

## **Modulation of transgene immune response is a requirement for successful gene therapy trials.**

Annoni A., Battaglia M., Gregori S., Naldini L., and Roncarolo M.G.

San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), Via Olgettina 58, 20132, MILANO

Gene therapy is a promising approach for the treatment of several genetic diseases. Efforts to develop clinical gene transfer protocols have been hampered by immune response to the transgene products, which leads to the clearance of transduced cells. To limit immune responses to transgenes, a number of strategies have been employed, among them: administration of immunosuppressive drugs, different routes of vector administration, tissue-specific promoters, and modifications of vector design. Alternatively, the induction of transgene-specific tolerance represents a novel approach for the prevention of immune response against genetically modified cells.

We developed an experimental mouse model to monitor the green fluorescence protein (GFP)-specific immune response following systemic administration of Lentiviral (LV) vector encoding GFP. In immunocompetent C57Bl/6 mice systemically injected with LV expressing GFP under the ubiquitous CMV promoter (LV-GFP), GFP expression was detected in the liver, bone marrow and spleen with a peak of expression at the second week after injection and decrease thereafter. A significant CD8<sup>+</sup> T cells infiltration was observed in the liver and spleen of injected mice. GFP-dependent IFN- $\gamma$  production by splenic CD8<sup>+</sup> T cells and anti-GFP antibodies in LV-GFP injected mice were observed starting two weeks after injection and gradually decreased over time. Overall these data indicate that immunocompetent mice injected with LV-GFP develop both cellular and humoral anti-GFP immune response that leads to clearance of the transgene expressing cells. Interestingly, when transgene expression was limited to liver cells using a tissue-specific promoter (ALB), a reduced immune response with the consequent long-term transgene expression was observed. Therefore, targeting transgene expression to specific tissues may facilitate long-term transgene expression. Alternatively, transgene-specific tolerance can be achieved by cellular therapy with tolerogenic cells. Both regulatory T cells and antigen presenting cells (APCs) can be used.

CD4<sup>+</sup>CD25<sup>+</sup> T regulatory (Tr) cells play a key role in peripheral tolerance. Among the different regulatory T cell subsets identified, naturally occurring CD4<sup>+</sup>CD25<sup>+</sup> Tr cells have been extensively characterized in pre-clinical and clinical models of T-cell mediated diseases. In the GFP model we investigated whether CD4<sup>+</sup>CD25<sup>+</sup> Tr cells could prevent GFP clearance *in vivo*. C57Bl/6 mice were co-injected with LV-GFP and CD4<sup>+</sup>CD25<sup>+</sup> Tr cells isolated from syngeneic wt mice (wt CD4<sup>+</sup>CD25<sup>+</sup> Tr). Clearance of GFP expressing cells and anti-GFP immune response occurred both in LV-GFP and LV-GFP + wt CD4<sup>+</sup>CD25<sup>+</sup> Tr cells

co-injected mice, suggesting that injection of wt CD4<sup>+</sup>CD25<sup>+</sup> Tr cells did not protect from the immune response to the transgene.

The Ag-specificity requirement of CD4<sup>+</sup>CD25<sup>+</sup> Tr cells in modulating the anti-GFP immune response was then investigated. C57Bl/6 mice were co-injected with LV-GFP and highly purified GFP Tg CD4<sup>+</sup>CD25<sup>+</sup> Tr (99,1%). Surprisingly, transfer of highly purified CD4<sup>+</sup>CD25<sup>+</sup> GFP Tg Tr cells did not modulate the immune response to GFP *in vivo*. This demonstrates that neither increasing the endogenous pool of CD4<sup>+</sup>CD25<sup>+</sup> Tr cells nor transferring CD4<sup>+</sup>CD25<sup>+</sup> Tr cells selected in a GFP<sup>+</sup> environment can induce sustained transgene expression.

On the contrary, the co-administration of LV-GFP and highly purified GFP-Tg APCs significantly modulated the anti-GFP immune response to the transgene, leading to stable GFP expression *in vivo*. GFP-tg APCs displayed a significant low level of activation markers compared to GFP<sup>+</sup>APCs generated after *in vivo* LV-GFP transduction.

These results indicate that the mode of transgene presentation by APCs is crucial to determine which kind of response (regulatory vs effector) can be induced. We can hypothesize that CD4<sup>+</sup>CD25<sup>+</sup> Tr cells, when activated in the appropriate way (i.e. absence of inflammatory signals) can play a pivotal role in the modulation of the immune response to the gene therapy derived product.

Overall these studies suggest that cellular therapy with tolerogenic cells can be a successful approach to modulate immune response to transgenes.