

● PERSPECTIVE

Soluble interleukin 23 receptor gene therapy with adeno-associated vectors for the treatment of multiple sclerosis

Gene therapy as strategy against autoimmune diseases: In the last century, human societies have witnessed remarkable improvements in wellness and life expectancy, thanks to the consolidation of modern science and medicine. But, while some infectious and other diseases, have been almost fully controlled, pathologies associated with intrinsic factors and longevity have increased in incidence. Cancer, degenerative and autoimmune diseases are the major current challenges. For example, net increase per year incidence and prevalence of autoimmune diseases worldwide during last 30 years has been estimated to be 19.1% and 12.5% respectively (Lerner et al., 2005).

Autoimmune diseases are characterized by the immune system attacking self. In other words, there is a breakdown of the complex equilibrium whereby the immune system fights against harmful intrusions, while minimizing damage to the organism's own structures. Usually this is initiated by a dysfunction in the adaptive immune system, leading to a hyperactivation of the innate response, thus producing adverse inflammation and tissue damage. In multiple sclerosis (MS), the inflammation affects the central nervous system (CNS) and axonal myelin sheath. Since the triggering stimuli, autoantigens, are endogenous, this self-destructive reaction cannot be easily stopped. Classic therapeutic strategies target the source of damage but, when this source is our own immune system, the situation is complex. For instance, anti-inflammatory compounds can mitigate part of the damage but when autoimmunity leads to chronic inflammation their efficiency is readily lost. Immunosuppressants may be effective but leave the organism defenseless against other pathologies and are not always well tolerated.

In this context, use of state-of-the-art strategies like antibody administration has proven effective in animal models for MS and other autoimmune diseases. Some have been approved for clinical use. One of the better known therapeutic antibodies used in MS is Natalizumab, targeted against alpha 4-integrin. Despite its undesirable effects associated with general immune downregulation, Natalizumab has shown better efficiency than most other approved drugs for MS. However, recent studies demonstrate a loss of efficiency associated with the generation of neutralizing antibodies (Vennegoor et al., 2013).

The possibility of such an acquired immune response is a key consideration when advanced therapies are designed. Monoclonal antibody therapy against interleukin (IL)-23/IL-12 p40, Ustekinumab, also lost efficacy in MS (Toussiot et al., 2013). To avoid this effect, recombinant human proteins should be chosen for which an immune response is minimal or absent. An example of such a protein is interferon-beta 1-alpha, a recombinant cytokine. It was the first treatment to be approved to modify the clinical course of MS and it has been used for decades without loss of efficiency. The main problem with this kind of treatments is the need for regular injections lifelong, which could provoke skin reactions, lipoatrophy and general unease in the patient.

Gene therapy allows producing the therapeutic molecule by the patient's own cells in such way that only one or few administrations may be sufficient to maintain a continued therapeutic effect. A good genetic engineering design should avoid an immune reaction as well. In this regard, the use of viral vectors such as adeno-associated vectors (AAVs) have led to great advances in gene therapy with an excellent safety profile (Vandamme et al., 2017).

Research in autoimmunity has shown the relevance of the balance between specific lymphocytic activation and immune response regulation. Following this rationale, two main approaches can be used for gene therapy: promotion of regulatory and immunomodulatory

pathways; and downregulation of pro-inflammatory factors. Th17 lymphocyte differentiation pathway sustained by IL-23 is a key target to combine these approaches. Gene therapy using soluble cytokine receptors provides an innovative and promising solution.

Key role of IL-23 pathway during Th17 mediated autoimmunity in multiple sclerosis: Different reports have shown the applicability of gene therapy in the experimental autoimmune encephalomyelitis (EAE) model of MS. Initial studies were based on interfering RNA targeting pro-inflammatory cytokines (Shu et al., 2015). This methodology could be effective but lacks easy applicability in clinical trials. More recent strategies are focused on the activation of Th2 or Treg responses (Shu et al., 2015), which could then deactivate the aggressive autoimmune response but may also lead to a general deregulation of the normal immune response. In comparison, inhibition of a particular stimulatory signal through truncated or modified soluble receptors has shown a good safety profile, effectiveness and specificity. For instance, modifying fibroblasts by retroviral *ex vivo* gene transfection with the IL1Ra gene - the "receptor antagonist" that sequesters IL-1 signaling has proven to be a successful therapeutic strategy for rheumatoid arthritis (Wehling et al., 2009) (Figure 1).

During MS neuropathology, antigen stimulated dendritic cells begin to secrete lymphocyte activator signals such as IL-23. IL-23, an interleukin member of the IL-12 family interacts with its membrane receptor to activate JAK kinases (JAK2 and TYK2) and then signal transducer and activator of transcription (STAT)3 and STAT4, whose action together with retinoid-related orphan receptor gamma t (ROR γ t) transcription factor leads to the differentiation and activation of the Th17 pathway. Th17 cells promote inflammatory effects through proinflammatory cytokines such as IL-17, tumor necrosis factor-alpha (TNF- α), IL-6, IL-21 and IL-22. Therefore, IL-23, as a molecule involved in the stabilization and amplification of the Th17 phenotype and induction of the inflammatory response, is considered an interesting therapeutic target to treat MS (Toussiot, 2012).

