Immune modulation and monitoring of cell therapy in inflammatory disorders
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6

General discussion

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Autoimmune and inflammatory disorders are caused by a disturbed and disbalanced immune system. Vital cells and tissues of the body are destroyed as consequence of excessive inflammation. In type 1 diabetes (T1D), insulin producing β-cells in the pancreas are destroyed by cytotoxic T-cells, which leads to the disruption of blood glucose homeostasis and a lifelong dependence on exogeneous insulin treatment. In **Chapter 2** and **Chapter 3**, we explored the immunopathology of T1D by investigating the human pancreas during the pre-diabetic phase and circulating autoreactive CD8 T-cells in patients with established T1D. Induced regulatory T-cells *in vitro* as mode of action of tolerogenic dendritic cell (tolDC) therapy were studied in **Chapter 4**, where we identified surrogate immune correlates of therapeutic efficacy. Lastly, we showed the *in vivo* immunological effects of tolDC and mesenchymal stromal cells as cellular immune intervention therapy in **Chapter 5**.

Insights into type 1 diabetes progression

Throughout the years, the pathological processes underlying T1D are becoming increasingly clear. It is now well established that T-cells are key in the progression to T1D; CD4 T-cells are essential to initiate autoimmune T1D, while cytotoxic CD8 T-cells are the pathological mediators that kill pancreatic β-cells (1-3). In addition, a range of T1D associated epitopes that can be targeted by autoreactive CD4 and CD8 T-cells have been discovered (4-7). The number of different circulating islet autoantibodies can reliably predict the risk for developing T1D, which is essential for early intervention therapies (8). β-cell function is rapidly declining around time of diagnosis, but interestingly, residual β-cells can still be detected even in patients with long standing disease (14, 15). These discoveries give considerably hope to find novel intervention strategies even in later disease stages. However, no cure for T1D has been found with long lasting effect. Growing evidence on inflammation and ER stress point towards an important role of β-cell dysfunction in the initiation and progression

of T1D (9-13). Still, the exact trigger that initiates autoimmunity in T1D is an area of speculation, warranting more study on events occurring in the pre-diabetic period to design preventive treatments. Because of the limited availability of pancreatic tissue from pre-diabetic individuals, early pathological events that occur before clinical T1D are less chartered territories. These events are steadily explored in the recent years as biobanks such as the Network for Pancreatic Organ Donors with Diabetes (nPOD) facilitates the collection of T1D pancreata from all stages of disease. As such, pancreatic sections from a double autoantibody positive (IA-2 and GAD) donor at high risk for developing T1D were studied in **Chapter 2**. Systemic histological analysis showed that islets from autoantibody positive individuals without glycemic dysregulation already show pathognomic signs of T1D: MHC-I hyperexpression and CD8 T-cell infiltrations. High expression of MHC-I presenting autoantigens renders β-cells susceptible to CD8 T-cell induced killing. This persists in insulin containing islets for several years after diagnosis, potentially expediting the continued destruction of β-cells which eventually diminishes (16). Interestingly, we observed a highly lobular pattern of MHC-I hyperexpression with generally higher levels of CD8 T-cell infiltration than normal islets, though areas of MHC-I hyperexpression without T-cell infiltration were also observed. This heterogeneous distribution of pathologic areas was also observed in pancreas of recent-onset T1D patients with islets in different disease stages (from unaltered phenotype till β-cell death) occurring alongside to each other (17). This also indicates a more protracted and potential reversible disease process, which provides a window of opportunity for intervention therapy. One hypothesis explaining this vitiligo-like distribution of immune lesions in T1D is a viral infection that could induce a lobular spread of MHC-I hyperexpression (18). However, no signs for such a viral cause were detected in the pancreas (by immunohistochemistry or PCR) of the donor studied in **Chapter 2**, although not all regions of the pancreas were analyzed. Neuroimmune interactions within the pancreas could also offer an explanation for this phenomenon. Recently, studies showed that interference of sympathetic signaling in the pancreas halts the onset of diabetes in mice (19, 20).

6

Finally, β-cells themselves could be the driving force for patch wise immune destruction in T1D, since increase of stress or metabolic demand could cause local alterations and inflammation. Elucidating the pattern of immune lesions will likely help us to better understand the etiology of T1D, while autoantibody positive cases might give insights into why these events are present in the earliest phase of disease pathology.

Cytotoxic CD8 T-cells targeting islet-autoantigens play an important role in T1D progression. Due to their very low frequencies in peripheral blood and the technical difficulties to identify them, autoreactive T-cells are generally poorly defined. In **Chapter 3**, these rare cells were examined by combining a detection method of antigen specific T-cells with MHC-I tetramers and mass cytometry (21). This study was a proof of concept showing a novel technique to identify and study such rare antigen-specific T-cells in T1D patients. Remarkable heterogeneity in this regard was observed in the CD8 T-cell compartment of T1D patients. This was also the case for the islet-specific CD8 T-cell pool with hardly shared marker expression patterns in the available study subjects. Interestingly, the intensity of tetramer signal of islet specific CD8 T-cells was low, which is in line with previous studies showing low-affinity binding TCR in autoreactive CD8 T-cells (4, 6). Two studies applied an analogous method to study autoreactive CD8 T-cells in T1D (22, 23), confirming our observed phenotypical heterogeneity of islet-specific CD8 T-cells at both the individual and population level. In addition, Ogura et. al. found increased ZnT8-reactive CD8 T-cells with CD57^{+}CD45RO^{+} phenotype in T1D patients, suggesting increased exposure to ZnT8 and activation of autoreactive CD8 T-cells in the studied patients (n=29) (22). Wiedeman *et al.* included a larger study group (n=46) where changes could be related to different progression rates. An activated memory phenotype (helios^{+}CD27^{+}) in islet-specific (PPI, GAD, IGRP, IAPP pooled) CD8 T-cells was linked to rapid progressors, whereas an exhaustion phenotype (eomes^{+}KLRG1^{+} TIGIT[+] PD1^{+}), was more prevalent in slow progressors and possibly signifies a more indolent autoimmune activity (23).

