521. AAV Immunotherapy Induces Functional Antigen Specific Regulatory T-Cells to a Neuroantigen: A Potential Treatment for MS

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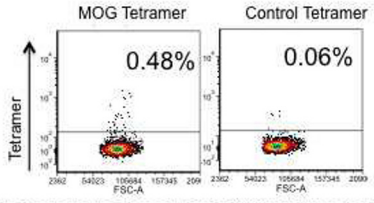
Immunotherapy for autoimmune disease aims at restoring a state of tolerance to the self-antigens that propagate disease. Multiple sclerosis (MS) is an immune-mediated disease of the CNS in which leads to demyelination and damage of axons. It is believed that a failure of central and peripheral mechanisms to maintain self-tolerance to auto-aggressive myelin-specific T cells plays a central role in the pathogenesis. It has been demonstrated that antigen (Ag)-specific regulatory T cells (Tregs) have a significant role in modulating autoimmune CNS disease and when used therapeutically, can be highly effective at treating MS. Thus, using AAV liver gene therapy to restore normal Treg function and re-establish immune tolerance to myelin proteins in early onset MS represents a novel treatment strategy for stabilization of disease progression.

The development of protocols that directly affect the expansion Ag-specific Treg numbers and/or enhances their function has become a significant focus in the research community. Unfortunately, successful therapeutic use of Tregs has been limited by the lack of safe and effective protocols for isolation and expansion that are suitable for translation. However, hepatic gene transfer with AAV vectors containing liver specific promoters has been shown to produce stable transgene expression and induce a robust antigen-specific immune tolerance to a variety of therapeutic proteins.

In a murine model for MS, we have previously demonstrated that liver directed AAV gene therapy represents a novel approach to not only prevent experimental autoimmune encephalomyelitis (EAE) disease, but more importantly, can also abrogate disease progression. The work presented here provides evidence that liver directed AAV gene therapy using the neuroantigen MOG does indeed produce functional Ag-specific Tregs. In a series of experiments, $B6^{FoxP3\text{-}GFP}$ mice were injected with AAV8-MOG via the tail vein. After 8 weeks, splenocytes were harvested and analyzed using I-Ab $MOG_{35\text{-}55}$ (MHC II) or control tetramers (provided by NIH Tetramer Core). The results revealed that mice receiving AAV8-MOG did indeed produce Ag-specific Tregs (Fig.1). Functionality was further determined using an in vitro Treg suppression assay. Briefly, $CD4^+CD25^+$ Tregs were magnetically sorted and co-cultured with fluorescently labeled MOG-specific TCR^+CD4^+ cells at varying concentrations and in the presence of $MOG_{35\text{-}55}$ antigen. FACS analysis confirmed that the Mice receiving AAV8-MOG did indeed produce Ag-specific Tregs and that they were functionally suppressive (Fig.2).

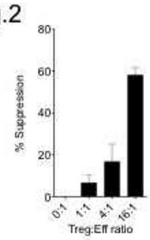
The work presented here shows that liver directed gene transfer using an AAV vector expressing a neuro-antigen is capable of inducing Ag-specific Tregs that are able to suppress Ag-specific effector CD4 cells. More importantly, this work continues to advance the therapeutic use of AAV for the treatment of auto-immune diseases such as MS.

Fig.1.



Tetramer expression on $FoxP3^{GFP+}CD4^+$ gated population

Fig.2



522. Targeting FVIII-Expression To Liver Sinusoidal Cells By Lentiviral Vectors Corrects the Bleeding Phenotype in Hemophilia A Overcoming Immunological Responses

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Hemophilia A (HA) is an X-linked bleeding disorder due to mutations in clotting factor (F) VIII gene. To date the treatment for preventing major bleeding episodes is represented by replacement therapy with recombinant or plasma-derived FVIII. The two major concerns are high cost and development of FVIII neutralizing antibodies in 20-30% of patients.

Several studies on gene transfer by direct injection of LV for HA have been recently published. Many efforts were focused on the improvement of LV, to obtain a selective targeting of transgene expression, or on the production of several bioengineered FVIII, in order to overcome some of the issues related to FVIII expression in HA animal models. However, in most cases, the immune responses associated with FVIII remain the major obstacle.

We prepared LVs containing the B-domain deleted (BDD) hFVIII under the control of PGK, VEC or CD11b promoters with or without the addition of the miRTs used for initial GFP expression studies, and we then injected HA mice with 109 TU/mouse of these LVs (3 mice for LV PGK-hFVIII ±142; 4-9 mice for the other vectors) and assessed FVIII activity by aPTT assay.

All mice injected with LV-VEC-hFVIII ± miRTs and LV-CD11b-hFVIII ± miRTs showed a FVIII activity between 3.5 and 5% one week after injection, while HA mice injected with LV-PGK-hFVIII±142 showed a FVIII activity £1%. Moreover, starting from 2 weeks after LVs injection we evaluated the presence of anti-FVIII antibodies by a direct ELISA. We detected the presence of anti-FVIII antibodies in the plasma of mice injected with LV-PGK-hFVIII±miRT-142 1 month after LV injection. Interestingly, the antibody titer was significantly lower in mice injected with LV-PGK-hFVIII-miRT-142-3p. In all mice injected with LV-VEC-hFVIII±miRT-122-142-3pwe detected hFVIII activity by aPTT assay up to 52 weeks after injection without production of anti-FVIII antibodies. HA mice injected LV-CD11b-hFVIII±miRT-126 showed hFVIII activity up to 52 w as well; interestingly, 60% of mice injected with LV-CD11b-hFVIII produced anti-FVIII antibodies 10-16 weeks after LV injection, while no anti-FVIII antibodies were detected in plasma of injected mice with LV-CD11b-hFVIII-miRT-126.

Genomic analysis on liver samples from mice 24 w after injection of LV-VEC-hFVIII±miRT-122-142-3p and LV-CD11b-hFVIII±miRT-126 demonstrated the presence of LV sequence integrated in the genome of injected mice. Immunofluorescence on liver sections showed that LSECs and KCs were positive for hFVIII. Next, to assess whether EC, in particular LSECs, are able to induce immunotolerance, we immunized mice with Refacto. Mice producing anti-FVIII Ab were then injected with 109 TU of LV-VEC-hFVIII-miRT-122-142-3p. We detected hFVIII activity in all injected mice and, noteworthy, antibody titer decreased over time in the plasma of these mice.

In conclusion, LV expressing FVIII under the control of VEC or CD11b promoters combined with miRTs combinations were able to overcome FVIII off-target expression limiting immune responses and providing phenotypic correction in treated HA mice with FVIII expression by sinusoidal cells.