In order to find specific therapeutic alternatives for the regulation of the Th17 pathway in MS, we blocked the interaction of IL-23 with its transmembrane receptor by intravenous administration of AAV8 encoding the soluble sequence of IL-23 receptor (IL-23R). The soluble IL-23R (sIL23R) isoform has been described as arising from an alternative splicing to the canonical protein in humans, but the existence of a murine isoform was unknown. We therefore designed murine sIL23R, mimicking the human sIL23R isoform. This was cloned into the AAV8 genome and administered in EAE animals for later functional and therapeutic characterization.

In vitro experiments showed first, that the soluble receptor was distributed evenly in the cytoplasm, whereas the transmembrane receptor, used as positive control, was located in the cell membrane. Secondly, sIL23R was functional since coinubation with IL-23 resulted in inhibition of STAT3 phosphorylation (Miralles et al., 2017). To assess the therapeutic efficacy of the receptor, EAE mice were injected intravenously with a 5×10^{11} vector genomes/mouse. Animals treated with AAV8/sIL23R showed a significant clinical improvement compared to mice treated with the null vector. This improvement was maintained stably until the end of the experiment. In addition, animals treated with the null vector were found to have a greater tendency to weight loss than animals treated with sIL23R. No adverse effects were observed in any of the EAE-animals, nor in non-EAE animals overexpressing sIL23R. Subsequent immunological analysis in sIL23R treated animals showed a significant decrease in Interferon gamma (IFN γ) levels accompanied by an increase in the production of granulocyte macrophage colony-stimulating factor. Histopathological CNS analysis showed no demyelination as well as reduced inflammatory infiltrates, at day 14 post-immunization (Miralles et al., 2017).

Future perspectives for vector mediated treatments for multiple sclerosis: More preclinical studies need to be performed in order to better understand the effects of the sIL-23R treatment and confirm the good safety profile reported. For human treatment, use of AAVs is highly recommended as they avoid insertional mutagenesis while

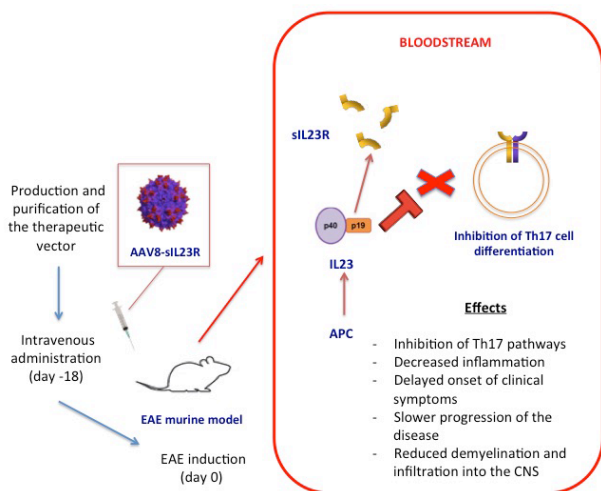


Figure 1 Diagram of the therapeutic strategy and the cellular and clinical effects observed.

EAE: experimental autoimmune encephalomyelitis; IL-23: interleukin 23; AAV: adeno-associated vector; siL23R: soluble IL-23 receptor; APC: antigen presenting cell; CNS: central nervous system.

providing long-term expression in non- or slowly-dividing cells without the risks of wild type virus infectious. Similarly, AAV genome size limitation should not be highly problematic due to the small size of interleukin soluble receptors. On the other hand, potential toxic effects associated with uncontrolled transgene expression can be avoided by using inducible promoters, while pre-existing neutralizing antibodies in the host, or potential CD8⁺ T cell responses induced by recombinant AAV vectors have not affected the success of clinical trials performed. Nevertheless, more studies in recombinant and wild type AAV related antigenicity and toxicity are highly needed since there is an important lack of consensual information (Vandamme et al., 2017). The AAV8 used in EAE mice showed high infectivity and expression of the siL-23R in the liver, which is ideal for a systemic outcome (Miralles et al., 2017). Nevertheless, a significant modification would be required for human use in recurrent diseases like remittent-recurrent MS. Namely a switch-off/switch-on system is required to silence gene expression when it is not necessary, permitting immune pathways to remain unaltered in the absence of autoimmune activation. Although new advances are being made in regulated gene activation, from Tet-on/off-like systems to optogenetics (Kolar and Weber, 2017), an inducible system associated directly with the clinical course of the disease itself would be highly attractive.

Soluble receptors used to downregulate IL-23 mediated Th17 activation pathways demonstrate high therapeutic performance in an elegant way that implies neither a counterproductive immunosuppression nor an antigenic rejection response. Furthermore, using synergic approaches in combination with classical medication and other advanced therapies could probably enhance efficiency. Thus, immunogenic inflammatory activation could be further downregulated by using other complementary cytokine soluble receptors in Th17 or Th1 signalling. Also, synergistic effects may be achieved by combining with direct anti-inflammatory mediators to stop the sustained activation of the autoimmunity as well as inhibition of already ongoing autoimmune damage, or by specific stimulation of Th2 or Treg cells through IL-2, IL-10, IL-37, transforming growth factor beta (TGF- β) or forkhead box P3 (FOXP3) pathways (Danikowski et al., 2017). However, the degree of immunosuppression generated in these strategies must be evaluated. Finally, taking into account that MS is a neuroimmune disease, a complementary approach to

soluble-receptors-based gene therapy would be to use neuronal enhancing factors with good profile in preclinical studies, such as Klotho (Chen et al., 2013; Massó et al., 2015). This should promote neuroprotection and neuroregeneration, thereby treating both the attacked and the aggressor systems

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