T cell differentiation in autoimmune diseases
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Chapter 1

Introduction
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Immunity

The human immunesystem consists of specific and non-specific subsystems that interact to defend the host against pathogens. The non-specific (innate) immunesystem consists of (1) phagocytes like macrophages, monocytes and neutrophilic granulocytes, (2) natural killer cells and (3) soluble components such as the molecules of the complement system. After pathogens have managed to cross the barriers of the human body (skin, mucous membranes), the innate immune system is able to act directly by opsonizing the pathogen with complement. This leads either to lysis of the microbe or to apoptosis of the intruder by recruited phagocytes. Additionally, pathogens can trigger pathogen recognition receptors on cells of the innate immune system by pathogen-associated molecular patterns. This induces endocytosis and, after binding to members of the Toll like receptor family, production of cytokines and membrane-bound co-stimulatory molecules that augments the innate immune response and activates the adaptive immune system. The specific (adaptive) immune system consists of lymphocytes subdivided in T cells and B cells. B cells can produce antibodies (immunoglobulins, Ig) that have direct effects like neutralizing bacterial toxins, interfering with bacterial adhesion molecules and preventing viruses to enter host cells. Antibodies bound to the pathogen can amplify the effect of the non-specific immune system by activation of the complement system and by binding to immunoglobulin receptors on phagocytes, thereby improving phagocytosis. Furthermore, through surface Ig, B cells can ingest antigens, which after intracellular protein processing, leads to presentation of antigenic peptides to T cells.

T cells can be divided in CD4 positive and CD8 positive cells. To participate in the defence mechanism they need to be recruited by the non-specific immune system. T cells can be activated if their specific T cell receptors (TCR) recognize antigen-derived peptides but only if they are presented to them by antigen presenting cells (APC) in HLA class I (CD8 cells) or HLA class II (CD4 cells) molecules. Once activated the response of T-cells depends on an interplay of:

1. The quality and quantity of antigen specific signal.
2. The quality and quantity of additional signals from cells of the non-specific immune system or B cells.
3. The stage of differentiation of the T cell.
Ad 1. Naïve T cells are only activated if their TCR has sufficient affinity for the particular peptide MHC complex and the density of peptides on APC has reached a threshold for activation.

Ad 2. Activation of naïve T cells by the T cell receptor (TCR) alone will not initiate productive T cell activation but rather leads to cell-death, anergy or ignorance. However simultaneous triggering of for instance CD27\textsuperscript{15}, CD28\textsuperscript{6,7}, or LFA-1\textsuperscript{18-20} by their specific ligands on APC results in T cell proliferation and differentiation.

Ad 3. During T cell differentiation the cellular activation requirements change. A repeated trigger of the TCR is sufficient for activation, and a co-stimulatory signal is less crucial. In addition activation of naïve T cells results in proliferation and IL-2 production, while reactivation of differentiated T cells results in the execution of effector functions.

Differentiated CD8 positive T cells have principally a cytotoxic effect on tissue cells presenting foreign antigen-derived peptides in HLA class I molecules. The presence of these peptides reveals that these cells are infected by pathogens. Although CD8 cells may develop in cytotoxic effector cells in the absence of CD4 positive T cells, they have been shown to be more effective when supported by CD4 positive T cells\textsuperscript{11}.

Differentiated CD4 positive T cells have predominantly immunomodulating effects. Dendritic cells presenting antigen to CD8 cells can do this more effectively when they have first had an interaction with specific CD4 T cells. In the interaction between CD4+ T cells and dendritic cells the CD40-CD40ligand interaction plays an essential role\textsuperscript{12,13}.

In addition, differentiated CD4 T cells provide help for B cells to produce antibodies with a higher affinity and induce a shift from production of IgM to IgG, A and E. Apart from these modulating effects on the specific immune system CD4 effector cells can activate cells of the non-specific immune system like macrophages via the production of interferon gamma (IFN-γ) or via direct cell contact (e.g. CD40-CD40ligand, ICAM-LFA1)\textsuperscript{14,15}

Observations by Mosmann and Coffman in 1986\textsuperscript{16} revealed that different functions of murine CD4+ T helper cells seem to be executed by different T cell subsets. These are distinguished on the basis of the production of different sets of cytokines. So-called type-1 helper cells produce IL-2, interferon-gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α), while type-2 CD4+ cells produce IL-4, IL-5 and IL-10. Later similar subsets were found in humans as well\textsuperscript{17}. Prototypically human type-1 T cells produce IFN-γ while type-2 cells produce IL-4. Functional studies have shown that type 2 cells are more effective in providing B cell help, while both type-1 and type-2 cells have a regulatory effect on cells of the non-specific immune system. Type-1 cells are proinflammatory as they can enhance the activation of macrophages and their forerunners monocytes, resulting in more effective killing of ingested bacteria as well as the production of proinflammatory cytokines like TNF-α, IL-1 and IL-6\textsuperscript{18-20}.
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The different compartments of T cell subsets

