Immune modulation and monitoring of cell therapy in inflammatory disorders

Suwandi, J.S.

Addendum

English summary
Nederlandse samenvatting
List of publications
Acknowledgments
Curriculum vitae

English Summary

The immune system consists of a complex network that protects the body from pathogens and cancer. Autoimmune diseases and inflammatory conditions are caused by a disturbed and unbalanced immune system in which the body's own cells are destroyed by immune cells as a result of an inflammatory reaction. In this thesis, I focused on type 1 diabetes as an autoimmune disease to investigate the derailment of the immune system and explored strategies involving cell therapy with tolerogenic dendritic cells to restore immune tolerance.

Type 1 diabetes is an autoimmune disease in which insulin-producing β-cells in the pancreas are destroyed by cytotoxic CD8 T-cells. As a result, patients with type 1 diabetes suffer from insulin deficiency, leading to an imbalance of glucose in the blood and, in the long term, an increased risk of vascular complications. Diabetes is a global problem and its prevalence is currently estimated at 463 million people, of which 5-10% is caused by type 1 diabetes and 90-95% by type 2 diabetes. Unlike type 1 diabetes, type 2 diabetes is caused by a combination of insulin resistance (becoming less sensitive to insulin) and a gradual loss of insulin production. Type 1 diabetes is the most common type of diabetes in children and adolescents and is predicted to grow in prevalence in the coming years, especially in young children aged 0-4 years. The cause of type 1 diabetes has not been fully elucidated; various factors could be involved. It is thought that environmental factors such as infections and gut bacteria may trigger an autoimmune reaction against β-cells in genetically predisposed individuals. However, little is known about how this disease process is initiated.

In **Chapter 2** of this thesis, we focussed on the disease lesion and studied pancreatic tissue from an individual at high risk of type 1 diabetes (characterised by the presence of multiple autoantibodies in the blood). We found characteristic signs of type 1 diabetes in the pancreatic tissue, such as hyperexpression of MHC class I molecules and infiltration of cytotoxic T cells. This shows that tissue abnormalities occur before

symptoms of type 1 diabetes become apparent. It was striking that the expression of MHC class I was irregular and showed a vitiligo-like pattern, which means that not all β-cells are affected proportionally. In addition, expression of MHC class I was associated with more infiltration of cytotoxic T cells. Autoreactive cytotoxic T cells in the blood of type 1 diabetic patients were investigated in **Chapter 3**. These T-cells occur in low frequencies in the blood and have been little studied to date. We used MHC class I tetramers to detect and subsequently characterise cytotoxic T cells directed against β cells by mass cytometry (CyTOF). CyTOF is a relatively new platform whereby cells are characterised by antibodies bound to metal isotopes, allowing more than 35 proteins to be measured simultaneously on a cell. We found remarkable heterogeneity in the phenotype of cytotoxic T cells from type 1 diabetes patients and did not find shared marker expression on auto-reactive T cells directed against type 1 diabetes-associated antigens within our study group. The phenotypic heterogeneity within and between patients could indicate variability in autoimmune responses in the course of type 1 diabetes.

To date, treatment for type 1 diabetes is mainly aimed at regulating the glucose balance by insulin administration, which places a considerable burden on the daily lives of patients. Much research is being done to investigate novel therapies that may inhibit the disease process. In the next section of this thesis, I focus on cell therapies that can induce or restore tolerance and inhibit autoimmune reactions. Chapter 4.1 describes how tolerogenic dendritic cells can intervene in the disease process of type 1 diabetes, but also rheumatoid arthritis, a disease that shows many similarities with type 1 diabetes. Tolerogenic dendritic cells are antigen-presenting (immune) cells that have been modulated *in vitro* to have a regulatory function. Tolerogenic dendritic cells have the capacity to balance the immune system by inhibiting inflammatory T cells and inducing/stimulating regulatory T cells.

The immune system can be specifically inhibited by loading tolerogenic dendritic cells with specific antigens that are involved in the disease process of type 1 diabetes. Protection against pathogens and cancer is thereby maintained. Regulatory T cells induced in vitro by tolerogenic dendritic cells are extensively characterised in **Chapter 4.2**. These regulatory T cells consisted of subpopulations with different phenotypes of which $CD45RA^{-}CD25^{hi}$ and $CD45RA^{-}CD25^{lo}$ were the most prominent. Both populations were able to inhibit CD4 T-cells, but only the $CD45RA^{-}CD25^{hi}$ population was able to stop CD8 T-cell lysis as well.

Chapter 5 describes the clinical applications of immune modulating cell therapy. **Chapter 5.1** describes methods to measure immunological effect in patients using the mechanism of action of tolerogenic dendritic cells as reference. These methods are applied in **Chapter 5.2**, which describes the immunological effects in a phase I clinical trial. Two doses of tolerogenic dendritic cells loaded with proinsulin peptide were administered intradermally in patients with type 1 diabetes. Antigen-specific responses were measured by proliferation (LST), cytokine (ELISPOT) and quantum dot (Qdot; quantification of auto-reactive T cells) assays. Patients who reacted against the vaccine peptide (proinsulin) prior to treatment showed a decrease in autoimmunity. Changes in immune populations in the blood were studied with flow cytometry and CyTOF. Two effector populations (Th17 and $CD103^{+}$ Trm CD4 T cells) peaked after treatment, with the Th17 population decreasing further than the baseline frequency. In addition, a specific regulatory T-cell population ($ICOS^{+}CCR4^{+}TIGIT^{+}$) increased in frequency. These findings indicate an immunological effect of tolerogenic dendritic cell therapy *in vivo*. Possible clinical effects will be studied in a follow-up study in patients with recently developed type 1 diabetes.

Finally, cell therapy is highlighted in another clinical setting in **Chapter 5.3**. In this chapter, the effect of mesenchymal stromal cell therapy is studied in children with graft-versus-host disease, in which immune cells originating from the stem cell donor

attack the tissue of the recipient. In particular, the skin, intestine and liver are affected in graft-versus-host disease. Mesenchymal stromal cells are undifferentiated connective tissue-like cells and, like tolerogenic dendritic cells, have a regulatory function on the immune system. In addition, mesenchymal stromal cells can differentiate into different cell types, thus promoting tissue repair. Because of these properties, mesenchymal stromal cell therapy is applied in various conditions, including graft-versus-host disease. Several studies have shown a positive effect of mesenchymal stromal cells in the recovery of graft-versus-host disease. We compared immune cells from patients who showed clinical improvement after mesenchymal stromal cell therapy with patients who did not show clinical improvement. Clinical improvement was characterized by decreasing effector T cells expressing receptors that allow migration to the gut and skin. An increase in these cells was observed in the group with no clinical improvement, accompanied by an increase in regulatory T cells. We also found differences between the treatment groups before start of the treatment. Patients with no clinical improvement showed signs of increased inflammation in several immune populations including specific T cells, B cells and myeloid cells. This implies that mesenchymal cells may not be effective enough in far advanced graft-versus-host disease and that early intervention is needed.

Immune modulating cell therapies such as tolerogenic dendritic cells and mesenchymal stromal cells are promising treatments for autoimmune and inflammatory diseases. Understanding disease processes and mechanism of action are crucial for the development and optimisation of novel cell therapies. Continued research will possibly lead to the application of cell therapy in regular practice as additional treatment option.