The phenotypical heterogeneity of islet-specific T-cells revealed in these studies could therefore be indicative of variability in islet-autoimmunity during disease onset and could have implications for progression of T1D as is suggested by the latter study. Nevertheless, utilizing the phenotype of autoreactive T-cells as biomarker to explain the immunologic mechanisms, predict disease progression or to monitor therapeutic efficacy are likely to remain challenging in light of their observed heterogeneity.

Immune monitoring tools for tolerogenic dendritic cell therapy

Immune intervention therapy with tolDC has been considered for the improvement of management or even cure of various autoimmune and inflammatory diseases. TolDCs in this regard possess the capacity to skew the immune system towards a more regulatory state and present an attractive intervention therapy to reduce autoimmunity in T1D and could also be applied to other autoimmune and inflammatory disorders such as Rheumatoid Arthritis (RA) as described in **Chapter 4.1**. Our group has shown that vitamin D and dexamethasone are suitable agents to induce tolDCs with a stable regulatory phenotype (24) while the mechanism of action of vitD-dex induced tolDC has been elucidated from extensive *in vitro* studies (24-30). **Chapter 5.1** describes the mechanism of action of tolDC, compares different outcomes with respect to different tolDC modulating agents and summarizes existing and potential monitoring methods to measure the effect of tolDC *in vivo*. The most important effects of tolDCs for the treatment of T1D are inhibition of antigen specific CD4 and CD8 T-cells and induction of Tregs. The application of selective tolerance by pulsing tolDC with target antigen(s) indeed enables specific disease-relevant immunomodulation without general immune suppression. To monitor the effect of tolDC, evaluating quantity of immune populations by phenotypical characterization (flow cytometry or mass cytometry) as well as functional characterization of T-cell responses (lymphoproliferative assay and ELISPOT) are essential. However, quantification of Tregs and specifically tolDC-induced Tregs is challenging since

markers delineating induced Tregs are lacking, although iTregs differ from nTregs in for instance FoxP3 demethylation and possibly reverse polarity of TCR/HLA interaction (25). Therefore, extensive investigation of the phenotype of tolDC-induced Tregs using mass cytometry with respect to functional properties was performed in **Chapter 4.2**. TolDC-induced Tregs consisted of subpopulations with distinct phenotypes of which CD45RA$^-$CD25^{hi} and CD45RA$^-$CD25^{lo} were the most prominent and suppressed CD4 T-cell proliferation, while CD45RA$^-$CD25^{hi} Tregs also suppressed CD8 T-cell induced killing. An additional population in tolDC-stimulated cultures lacking suppressive capacity retained a naïve-like phenotype likely as consequence of inhibitory factors from tolDCs or tolDC-induced Tregs. CD45RA$^-$CD25^{hi} Tregs seem to prevail in a more activated state by expressing increased activation markers such as ICOS, CCR4, CD38 and FoxP3 and showing increased cytokine production compared to CD45RA$^-$CD25^{lo} Tregs. Suggesting that such activation markers could be indicators for Treg functionality. These markers, however, are not exclusively present on tolDC-induced Tregs. As such, tolDC-Tregs share markers with induced Tr1 Tregs (31, 32) as well as thymic derived (nTregs) (33-36) and may be detected within the nTreg pool when using the above mentioned markers to discriminate them (Table 1). It is therefore necessary to explore novel markers as well as investigate the antigen specificity to delineate induced Tregs. For now, classical Treg markers such as CD25 and CD127 should suffice to identify a bigger pool of Tregs including nTregs and induced Tregs.

Table 1. Characteristics tolDC-induced Tregs

	CD25^{hi} tolDC-Treg	CD25^{lo} tolDC-Treg	nTreg (33)	Tr1-Treg (31, 32)
Inhibition CD4 T-cell proliferation	+	+	+	+
Inhibition CD8 T-cell killing	+	-	+	+
Cytokine production[1]				
IL-10	low	low	+	+
TGF-β	*n/a*	*n/a*	+	+
IL-13	++	+	+	*n/a*
IFN-γ	++	+	+	++
TNF-α	++	+	+	*n/a*
Surface markers[1]				
CD45RA	-	-	+	-
CD25	++	dim	++	*n/a*
CD127	dim	-	-	*n/a*
TIGIT	+*	-*	+	*n/a*
Lag-3	-	+	+	+
CD49b	dim	-	-	+
PD-1	+	-	+	+
CTLA4	-	-	-	*n/a*
HLA-DR	+	+	+	*n/a*
ICOS	+	dim	+	*n/a*
CD38	+	dim	+	*n/a*
CD39	+	dim	+	+
CD69	+	dim	+	*n/a*
CCR4	+	dim	+	*n/a*
CD161	+	dim	+	*n/a*
Intracellular markers[1]				
FoxP3	++	dim	++	-

[1]Cytokine production and marker expression after stimulation (tolDC-Tregs)

