ImmunoTools *special* Award 2014

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'Lentiviral vectors encoding IL-12 for antitumor immunotherapy: to target or not to target'

Introduction

In antitumor immunotherapy, antigen-presenting cells (APCs), play a key role as they are able to turn T lymphocytes into tumor-associated antigen (TAA) specific cytotoxic T cells (CTLs). To ensure proper antitumor immunity, it is detrimental to correctly activate these APCs as otherwise a tolerogenic status could be induced. Therefore, co-delivering the TAA(s) together with one or more maturating signals is recommended. A straightforward way to achieve this, is by using a lentiviral vector (LV) encoding the TAA(s) of choice like Tyrosinase related protein 2 (Trp2). While the TAA is delivered, the LV activates the APC as the vector is intrinsically immunogenic and consequently induces the correct $CD4^+$ T_H1 polarizing environment to stimulate potent $CD8^+$ CTLs.

Although numerous antitumor vaccination strategies in mice resulted in very promising date, their potential for cancer patients is less obvious. Although TAA-specific CTLs can be detected after therapeutic delivery of TAAs with or without maturating signals, the overall improved survival of the patients is low. These observations result in at least two highlights in antitumor immunotherapy research. First, it is important to use a more humanized setting as the immune system of mice and men shows a lot of evolutionary differences which hamper the translation of mouse data to clinical trials. Secondly, antitumor immunotherapy in human beings needs to be optimized since the therapeutic vaccinations of today don't give the envisaged results. An interesting candidate for the latter, is the proinflammatory cytokine IL-12. It was shown in several comparative studies that murine IL-12 is one of the most effective cytokines to eradicate experimental tumors, to prevent metastases and induce long-term antitumor immunity in mice. Importantly, it has also been demonstrated that the best results were obtained if IL-12 was delivered together with the TAA into APCs.

Therefore we propose to evaluate the immune response in humanized mice after they are injected with a broad tropism or APC-targeted LV encoding the TAA Trp2 together with or without hu IL-12.

Project set up

Four weeks old NOD.Rag1$^{-/-}$.IL-2rc$^{-/-}$ (NRG) mice will be reconstituted with human cord blood derived CD34^{+} hematopoietic stem cells. Four weeks later, a cocktail of recombinant human (rh) cytokines (500µg/kg) will be administered every three days for two weeks. These include **IL-3, GM-CSF, FLT3-L, IL-7** and **IL-15**. Another two weeks later, the reconstituted NRG mice will be immunized with PBS, $10^{E}8$ TUs of a broad tropism LV or APC-targeted LV encoding eGFP and Trp2 or IL-12 and Trp2, and this two times intravenously with a seven-day interval. Five days after the second immunization, spleens will be isolated and processed to single cell suspensions. Next the hu CD4 or CD8^{+} T cells will be enriched and subsequently pretreated for two days with **rh IL-2** while the hu CD4 or CD8^{-} fraction, containing APCs, will be loaded with or without Trp2 peptide (immunodominant MHC class I or II epitope for CD8 and CD4^{+} T cell restimulation respectively). Two days later, the peptide loaded hu CD4 or CD8^{-} fraction will be used to restimulate the preactivated hu CD4 or CD8^{+} fraction at a 1:10 ratio for another five days. Finally, cells and supernatants will be evaluated. For flow cytometric analysis, the cells will be washed and stained with **hu CD4 or CD8 APC** respectively, **hu CD3 FITC**, **hu CD45 PerCP** and **hu IFNγ or hu IL-8 PE**. Furthermore supernatants will be screened for their presence of hu IFNγ, **IL-4, IL-6, IL-8**, **TNFα** and **IL-12p40** using the available **ELISA kits**.

These experiments will give us a first clue if: (1) hu IL-12 is able to increase the immune stimulatory potential of the LV-based vaccine in a humanized mouse model and (2) if hu IL-12 needs to be delivered exclusively to APCs or also to non-APCs in order to obtain a good immune response.