailing an increased proliferation capacity and a superior antitumor effector function [7]. In order, to investigate several non-viral vectors, T cells are isolated from healthy donors and activated for proliferation and expansion. We are investigating different interleukin concentrations (IL-2, IL-7, IL-15 and IL-21) to address a T_{SCM} phenotype by monitoring T cell activation marker and Wnt-B-catenin signalling pathway [8]. Two different approaches are currently under investigation to reach high transfection of T cells. The first relies on the employment of polymeric nanovectors characterized by high degree of biocompatibility and hemocompatibility; the second one embraces a biomimetic coating consisting of either membranes derived from red blood cell (RBC) functionalized or not with DOPE (1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine), or a coating derived from peripheral blood mononuclear cell (PBMC) membranes. From preliminary results we obtained a good degree of balance between biocompatibility and transfection with a polymeric nanoparticle (around 13 %, EGFP as reporter gene). However, some nanovectors induce cytotoxicity despite single constituents are biocompatible (Fig.1), thus opening questions about the role of nanostructuration and its influence on T cells. The employment of membranes-coated nanovectors resulted in poor improvements on transfection efficiency. Interestingly, by exploring PBMCs-membranes coating from cells stimulated with a lipopolysaccharide to increase the superficial interaction receptors, we noticed that the autologous targeting of stimulated-PBMCs vehicles is higher than unstimulated PBMCs- and RBCs- membranes vehicles (Fig. 2).
In conclusion, the development and the assessment of T_{SCM} along with the design of biocompatible and selective non-viral nanovectors will allow us to pave the way for the generation of highly efficient nanovectors for nucleic acids delivery into immune cells.

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Figures

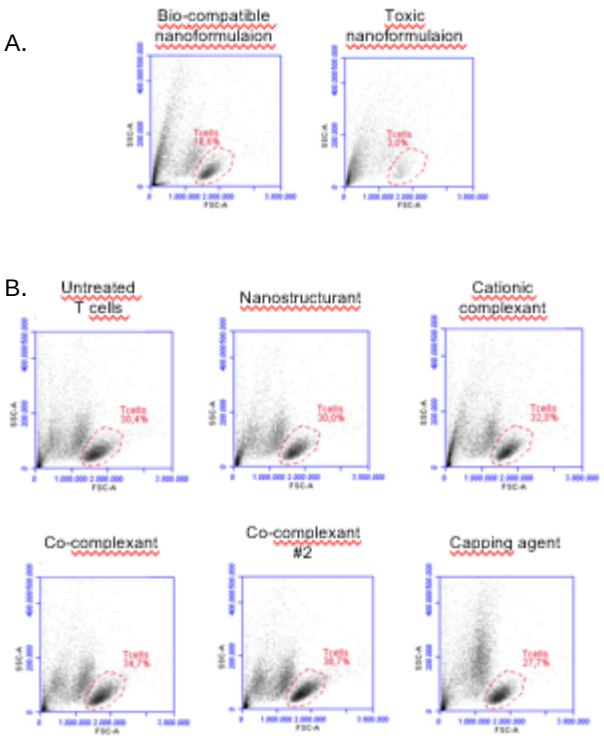

Figure 1. Primary T cells treatment with (A) two different polymeric nanostructured complexes and (B) complexes' single component.

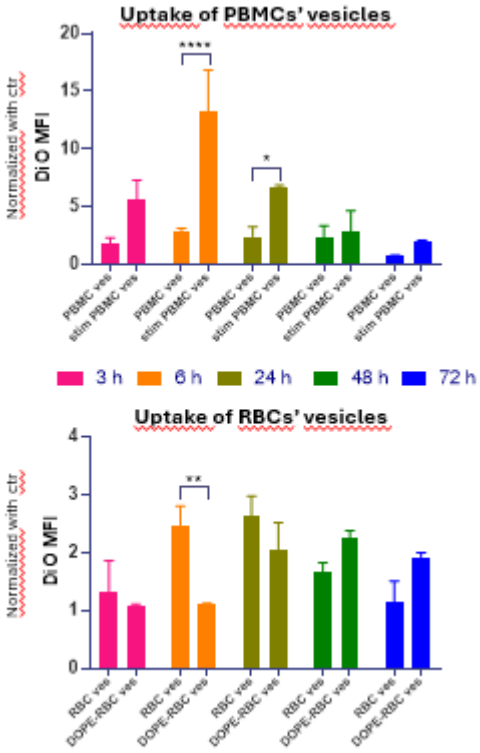

Figure 2. Uptake experiment to detect the targeting of PBMCs' vehicles (stimulated and not) and RBCs' vehicles (functionalized and not) at different time points.