*Data not shown in chapter 4.3

Tolerogenic dendritic cells in clinical trials

Phase I clinical trials with tolDC generated with various modulatory agents have been completed in rheumatoid arthritis (37), Crohn's disease (38), multiple sclerosis (39) and T1D (40) (reviewed in **Chapter 4.1** and **Chapter 5.1** and demonstrated the safety and feasibility of tolDC therapy. The different methods of generating tolDC, however, surely affect their regulatory function. For instance, tolDC generated with vitD induce Tregs which exhibited antigen specificity, while tolDC generated with dexamethasone induce non-specific Tregs (30). Knowledge on autoantigens involved in the disease

pathogenesis facilitates the application of targets for tolDC therapy, but this is not established in all autoimmune or inflammatory diseases (**Chapter 4.1**). For T1D, a considerable number of associated epitopes has been identified, and vaccines based on these peptides have been tested in clinical trials. Immunotherapy with proinsulin (C19-A3) peptide induced favorable metabolic and immune effects in individuals with new-onset T1D (41, 42). Giannoukakis *et al.* reported the first trial of genetically engineered tolDC in T1D patients, albeit without the addition of target autoantigen, and demonstrated an increase of Bregs after tolDC administration (40). We reported the first clinical trial with combined treatment with tolDC pulsed with proinsulin peptide in a placebo-controlled, dose escalation phase 1 clinical trial with T1D patients and clinical release criteria were met for all generated tolDC products (43). The importance of quality control markers for the regulatory function of tolDC was furthermore demonstrated in **Chapter 4.2**, as tolDC with aberrant phenotype and increased expression of CD86 induced T-cells lacking immune suppressive activity. Contrary to the study of Giannoukakis, we did not detect circulating Bregs expressing B220. The immune modulatory effects of proinsulin-pulsed tolDC *in vivo* were reported in **Chapter 5.2**. Using the knowledge on mechanisms of action as described in **Chapter 5.1**, various analysis methods were used to measure antigen-specific responses as well as to phenotypically characterize the immune subsets in peripheral blood. The application of mass cytometry including a broad panel of T-cell markers, furthermore enabled identification of 53 CD4 T-cell and 42 CD8 T-cell subclusters. Th17-like and CD4 T-cells with tissue resident (Trm) phenotype showed a transient increase after tolDC injection (Figure 1), while major leukocyte subsets remained stable. Interestingly, Th17-like CD4 T-cells declined to frequencies below baseline levels, which has a potential therapeutic implication since Th17 cells play a role in T1D pathogenesis (44). This short-lived immune activation may reflect therapeutic induction of immune tolerance, which is an active process that needs a preceding immunization. Furthermore, a transient increase of Tregs expressing activation markers (ICOS$^+$CCR4$^+$TIGIT$^+$) was observed. This temporary increase possibly reflects

Treg migration to lymph nodes or peripheral tissues, which could be facilitated by the expression of chemokine receptors such as CCR4. These Tregs much resemble the *in vitro* tolDC-induced CD45RA⁻CD25^{hi} -Tregs as reported in **Chapter 4.1**, suggesting that Tregs with CD4 as well as CD8 inhibiting capacity might also be induced *in vivo*. On the other hand, it could also signify activation of thymic derived nTregs. Unfortunately, the antigen-specificity of affected T-cell populations was not determined, though a separate antigen-specific analysis demonstrated that proliferative and IFNγ responses against vaccine proinsulin peptide reduced in patients with pre-existing responses. Most of the changes after tolDC injections were not related to the administered dose, suggestive of a low dose effect of tolDCs.
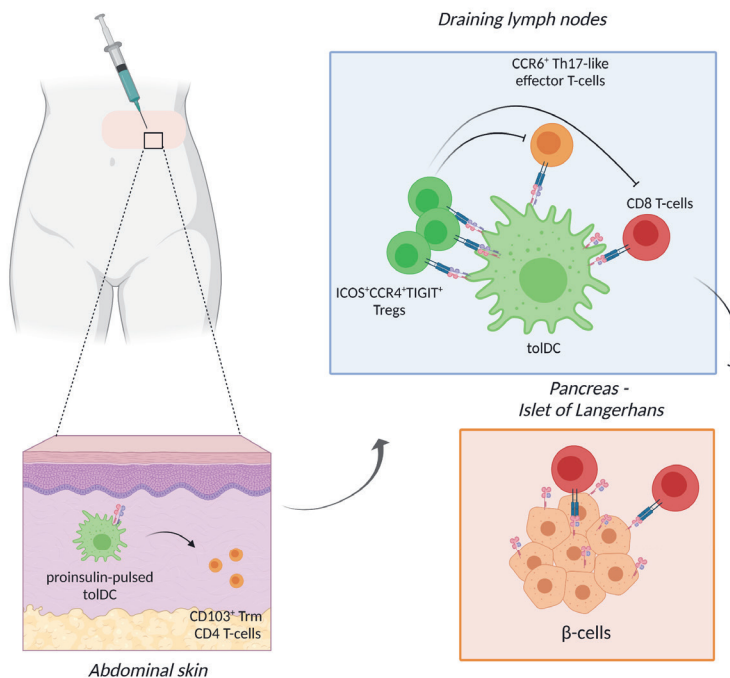

Figure 1. Mechanism of action of intradermal injection of proinsulin-pulsed tolDC as therapy for type 1 diabetes. TolDC are injected intradermally in the upper left abdominal quadrant. CD103⁺ tissue resident CD4 T-cells are stimulated upon tolDC injection. Upon migration to draining lymphnodes, tolDC induce Tregs with ICOS⁺CCR4⁺TIGIT⁺ phenotype and inhibit/eliminate effector Th17-like T-cells. Created in BioRender.com.

