ImmunoTools *special* Award 2018

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Targeted tolerance in multiple sclerosis: Development of transgenic T cell receptor-engineered regulatory T cells recognizing myelin basic protein

Background: Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS). This demyelinating neurodegenerative disorder is considered to be a T cell-mediated autoimmune disease, in which autoreactive T cells specific for myelin-derived antigens cause inflammation and tissue damage in the CNS. Nevertheless, the disease pathogenesis is complex with both genetic and environmental factors contributing to the autoimmune reaction. For instance, DRB1*15:01 is defined as a genetic risk factor for MS, not only in the Caucasian population but also in Japanese and Middle Eastern populations[1]. The therapeutic landscape of MS is constantly evolving, and one could pose the question if we still have unmet needs for the treatment of MS? Regardless the availability of improved therapies and the significant advances in the understanding of what triggers disease, patients continue to experience relapses and, in some cases, are exposed to potential life-threatening side-effects. Hence, current challenge is to balance the need to modify the underlying disease pathogenesis and the long-term risks. In this perspective, immune-modulatory cell therapy has brought a new hope for a wide spectrum of diseases as it is one of the most promising future clinical options in the medical arsenal for the treatment of patients suffering from serious conditions where unmet medical needs exist. This strategy has demonstrated to be safe and effective in numerous clinical trials[2,3]. With this in mind, the essential role of regulatory T cells (Tregs) in preventing autoimmunity and controlling responses to self-antigens is well established. Multiple Treg-based cell therapy approaches are now being tested in the clinic, and the results of the early phase I clinical trials indicate that Treg therapy is well tolerated[4-8]. Yet, infusions of polyclonal Tregs have poor specificity, thereby causing a transient risk of generalized immunosuppression[9]. However, considering the low precursor frequency of Tregs, identifying, isolating, and expanding antigen-specific cells for immunotherapy is technically challenging. Tregs exert their immunosuppressive functions (i) via secretion of inhibitory cytokines, (ii) by interfering with the metabolism of T cells, and/or (iii) in an undetermined contact-dependent manner. Furthermore, (iv) Tregs block T cell activation indirectly via their interaction with antigen-presenting cells (APCs), such as dendritic cells (DCs), preventing the upregulation of the expression of co-stimulatory molecules and the secretion of pro-

inflammatory cytokines. Tregs offer the opportunity to target cells that are potentially involved in the disease progress. Whether Tregs act in an antigen-specific manner remains elusive. Hence, despite the potential that Treg therapy holds, there are still some challenges, not in the least to direct the interaction of Tregs with key disease-associated immune cells in an antigen-specific manner.

Research hypothesis and objectives: Since we[10] and others[11,12] have demonstrated that DCs of patients with multiple sclerosis (MS) reside in a "hyper"-activated state, as evidenced by a higher expression of the co-stimulatory molecule CD86 and a higher induction of IL-12 following TLR ligation, we aim here to develop "designer" Tregs expressing a transgenic T cell receptor (TCR) recognizing myelin basic protein (MBP). In doing so, we anticipate enforcing the interaction of Tregs with DCs expressing the MBP antigen in a major histocompatibility complex (MHC)-dependent manner, regulating ongoing immune reactions and ultimately controlling autoimmunity. The following objectives have been set forth in current project proposal: Our first objective is to select antigen-specific effector T cells by means of tetramer analysis, thereby identifying and cloning a myelin-recognizing TCR. Secondly, we will optimize a clinically safe mRNA electroporation protocol to induce expression of mRNA encoding the TCR in freshly-isolated and expanded Tregs from MS patients. Thirdly, we ensure the stability of the phenotype and suppressive function of TCR-engineered Tregs. In doing so, we will deliver in vitro proof-of-concept of the safety of the approach which is especially important when administering the cells in an inflammatory disease-driven microenvironment. Finally, we will investigate if TCR-transgenic Tregs can modulate ongoing disease processes by investigating their effect on the phenotype and function of DCs from healthy volunteers and MS patients. Ultimately, we envisage that this will foster a durable clinical application of this technology without the risk for general immunosuppression.

Methods implementing ImmunoTools reagents.