Pathogens usually enter the body through the skin or mucous membranes. Locally an inflammatory response is generated by the innate immune system. If the pathogen is unknown there will be no production of specific effector T cells. To generate these, antigens have to be presented to naïve T cells by APC. Naïve human T cells can be identified by the expression of the CD45RA isoform and are receptive to costimulatory signals as they express CD27 and CD28. After their generation in the thymus they recirculate between the blood and lymphoid tissue using their homing receptors CD62L and CCR7. Professional APC are present both in peripheral tissues as well in T cell areas of the lymphoid tissue. The differentiation of pathogen-specific T cell effectors from naïve T cell takes place in these T cell areas when antigen is drained from the site of entry by the blood or lymph and is filtered by the lymph nodes or spleen, respectively when professional APC like dendritic cells pick up pathogens/antigens in the peripheral tissue and migrate to the nearest lymph node. Circulating naïve T cells will be trapped in the lymph node if their T cell receptor recognizes specific peptide/MHC complexes present on the APC. When adequately activated by the APC by additional costimulatory signals these cells will expand and differentiate into effector T cells. During this process the CD45RA isotype is downregulated while the CD45R0 isotype is upregulated. In parallel, molecules responsible for recirculation into the lymphnodes are diminished, while adhesion molecules and chemokine receptor molecules involved in homing to inflamed tissues are upregulated: alpha1, 2 integrins, CCR5 and CXCR-3 on type-1, CCR3 and CCR8 on type-2 T cells.

Whether T cells develop into a type-1 or type-2 phenotype depends on signals like IL-12 and IL-18 for type-1, and IL-4 for type-2 T cells. In turn whether APC produce type-1 or type-2 directing signals depends on properties of the pathogen i.e. binding to particular PRR as well as the innate defence mechanism of the host. Positive and negative feedback loops exist in T cell differentiation. Type-1 cells can amplify type-1 responses by producing IFN-γ and inhibit type-2 responses, whereas type-2 T cells can do the opposite.

Rheumatoid Arthritis

Rheumatoid arthritis is a syndrome of symmetric polyarthritis mostly involving the small joints of hands and feet. Although spontaneous remission can occur, it has the tendency to become chronic resulting in progressive damage to the cartilage and underlying bone structures of the joints. It can be concluded from incidence numbers that both genetic and environmental factors are involved in the pathogenesis. In the general population rheumatoid arthritis is found in 0.5% to 1% of individuals, while it can be found in 2% to 4% of people who have a sibling with rheumatoid arthritis. That also environmental factors are needed for the disease to develop can be suspected from the fact that it is only found in 12% to 15% of people who have an identical twin with rheumatoid arthritis.
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**Clues for the pathogenesis**

The underlying mechanisms resulting in development of rheumatoid arthritis are largely unknown. It can be considered that rheumatoid arthritis is an autoimmune disease since constant or intermittent inflammation is present in the synovium without evidence of an initiating pathogen. However, an autoantigen at which the autoimmune reaction is directed has never been established.

A clue for the involvement of the specific immune system in the pathogenesis of RA is the association of the disease with the "shared epitope". The shared epitope is a homologous sequence of amino acids in the antigen binding groove of several MHC class II molecules that are associated with the disease $^{36}$.

Clinical responses of the disease to TNF-α neutralizing therapies imply that TNF-α plays a substantial role in generation of the symptoms $^{37}$. TNF-α in the synovium is predominantly produced by synoviocytes and macrophages. Still it is not known what factors are initiating and sustaining the unbalanced production of this pro-inflammatory cytokine.

Differentiated T cells are capable of regulating inflammatory responses. Type-1 T cells which are abundantly found in the synovium can activate macrophages to produce inflammatory cytokines like TNF-α, while Type-2 cells can inhibit this effect. IL-4 producing cells are only found in small amounts in the joint, which has lead to the hypothesis that an imbalance between type-1 and type-2 T cells can contribute to the excessive TNF-α production in the synovium. Since it is not known whether an autoantigen is involved in the pathogenesis of rheumatoid arthritis, uncertainty exists about whether synovial T cells are antigen specific or just bystanders that are trapped by the abundance of TNF-α induced adhesion molecules and chemokines.

**Aim of the thesis**

In analogy to what has been found in animal models, it has been hypothesized that the balance between pro-inflammatory T$_{HELPER}^1$ and anti-inflammatory T$_{HELPER}^2$ cells plays an important role in (1) sensitivity for and (2) clinical course of autoimmune disease in humans. The experiments described in this thesis aimed to scrutinize this hypothesis in Rheumatoid Arthritis. For this a cohort study was performed in which T cell functions were analyzed longitudinally in patients who visited the Early Arthritis Clinic of the *Jan van Breemen Institute*.

The studies have focussed on five separate aspects:

1. Compartmentalization of T$_{HELPER}^1$ and T$_{HELPER}^2$ cells in inflamed synovium of RA patients (Chapter 2).
2. Markers of T cell differentiation in patient groups with other autoimmune diseases, i.e. SLE (Chapter 3) and M. Bechterew (Chapter 4).
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3. Effects of DMARD’s and anti-TNFα treatment on the T\(_{h1}\) and T\(_{h2}\) balance in the circulation (Chapters 5 and 6).

4. Relation of disease activity with markers of T-cell differentiation (Chapter 2, 8).

5. Predictive value of the T\(_{h1}\) and T\(_{h2}\) balance for disease progression (Chapter 7, 8).

A summary and conclusion of the experimental chapters will be given in Chapter 9.

References