This phase I trial with promising results regarding immunological efficacy of proinsulin-pulsed tolDC was not designed to show clinical improvement, since the participating patients had long standing T1D with mostly undetectable c-peptide levels. Assessment of clinical efficacy of tolDC intervention therapy therefore heavily relies on the resilience of residual β-cells and early intervention is key in order to preserve as many functional β-cells as possible. In this light, patients that develop T1D at a young age seem to have a more fulminant disease with higher amounts of islet-infiltrating immune cells and more severe loss of β-cells. In contrast, patients that develop T1D as teenager retain more β-cells (>40-60% insulin containing islets) (45). Furthermore, patients with insufficient residual β-cells might benefit from an additional β-cell replacement therapy with islet transplantation or β-cells derived from embryonic or induced pluripotent stem cells (46, 47). Future studies including patients with recent onset disease should determine the clinical efficacy of tolDC therapy and correlate immunological efficacy with clinical efficacy endpoints. For the trial reported in **Chapter 5.2**, proinsulin peptide binding to HLA-DR4 was used as target antigen for tolDC. Although the majority of T1D patients are HLA-DR4^{+}, around 30% of patients lack HLA-DR4 and are therefore not eligible for this personalized therapy. To extend tolDC therapy to all patients, other target antigens binding to different HLA types would be needed, while also loading tolDC with multiple antigens might broaden the Tregs' target potential by the additional antigen-specificities presented by tolDC. On the other hand, this might not be necessary since tolDC were shown to act through so called 'infectious tolerance' and 'linked suppression'. Indeed, tolDC-induced Tregs can alter conventional DCs to adopt an anti-inflammatory phenotype (infectious tolerance), while these modulated DC in turn can prime Tregs of different specificities (linked suppression) (27). Therefore, the suppressive effect of tolDCs is not limited to the selected vaccination peptide, but spreads to other epitopes present in the encountered target tissue. First signs of this phenomenon were observed in our clinical trial **(Chapter 5.2)**, as PPI-specific responses were additionally reduced in 7 out of 9 patients. In any which way, the tolDC presented

peptide could be aligned to the type of pre-existing autoimmune responses. Further research into novel T1D associated antigens as well as investigation of alternative tolDC antigens should first show whether infectious tolerance will lead to clinical efficacy or that multiple antigen targeting is needed.

Mesenchymal stromal cell therapy in acute graft-versus-host disease

Mesenchymal stromal cells (MSC), like tolDCs, have been extensively investigated as intervention therapy. The ability of MSCs to differentiate into various cell types and modulate immune responses *in vitro* renders therapy with MSC suitable to support inflammatory conditions with tissue injury such as graft-versus-host disease (aGvHD), but also Crohn's disease, cardiovascular disease and several neurological diseases (48). Promising clinical effects for example have been reported in patients with steroid-refractory aGvHD, especially in pediatric patients. More than half of included patients showed clinical response to MSC therapy with a more than 2 years overall-survival after stem-cell transplantation (49). MSCs *in vitro* inhibit T-cells (50), modulate myeloid cells (51) and induce Tregs (51, 52), mainly via paracrine factors such as IDO. It was first thought that intravenously administered MSCs *in vivo* migrate to inflamed tissue and differentiate to support tissue regeneration and immune regulation (53). Later studies, however, showed that MSCs are short-lived and do not migrate beyond the lungs (54). Yet, Galleu *et al.* showed that phagocytes take up apoptotic MSC and produce IDO (55), which could explain that MSC are barely detected after infusion even in case of a clinical effect. Though *in vitro* observations show clear immunomodulatory properties of MSC, little is known about the MSC-induced immune modulation in clinical responding patients. In **Chapter 5.3**, using CyTOF to analyze peripheral blood of children with aGvHD, we could show changes within the T-cell compartment that were associated with clinical outcome of steroid-refractory aGvHD after MSC infusion. Effector T-cells with high expression of gut and skin homing markers (CXCR3, CCR9 and CCR10) declined in clinical responders, while

6

an increase was observed in non-responders. These T-cells had inflammation-promoting properties, as they were capable of releasing factors such as IFNγ, TNF and Granzyme B. Interestingly, this increase of effector T-cells in non-responders was accompanied with an increase of CD25^{++}CD127^{-} Tregs, some of which also expressed gut and skin homing markers. Although high frequencies of Tregs at aGvHD onset have been associated with increased survival (56, 57), our study demonstrates that circulating Tregs in aGvHD follow the trend of effector T-cells and are reducing in clinically improving patients. Our findings might not be contradicting previous observations, since Treg frequencies at aGvHD onset were not measured, Instead, Treg frequencies may adjust to the degree of inflammation – i.e. the activity of GVHD-present. MSC hence might mediate inhibition of effector T-cells, induce expansion of Tregs, while migration of Tregs from circulation into inflammatory lesions (leading to their decline in peripheral blood) is also conceivable. Notably, the sole long-term survivor within the MSC non-responders showed an effector T-cell signature more similar to responders (decreasing frequencies of effector T-cells and Tregs expressing CXCR3/CCR9/CCR10), supporting the clinical association with this T-cell signature.

