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Scope & Outline

Breakdown of natural mechanisms of immune tolerance towards "self" antigens can result in autoimmune or allergic disease development. There is considerable experimental evidence that the response to antigen which might include full immunity or immune tolerance induction is dependent on the activation status of the antigen presenting dendritic cells. Current therapeutic options deplete immune cell populations, interfere with immune cell trafficking to the tissues, or inhibit inflammatory cytokine function and lymphocyte signaling. Although these treatments can be effective for certain diseases, they can also cause significant adverse effects and it is unlikely that they could induce stable, long-term immune tolerance. Cellular therapies using either tolerogenic dendritic cells or regulatory T cells may be able to achieve it. This thesis is focusing on cell-mediated induction of immune tolerance and consists of two parts. The studies described in **Part I** report the development of strategies for possible treatment of Inflammatory Bowel Diseases (IBD). Induction of immune tolerance, in IBD mouse model, with the use of regulatory T (Treg) cells generated *in vitro* by specific activation of naive T cells was achieved. Additionally Treg cells were also shown to be induced *in vivo* and restore intestinal immune tolerance with the use of adeno-associated virus (AAV) vector-based gene delivery. In relation to the use of AAV vector, **Part II** of this thesis is addressing the possibilities of tolerance induction to AAV capsid or transgene specific immune responses which can develop after AAV -based therapy.

Part I

Development of new treatment strategies for Inflammatory Bowel Diseases, which is a group of diseases considered to be autioimmune, is of great interest as currently there is no curative treatment. Cell and gene therapy approaches have been recently studied in relation to the inhibition of inflammation in the gastrointestinal tract. Treg cells have the ability to suppress immune responses and to sustain systemic immune balance. Therefore Treg cells have the potential to prevent inflammatory disorders by induction of immune tolerance. The major limitation to the use of natural Treg (nTreg) cells is their low availability

and unstable phenotype profile upon *ex vivo* expansion. Hence, use of *in vitro* generated induced Treg (iTreg) cells represents a good alternative. Currently, there are several techniques available to generate induced T regulatory (iTreg) cells, nevertheless all of them are to some extend impended with their own limitations. Therefore, establishing new, improved methods of iTreg generation is of great interest. The goal of the experiments presented in **Chapter 2** was to develop a new, straightforward method to generate *in vitro* functional and stable iTreg cells from CD4+CD25- human cells. Generated iTreg cells (TregPMA) proved to be functional *in vitro* in a mixed lymphocyte reactions (MLR's) as they suppressed proliferation of responder cells in a dose dependent manner. The protocol to generate TregPMA *in vitro* was also applied to murine cells. It has been described in **Chapter 3**. Functionality of the generated murine iTreg was demonstrated by amelioration of experimental colitis *in vivo* in a mouse model of IBD.

Also gene and cell therapy approaches have been recently studied in order to induce regulatory T (Treg) cells that would be able to inhibit inflammation in different tissues. In **Chapter 4** a gene delivery approach to promote Treg cells *in vivo* was explored. The delivery of regulatory T-cell epitope 167 (Tregitope 167) by adeno-associated virus (AAV) vector proved to induce Treg cells *in vivo* and ameliorated the experimental colitis. This study identifies AAV-based Tregitope 167 delivery as a new anti-inflammatory approach for induction of immune tolerance by Treg cells and in consequence possible treatment of autoimmune and inflammatory disorders on an example of IBD model.

Part II

The main obstacle in AAV-based gene delivery is the humoral immune response against AAV vector capsid that appears after primary AAV delivery. The neutralizing antibody (NAB) level that rises against AAV capsid, inhibit the AAV vector transduction upon re-administration of the AAV vector of the same serotype. In **Chapter 6** we demonstrate in a murine model that cross administration of AAV serotypes 5 and 1 can be an alternative for re-administration due to the lack of cross-reactivity of the NAB. Additionally, in **Chapter 7**, we explore different immune suppressive regimens for their ca-

pacity to decrease circulating anti-AAV NAB level that rises after primary AAV vector gene delivery. The aim of this study was to define the immune suppression strategy and time frame in which the decrease of the anti-AAV NAB level would allow the AAV re-administration.

Another concern in AAV vector-based gene therapy is the potential development of immune responses against the transgene product which might lead to loss of expression of the therapeutic transgene. Therefore, strategies to induce tolerance towards the transgene product are needed. In **Chapter 8**, we demonstrate the feasibility to use mir-142-3p target sequences to prevent immune responses against the transgene product after intramuscular AAV vector delivery.