T cell immunity to islets of Langerhans : relevance for immunotherapy and transplantation to cure type 1 diabetes

Huurman, V.A.L.

SUMMARY

BACKGROUND

Type 1 diabetes (T1D) is an autoimmune disease in which the insulin-producing β-cells in the pancreas are selectively destroyed. This results in insufficient production of insulin leading to a disturbed glucose metabolism that can lead to severe short- and long-term complications. In *Chapter 1*, different aspects of the disease are introduced and several therapeutic options are described. Since the introduction of exogenous insulin therapy, the majority of patients can be successfully treated. However, the cause of the disease, the T cell-driven autoimmune process, is not countered by this treatment. Furthermore, a substantial subgroup of patients cannot be sufficiently treated with insulin, so novel therapeutic strategies are needed. Two main possibilities are pursued: immunotherapy that tries to counter autoimmune T cells in patients with remaining β-cell function, and transplantation-based treatments that replace the destroyed β-cells while attempting to prevent both allograft rejection and recurrence of autoimmunity. This thesis studies the role of T cells in both of these novel types of treatment for T1D.

IMMUNOTHERAPY

Use of the costimulatory molecule CTLA4, genetically linked to the development of T1D, could prevent allograft rejection in transplantation models and has therefore been proposed for immunotherapeutic use. In *Chapter 2* the mechanism of action of this molecule is studied; in an animal cardiac allograft model it was shown that CTLA4 does not only work by inhibiting CD28/B7 interactions, but also by intracellular signaling. Subsequently, it was shown that CTLA4Ig (a fusion protein successful in inhibiting allograft rejection) could prevent activation of native T-cells, but was unable to inhibit committed autoreactive human T cells *in vitro*. Intervention in such pre-existent reactivity proved more difficult than prevention of a naïve response and therefore may require distinct immunotherapy, possibly because co-stimulation is less important in case of pre-immunized autoimmune T cells.

Instead of inhibiting pro-inflammatory T cells, autoimmunity may presumably be countered by induction of autoantigen-specific T cells with regulatory properties (Tregs). This may be achieved by immunization with auto-antigens such as hsp60 peptide p277, described in *Chapter 3*. In a phase Ib/II clinical trial, immunization of recent-onset T1D patients with this peptide was shown to be safe and led to a small but significant preservation of C-peptide

production when compared to placebo. For the first time in clinical intervention studies in T1D, immunological studies on mechanisms safety and efficacy were performed that offered informative correlates. Preservation of β-cell function was associated with p277-specific production of IL10 prior to therapy and a decreasing cellular response to p277 during therapy. Our studies confirm the potential of therapeutic immunization providing a basis for future, more extensive trials, and demonstrate the reward of immunological monitoring. Furthermore, our studies identified, developed and validated robust and reliable tools to assess immunological efficacy in relation with clinical efficacy of immune intervention at diagnosis that may also prove suitable in the context of β-cell replacement therapy.

TRANSPLANTATION

Transplantation-based therapy for T1D has the advantage of introducing a completely new source of insulin production. However, patients need to undergo an invasive procedure and are subjected to immunosuppression that needs to prevent both allograft rejection and recurrence of autoimmune β-cell destruction. Although the procedure is associated with less complications, clinical outcome of transplantation of isolated islets of Langerhans is presently worse than that of whole pancreas transplantation. Factors influencing islet transplant survival conceivably relate to the transplanted islets, the immune system of the recipient and the immunosuppressive regimen. In *Chapter 4*, a standardized cohort of islet transplant recipients (under ATG-tacrolimus-MMF immunosuppression, the 'Brussels protocol') was analyzed regarding a number of immune factors. Pre-existent and post-transplant cellular autoimmune reactivity as well as graft size were significantly associated with worse transplantation outcome, while the presence of $CD8^+$ alloreactive cytotoxic T cells (CTLs) or autoantibodies was not. In an extended cohort, lymphocyte count was identified as an independent and straightforward alternative measure discerning successful from non-successful transplantations. Counteracting both pre-existent autoimmunity and lymphocytosis may therefore significantly improve pre-transplantation conditioning.

While the presence of alloreactive CTLs was not informative in the ATG-tacrolimus-MMF cohort, it was strongly associated with loss of graft function in patients transplanted under sirolimus-based immunosuppression (*Chapter 5*). A breakthrough achievement was the definition of an immune correlate that acted as endpoint of successful and persistent islet allograft function. Cytokine profiles of allograft-specific T cells were distinctly different between patients reaching insulin independence and patients that still required insulin after transplantation. Especially the induction of the Treg-associated cytokine IL10 against the islet allograft upon transplantation was related to less allo-specific proliferation and better transplant outcome. This indicates that there is indeed a role for pro-inflammatory as well as regulatory alloreactive T cells after transplantation and that IL10 may be an indicator for

transplant success. *Chapter 6* describes a pilot study in which immune factors of five islet transplant recipients during tapering of immunosuppression were analyzed and related to clinical follow-up. Patients showed increases in autoreactivity, alloreactivity or combinations thereof. The percentage of highly avid alloreactive CTLs increased significantly during tapering. These changes in immune reactivity were concordant with overall decreases in β-cell function. In this pilot study, no evidence for tolerance to the graft could be observed. Nonetheless, our *in vitro* mechanistic studies indicate that tapering of tacrolimus is associated with changes in immune reactivity and tapering may benefit from close follow-up of immune factors.

The above studies indicate that insufficient immunosuppression may directly affect transplant outcome. However, immunotherapy should be applied with caution because it may lead to an increase in infectious complications. An example of this is given in *Chapter 7*, where the influence of two types of immunosuppressive induction therapy (ATG and daclizumab) on the occurrence of cytomegalovirus (CMV) infections in patients receiving whole pancreas transplantation is studied. While both induction therapies effectively preserved allograft function, T cell depleting, non-specific ATG leads to earlier and more severe CMV infections than daclizumab, which selectively attacks only activated T cells. These infections were related to a significantly lower amount of circulating CMV-specific memory T cells, indicating that non-specific depletion of the immune system may lead to unwanted side effects after transplantation.

CONCLUSIONS

The major implications of the research described in this thesis are discussed in *Chapter 8*. T cells are major players in T1D pathogenesis and should therefore be considered the foremost target for disease intervention. However, prevention of naïve (alloimmune) responses proves to be different from intervention in a pre-existing autoimmune T cell response and the potential of possible immunotherapeutics should be interpreted accordingly. The present regimens are insufficient to counter T cell autoreactivity after islet cell transplantation and also in this context novel strategies are needed. Alloreactivity can be detrimental after islet transplantation but mainly when immunosuppression is inadequate. Several useful biomarkers are identified in the context of T1D therapy that may lead to individual conditioning of patients receiving novel therapies. However, significant improvements especially for countering T cell autoreactivity are needed before a definitive cure for T1D comes in sight.

Suitable tools were developed and validated in this thesis, which provided compelling evidence of the feasibility of immune monitoring to define causes of failures and correlates of success. These helped revise and improve immune suppressive therapy at diagnosis of T1D or after β-cell replacement.