Additionally, we identified distinct populations within the myeloid and B-cell compartment that distinguished patients not responding to therapy. Class-switched plasmablasts, CD163^{+} monocytes and CD163^{+} DCs as well as CD56^{+} pDCs in this light were significantly increased in therapy-refractory aGvHD patients early after start of first line immune suppressive therapy and before the introduction of MSC therapy. CD163^{+} myeloid cells are specifically recruited to tissues with ongoing inflammation, where they develop into tissue-resident inflammatory macrophage-like cells that can attract neutrophilic granulocytes (58-60). Indeed, our study reveals that CD163^{+} myeloid cells are abundant in GI tract and skin biopsies collected from patients with locally active aGvHD. Interestingly, before MSC therapy, PD-1 expression was particularly high in TCRγδ[+] T-cells of non-responders. Considering the major role of TCRγδ[+] T-cells in the host defense of epithelial barrier tissues, this might point to

concurrent extensive damage in the GI tract or skin leading to activation of TCRγδ$^+$ T-cells. We did not observe specific changes after MSC therapy within the non-T cell compartment. Altogether, our data suggest that non-response to MSC therapy is associated with increased pro-inflammatory immune subsets likely reflecting escalating aGvHD immune reactivity, which was already apparent before introduction of MSC. Furthermore, we could hypothesize that early intervention with MSC, before progression to severe advanced aGvHD, could lead to better clinical response.

Table 2. Immunomodulatory effect of tolDC and MSC in this thesis

	Proinsulin-pulsed TolDC therapy in adults with T1D (Chapter 5.2)	MSC therapy in children with severe aGvHD (Chapter 5.3)
Inhibition of effector T-cells	Decrease of Th-17 like T-cells	Decrease of CD4$^+$ and CD8$^+$ effector T-cells with gut and skin homing markers
Induction of Tregs	Transient increase of ICOS$^+$CCR4$^+$TIGIT$^+$ Tregs	Decrease of Tregs with gut and skin homing markers
Modulation of B-cells, myeloid cells, NK cells	No specific changes observed	No specific changes observed
Modulation of antigen-specific responses	Reduction of PPI-specific response	Not studied, *in vitro* modulation of antigen-specific T-cell response shown in (133)
Clinical benefit	Not yet determined	Resolving aGvHD in a subset of patients

Evidently, identification of biomarkers that predict response to MSC therapy would greatly benefit application of MSCs in clinical practice. Serum biomarkers for tissue inflammation and endothelial cell damage have been used as tool for monitoring response to aGvHD treatments. Indeed, concentrations of tissue inflammation markers such as TNFR1 were predictive of response to MSCs in steroid-resistant aGvHD, but lacked specificity and sensitivity (61). Combining serum biomarkers with immunological signatures could provide a solution. In **Chapter 5.3**, mass cytometry

revealed specific changes in effector T-cell populations associated with clinical response. Before MSC infusion, non-responding patients showed signs of increased inflammation in the T-cell compartment as well as in the myeloid and NK compartment. These first results will be validated in a larger randomized cohort and could eventually provide alternative biomarkers to predict therapy response in steroid-resistant aGvHD patients.
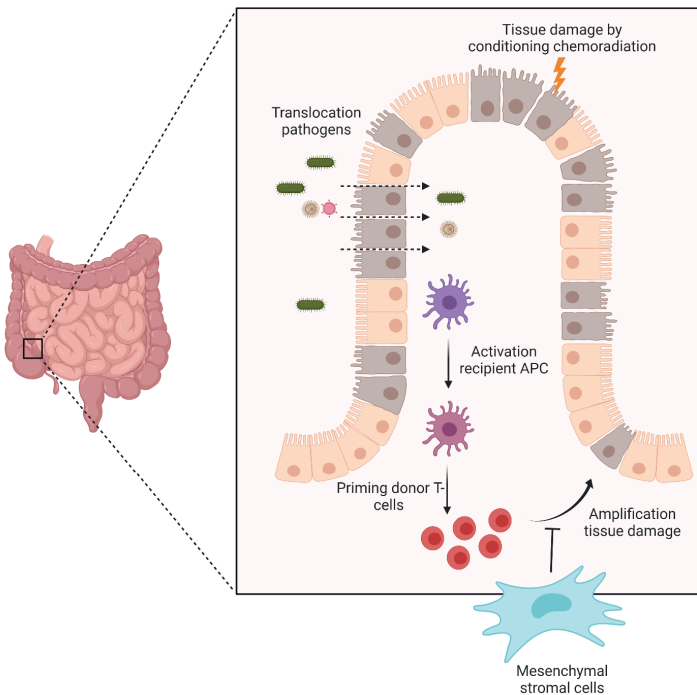

Figure 2. Mesenchymal stromal cells for the treatment of acute graft-versus-host disease. Conditioning regimens prior to graft infusion cause damage to the epithelial barrier in the intestines. Danger signals released upon tissue damage activate antigen presenting cells (APC) residing locally and prime allogeneic T-cells, which in turn cause additional tissue damage. Mesenchymal stromal cells are infused in the circulation and inhibit allogeneic T-cells and support regeneration of tissue damage, thereby ameliorating clinical symptoms of aGvHD. Created in BioRender.com.