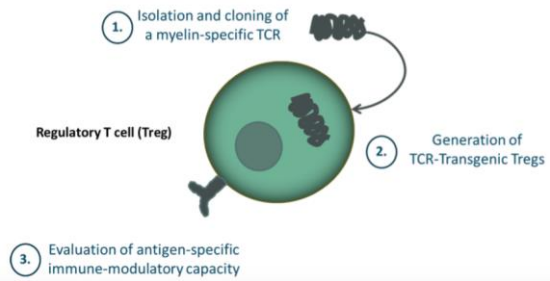

Figure 1: Development of "designer" Treg enforces the interaction with DC in a MHC-dependent manner. T cells recognize peptide fragments of an antigen by means of the TCR. These fragments of antigen are processed by APC before they are presented in association with MHC molecules to the T cell. Altogether, these data have prompted us to use DRB1*15:01/MBP_{83-101} tetramers to isolate autoreactive T cells. MBP_{83-101}-eactive $CD4^+$ T cells will be selected using DRB1*15:01/MBP_{83-101} tetramers by means of FACS and subsequently used for PCR-based cloning of the TCRαβ-genes.

Peripheral blood mononuclear cells (PBMC) will be isolated from DRB1*15:01-positive patients diagnosed with MS according to the revised McDonald criteria[13] using density gradient centrifugation, and subsequently stimulated in vitro in the presence of the MBP83-101 peptide. After 1 week of co-culture, stimulated lymphocytes will be harvested and stained with tetramers in combination with antibodies to CD3 and CD4 and isolated by means of FACS. Sorted T cells will be expanded using irradiated MBP83-101-pulsed DRB1*15:01+ Priess B cells. Among the clones obtained, we will randomly choose clones to examine their antigen reactivity by IFN-γ secretion by means of ELISA. Subsequently, these autoreactive cells for MBP will be used for PCR-based cloning of the TCRαβ genes. The TCRαβ genes will be codon-optimized, subcloned into an in vitro transcription vector and in vitro validated following mRNA electroporation of MBP83-101 TCR constructs into a TCRαβ-deficient CD4+ Jurkat cell line (figure 1 step 1).

$CD25^{high}FOXP3^{+}CD45RA^{+}$ Tregs will be isolated and expanded as previously described by the group of Trzonkowski et al.[14]. Briefly, PBMC will be isolated by density gradient centrifugation, and stained with the following fluorochrome-labeled antibodies: anti-CD4, anti-CD25, anti-CD127, anti-CD45RA and anti-CD3. Cells will be sorted. After sorting, the cells are expanded in vitro using beads coated with anti-CD3 and anti-CD28-specific antibodies and recombinant human IL-2. This results in a consistent expansion greater than 600-fold of the Treg population[15].
Subsequently, transfection of the $CD25^{high}CD45RA^{+}$ Tregs is performed with the mRNA encoding the autoreactive TCR to end up with transgenic TCR-engineered regulatory T cells recognizing myelin basic protein (figure 1 step 2).

Finally, to assess the evaluation of the transgenic Treg phenotype, monocyte-derived dendritic cells (mo-DC) from DRB1*15:01-positive healthy volunteers will be generated in the presence of GM-CSF and IL-4. On day 6, mo-DC will be stimulated with pro-inflammatory molecules and pulsed with the MBP83-101 peptide in the presence or absence of TCR-transduced Tregs. After 24-48 hours, DC will be harvested for flow cytometric phenotype assessment using mAbs against HLA-DR, CD40, CD80, and CD86. Simultaneously, supernatants will be collected for analysis of the cytokine profile by means of ELISA (IL-12p40 and TNF-alpha). As a control, mo-DC pulsed with an irrelevant peptide will be used. Ultimately, a similar procedure will be followed for circulating DC isolated from DRB1*15:01-positive MS patients. Expression of activation markers on DCs is assessed before and after co-culture with TCR-transduced Tregs to confirm an antigen-specific modulatory effect of TCR-transduced Tregs. As a control, conventional DC isolated from DRB1*15:01-negative MS patients will be used (figure 1 step 3).

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