Conclusion

Immune modulatory cell-based therapy such as tolDC and MSC offer promising treatment modalities for autoimmune and inflammatory diseases and have reached the bedside of patients with T1D and aGvHD, as discussed in this thesis. Understanding the pathophysiology of the disease in question and mechanism of action of our immunomodulatory cell therapies is critical for further development and optimization of the latter and proved greatly facilitated by the application of mass cytometry as discovery tool. Advantageous properties of tolDC and MSC on immune regulation make them suitable treatment options in a variety of clinical conditions and should be further explored. Indeed, MSC are currently investigated as treatment approach in T1D (62, 63) while tolDC are considered for the treatment of aGvHD (64). Continued investigation on the efficacy of innovative cell-based treatment approaches may lead to their implementation in regular clinical practice, adding personalized medicine to intervene in inflammatory disorders as immune modulation and regenerative therapy.

References

1.	Richardson SJ, Willcox A, Bone AJ, Morgan NG, Foulis AK. Immunopathology of the human pancreas in type-I diabetes. Semin Immunopathol. 2011;33(1):9-21.
2.	Tan S, Li Y, Xia J, Jin CH, Hu Z, Duinkerken G, et al. Type 1 diabetes induction in humanized mice. Proc Natl Acad Sci U S A. 2017;114(41):10954-9.
3.	Unger WW, Pearson T, Abreu JR, Laban S, van der Slik AR, der Kracht SM, et al. Islet-specific CTL cloned from a type 1 diabetes patient cause beta-cell destruction after engraftment into HLA-A2 transgenic NOD/scid/IL2RG null mice. PLoS One. 2012;7(11):e49213.
4.	Abreu JR, Martina S, Verrijn Stuart AA, Fillie YE, Franken KL, Drijfhout JW, et al. CD8 T cell autoreactivity to preproinsulin epitopes with very low human leucocyte antigen class I binding affinity. Clin Exp Immunol. 2012;170(1):57-65.
5.	Ouyang Q, Standifer NE, Qin H, Gottlieb P, Verchere CB, Nepom GT, et al. Recognition of HLA class I-restricted beta-cell epitopes in type 1 diabetes. Diabetes. 2006;55(11):3068-74.
6.	Unger WW, Velthuis J, Abreu JR, Laban S, Quinten E, Kester MG, et al. Discovery of low-affinity preproinsulin epitopes and detection of autoreactive CD8 T-cells using combinatorial MHC multimers. J Autoimmun. 2011;37(3):151-9.
7.	McGinty JW, Marre ML, Bajzik V, Piganelli JD, James EA. T cell epitopes and post-translationally modified epitopes in type 1 diabetes. Curr Diab Rep. 2015;15(11):90.
8.	Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA. 2013;309(23):2473-9.
9.	Thomaidou S, Zaldumbide A, Roep BO. Islet stress, degradation and autoimmunity. Diabetes Obes Metab. 2018;20 Suppl 2:88-94.
10.	Thomaidou S, Kracht MJL, van der Slik A, Laban S, de Koning EJ, Carlotti F, et al. Beta-Cell Stress Shapes CTL Immune Recognition of Preproinsulin Signal Peptide by Post-Transcriptional Regulation of Endoplasmic Reticulum Aminopeptidase 1. Diabetes. 2020.
11.	Roep BO, Kracht MJ, van Lummel M, Zaldumbide A. A roadmap of the generation of neoantigens as targets of the immune system in type 1 diabetes. Curr Opin Immunol. 2016;43:67-73.
12.	Kracht MJL, Zaldumbide A, Roep BO. Neoantigens and Microenvironment in Type 1 Diabetes: Lessons from Antitumor Immunity. Trends Endocrinol Metab. 2016;27(6):353-62.
13.	Kracht MJ, van Lummel M, Nikolic T, Joosten AM, Laban S, van der Slik AR, et al. Autoimmunity against a defective ribosomal insulin gene product in type 1 diabetes. Nature medicine. 2017;23(4):501-7.
14.	Sosenko JM, Palmer JP, Greenbaum CJ, Mahon J, Cowie C, Krischer JP, et al. Patterns of metabolic progression to type 1 diabetes in the Diabetes Prevention Trial-Type 1. Diabetes Care. 2006;29(3):643-9.
15.	Rodriguez-Calvo T, Atkinson M, von Herrath MG. Increase in Pancreatic Proinsulin and Preservation of beta-Cell Mass in Autoantibody- Positive Donors Prior to Type 1 Diabetes Onset. Diabetes 2017; 66: 1334-1345. Diabetes. 2017;66(9):E10-E1.
16.	Richardson SJ, Rodriguez-Calvo T, Gerling IC, Mathews CE, Kaddis JS, Russell MA, et al. Islet cell hyperexpression of HLA class I antigens: a defining feature in type 1 diabetes. Diabetologia. 2016;59(11):2448-58.
17.	Damond N, Engler S, Zanotelli VRT, Schapiro D, Wasserfall CH, Kusmartseva I, et al. A Map of Human Type 1 Diabetes Progression by Imaging Mass Cytometry. Cell Metab. 2019;29(3):755-68 e5.
18.	Capua I, Mercalli A, Pizzuto MS, Romero-Tejeda A, Kasloff S, De Battisti C, et al. Influenza A Viruses Grow in Human Pancreatic Cells and Cause Pancreatitis and Diabetes in an Animal Model. J Virol. 2013;87(1):597-610.
19.	Christoffersson G, Ratliff SS, von Herrath MG. Interference with pancreatic sympathetic signaling halts the onset of diabetes in mice. Sci Adv. 2020;6(35).
20.	Guyot M, Simon T, Ceppo F, Panzolini C, Guyon A, Lavergne J, et al. Pancreatic nerve electrostimulation inhibits recent-onset autoimmune diabetes. Nat Biotechnol. 2019;37(12):1446-51.
21.	Newell EW, Sigal N, Nair N, Kidd BA, Greenberg HB, Davis MM. Combinatorial tetramer staining and mass cytometry analysis facilitate T-cell epitope mapping and characterization. Nat Biotechnol. 2013;31(7):623-9.

22.	Ogura H, Preston-Hurlburt P, Perdigoto AL, Amodio M, Krishnaswamy S, Clark P, et al. Identification and Analysis of Islet Antigen-Specific CD8(+) T Cells with T Cell Libraries. J Immunol. 2018;201(6):1662-70.

23.	Wiedeman AE, Muir VS, Rosasco MG, DeBerg HA, Presnell S, Haas B, et al. Autoreactive CD8+ T cell exhaustion distinguishes subjects with slow type 1 diabetes progression. J Clin Invest. 2020;130(1):480-90.

24.	Ferreira GB, Kleijwegt FS, Waelkens E, Lage K, Nikolic T, Hansen DA, et al. Differential protein pathways in 1,25-dihydroxyvitamin d(3) and dexamethasone modulated tolerogenic human dendritic cells. J Proteome Res. 2012;11(2):941-71.

25.	Beringer DX, Kleijwegt FS, Wiede F, van der Slik AR, Loh KL, Petersen J, et al. T cell receptor reversed polarity recognition of a self-antigen major histocompatibility complex. Nat Immunol. 2015;16(11):1153-61.

26.	Kleijwegt FS, Jansen DT, Teeler J, Joosten AM, Laban S, Nikolic T, et al. Tolerogenic dendritic cells impede priming of naive CD8(+) T cells and deplete memory CD8(+) T cells. Eur J Immunol. 2013;43(1):85-92.

27.	Kleijwegt FS, Laban S, Duinkerken G, Joosten AM, Koeleman BP, Nikolic T, et al. Transfer of regulatory properties from tolerogenic to proinflammatory dendritic cells via induced autoreactive regulatory T cells. J Immunol. 2011;187(12):6357-64.

28.	Kleijwegt FS, Laban S, Duinkerken G, Joosten AM, Zaldumbide A, Nikolic T, et al. Critical role for TNF in the induction of human antigen-specific regulatory T cells by tolerogenic dendritic cells. J Immunol. 2010;185(3):1412-8.

29.	Kleijwegt FS, Roep BO. Infectious tolerance as candidate therapy for type 1 diabetes: transfer of immunoregulatory properties from human regulatory T cells to other T cells and proinflammatory dendritic cells. Crit Rev Immunol. 2013;33(5):415-34.

30.	Unger WW, Laban S, Kleijwegt FS, van der Slik AR, Roep BO. Induction of Treg by monocyte-derived DC modulated by vitamin D3 or dexamethasone: differential role for PD-L1. Eur J Immunol. 2009;39(11):3147-59.

31.	Gagliani N, Magnani CF, Huber S, Gianolini ME, Pala M, Licona-Limon P, et al. Coexpression of CD49b and LAG-3 identifies human and mouse T regulatory type 1 cells. Nature medicine. 2013;19(6):739-46.

32.	Roncarolo MG, Gregori S, Bacchetta R, Battaglia M, Gagliani N. The Biology of T Regulatory Type 1 Cells and Their Therapeutic Application in Immune-Mediated Diseases. Immunity. 2018;49(6):1004-19.

33.	Mason GM, Lowe K, Melchiotti R, Ellis R, de Rinaldis E, Peakman M, et al. Phenotypic Complexity of the Human Regulatory T Cell Compartment Revealed by Mass Cytometry. J Immunol. 2015;195(5):2030-7.

34.	Baecher-Allan C, Wolf E, Hafler DA. MHC class II expression identifies functionally distinct human regulatory T cells. J Immunol. 2006;176(8):4622-31.

35.	Dwyer KM, Hanidziar D, Putheti P, Hill PA, Pommey S, McRae JL, et al. Expression of CD39 by human peripheral blood CD4+ CD25+ T cells denotes a regulatory memory phenotype. Am J Transplant. 2010;10(11):2410-20.

36.	Ito T, Hanabuchi S, Wang YH, Park WR, Arima K, Bover L, et al. Two functional subsets of FOXP3+ regulatory T cells in human thymus and periphery. Immunity. 2008;28(6):870-80.

37.	Benham H, Nel HJ, Law SC, Mehdi AM, Street S, Ramnoruth N, et al. Citrullinated peptide dendritic cell immunotherapy in HLA risk genotype-positive rheumatoid arthritis patients. Sci Transl Med. 2015;7(290):290ra87.

38.	Jauregui-Amezaga A, Cabezon R, Ramirez-Morros A, Espana C, Rimola J, Bru C, et al. Intraperitoneal Administration of Autologous Tolerogenic Dendritic Cells for Refractory Crohn's Disease: A Phase I Study. J Crohns Colitis. 2015;9(12):1071-8.

39.	Zubizarreta I, Florez-Grau G, Vila G, Cabezon R, Espana C, Andorra M, et al. Immune tolerance in multiple sclerosis and neuromyelitis optica with peptide-loaded tolerogenic dendritic cells in a phase 1b trial. Proc Natl Acad Sci U S A. 2019;116(17):8463-70.

40.	Giannoukakis N, Phillips B, Finegold D, Harnaha J, Trucco M. Phase I (safety) study of autologous tolerogenic dendritic cells in type 1 diabetic patients. Diabetes Care. 2011;34(9):2026-32.

41.	Thrower SL, James L, Hall W, Green KM, Arif S, Allen JS, et al. Proinsulin peptide immunotherapy in type 1 diabetes: report of a first-in-man Phase I safety study. Clin Exp Immunol. 2009;155(2):156-65.

6

42.   Alhadj Ali M, Liu YF, Arif S, Tatovic D, Shariff H, Gibson VB, et al. Metabolic and immune effects of immunotherapy with proinsulin peptide in human new-onset type 1 diabetes. Sci Transl Med. 2017;9(402).

43.   Nikolic T, Zwaginga JJ, Uitbeijerse BS, Woittiez NJ, de Koning EJ, Aanstoot HJ, et al. Safety and feasibility of intradermal injection with tolerogenic dendritic cells pulsed with proinsulin peptide-for type 1 diabetes. Lancet Diabetes Endocrinol. 2020;8(6):470-2.

44.   Ferraro A, Socci C, Stabilini A, Valle A, Monti P, Piemonti L, et al. Expansion of Th17 cells and functional defects in T regulatory cells are key features of the pancreatic lymph nodes in patients with type 1 diabetes. Diabetes. 2011;60(11):2903-13.

45.   Leete P, Willcox A, Krogvold L, Dahl-Jorgensen K, Foulis AK, Richardson SJ, et al. Differential Insulitic Profiles Determine the Extent of -Cell Destruction and the Age at Onset of Type 1 Diabetes. Diabetes. 2016;65(5):1362-9.

46.   Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazer S, et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol. 2008;26(4):443-52.

47.   Rezania A, Bruin JE, Arora P, Rubin A, Batushansky I, Asadi A, et al. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. Nat Biotechnol. 2014;32(11):1121-33.

48.   Squillaro T, Peluso G, Galderisi U. Clinical Trials With Mesenchymal Stem Cells: An Update. Cell Transplant. 2016;25(5):829-48.

49.   Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet. 2008;371(9624):1579-86.

50.   Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood. 2002;99(10):3838-43.

51.   Francois M, Romieu-Mourez R, Li M, Galipeau J. Human MSC suppression correlates with cytokine induction of indoleamine 2,3-dioxygenase and bystander M2 macrophage differentiation. Mol Ther. 2012;20(1):187-95.

52.   Chen PM, Liu KJ, Hsu PJ, Wei CF, Bai CH, Ho LJ, et al. Induction of immunomodulatory monocytes by human mesenchymal stem cell-derived hepatocyte growth factor through ERK1/2. J Leukoc Biol. 2014;96(2):295-303.

53.   Rustad KC, Gurtner GC. Mesenchymal Stem Cells Home to Sites of Injury and Inflammation. Adv Wound Care (New Rochelle). 2012;1(4):147-52.

54.   Eggenhofer E, Benseler V, Kroemer A, Popp FC, Geissler EK, Schlitt HJ, et al. Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. Front Immunol. 2012;3:297.

55.   Galleu A, Riffo-Vasquez Y, Trento C, Lomas C, Dolcetti L, Cheung TS, et al. Apoptosis in mesenchymal stromal cells induces in vivo recipient-mediated immunomodulation. Sci Transl Med. 2017;9(416).

56.   Cohen JL, Boyer O. The role of CD4+CD25hi regulatory T cells in the physiopathogeny of graft-versus-host disease. Curr Opin Immunol. 2006;18(5):580-5.

57.   Magenau JM, Qin X, Tawara I, Rogers CE, Kitko C, Schlough M, et al. Frequency of CD4(+)CD25(hi)FOXP3(+) regulatory T cells has diagnostic and prognostic value as a biomarker for acute graft-versus-host-disease. Biol Blood Marrow Transplant. 2010;16(7):907-14.

58.   Nishiwaki S, Terakura S, Ito M, Goto T, Seto A, Watanabe K, et al. Impact of macrophage infiltration of skin lesions on survival after allogeneic stem cell transplantation: a clue to refractory graft-versus-host disease. Blood. 2009;114(14):3113-6.

59.   Olaloye OO, Liu P, Toothaker JM, McCourt BT, McCourt CC, Xiao J, et al. CD16+CD163+ monocytes traffic to sites of inflammation during necrotizing enterocolitis in premature infants. J Exp Med. 2021;218(9).

60.   Terakura S, Martin PJ, Shulman HM, Storer BE. Cutaneous macrophage infiltration in acute GvHD. Bone Marrow Transplant. 2015;50(8):1135-7.

61.   Calkoen FG, Jol-van der Zijde CM, Mearin ML, Schweizer JJ, Jansen-Hoogendijk AM, Roelofs H, et al. Gastrointestinal acute graft-versus-host disease in children: histology for diagnosis, mesenchymal

stromal cells for treatment, and biomarkers for prediction of response. Biol Blood Marrow Transplant. 2013;19(11):1590-9.

62.   van Megen KM, van 't Wout ET, Lages Motta J, Dekker B, Nikolic T, Roep BO. Activated Mesenchymal Stromal Cells Process and Present Antigens Regulating Adaptive Immunity. Front Immunol. 2019;10:694.

63.   Wu H, Mahato RI. Mesenchymal stem cell-based therapy for type 1 diabetes. Discov Med. 2014;17(93):139-43.

64.   Stenger EO, Turnquist HR, Mapara MY, Thomson AW. Dendritic cells and regulation of graft-versus-host disease and graft-versus-leukemia activity. Blood. 2012;119(22):5088-103.

6