Targeting intracellular signaling pathways at the interface of T lymphocyte and innate immunity in immune-mediated inflammatory diseases
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Chapter 1

Introduction
Introduction

Rheumatoid arthritis

Rheumatoid arthritis (RA) is the most common inflammatory arthropathy with a prevalence of approximately 1% worldwide. It is a chronic systemic immune-mediated inflammatory disease which is clinically characterized by chronic (> 6 weeks duration) tenderness and swelling of primarily the small joints of the hand and feet, although other joints in the body are usually also affected. These symptoms, together with an elevated acute phase response and seropositivity for rheumatoid factor and/or anti-citrullinated protein antibodies (ACPA) constitute major components of the revised classification criteria for the diagnosis RA. The affected joints of RA patients are characterized by synovitis, which is a chronic inflammation of the synovial membrane. The synovial membrane in RA patients is characterized by a hypertrophic, highly vascularized layer with activated macrophages, lymphocytes, plasma cells and fibroblast-like synoviocytes (FLS). Due to chronic inflammation of the synovial tissue, associated with prominent angiogenesis, destruction of the adjacent cartilage and bone may develop, shown on radiographic images as erosions. Since the introduction of potent disease modifying antirheumatic drugs (DMARDs) and novel immune modulating therapies this process can be delayed or stopped in many patients. However, RA is still an important cause of long-term morbidity and early mortality.

Pathogenesis of RA

The exact aetiology of RA is unknown. It is a heterogenous disease in which, in addition to genetically inherited and acquired factors (e.g., infection or trauma), also environmental factors play an important role. The development of autoimmune disease requires the breakdown of immunologic self-tolerance that usually controls self and non-self discrimination, for example in auto-immune diabetes. In RA, a very important etiological factor is the presence of circulating ACPA in the serum of patients, autoantibodies directed against a diverse number of citrullinated proteins. Citrullination is a posttranscriptional enzymatic modification of the amino acid arginine. The first proteins that were discribed to be citrullinated were fibrin and fibrinogen but more recently citrullinated collagen type I and II, vimentin and histones have been identified. Citrullination of peptides is a physiological process which normally does not lead to chronic inflammation. Citrullinated peptides can be found in any form of inflammation, including different forms of arthritis. However in the context of a specific genetical predisposition this may lead to autoimmunity. Some individuals bear specific alleles of the human leukocytes antigen (HLA) -molecules (more specifically, DRB1 and DR4 alleles), which are able to present citrullinated protein fragments to T-cells more efficiently than others. This may provoke an immune response and may ultimately lead to the generation of anti-antibodies.
against this citrullinated protein. When ACPAs are detected using the cyclic citrullinated peptide (CCP) test, they are termed anti-CCP antibodies. Cigarette smoking is one of the important environmental risk factors for the development of these autoantibodies: carriers of HLA-DRB1 alleles have a more than 15x higher chance of generating ACPA when they are smokers. In about 75% of the established RA patients, ACPA are present in the serum, have a very high specificity (92-96%) for RA, and, interestingly, can be detected in the serum of RA patients years before the onset of clinical disease. However, not all patients with RA develop ACPA. Taken together, major contributors to the onset of RA are genetic factors in combination with other factors such as smoking and obesity which lead to longstanding autoantibody production against certain citrullinated proteins present in the synovium of patients.

Synovial inflammation
The synovial membrane, which is the principal site of pathological changes in patients with RA, is normally < 100 um thick and consists of 2 distinct compartments: a thin intimal lining layer and a thicker sublining layer. The intimal lining layer is in direct contact with the intraarticular cavity and plays an important role in the production of synovial fluid. Under normal conditions, this layer is only one to three cell layers thick and consists of macrophages and fibroblast-derived synoviocytes. The synovial sublining consists of connective tissue, synovial blood vessels and normally contains very few cells. In RA however, the sublining layer is heavily infiltrated with inflammatory cells such as macrophages, T/B lymphocytes, plasma cells, dendritic cells, natural killer cells, dendritic cells, neutrophils, mast cells, and endothelial cells. The activated immune cells produce high quantities of proinflammatory cytokines (eg., tumor necrosis factor (TNF)-alpha, interleukin (IL)-1, IL-12, IL-23), chemokines and matrix-degrading enzymes, such as matrix metalloproteinases (MMPs), leading to inflammation. In chronic inflammatory conditions, the synovium becomes hypertrophic (also called pannus at the site of invasion) and promotes cartilage degradation and bone destruction. Enhanced angiogenesis, the formation of new blood vessels, is crucial in this inflammatory process, providing sufficient nutrients and recruiting new cells into the synovium.

Treatment targets in RA
The treatment options for RA have changed dramatically over time. Before the introduction of corticosteroids in the 1950s, patients with RA were treated with gold salt injections, blood letting, fasting and even electric convulsion therapy. Morbidity and mortality improved significantly after the introduction of DMARDs. There are several conventional DMARDs, of which methotrexate is most widely used. It was developed as a chemotherapeutical agent to treat acute leukemia; in much lower doses it is beneficial and cost-effective for patients with RA. However, it is not effective for every patient, there is often not sufficient inhibition of progression of joint destruction.
and, due to its gastro-intestinal side effects, many patients do not tolerate the drug. A new exciting development within rheumatology was the introduction of biologic agents in 1998. These are produced using bioengineering techniques and are characterized by a targeted mechanism of action. Currently used biologic agents act as inhibitors of IL-6 (tocilizumab) or TNF (infliximab, adalimumab, etanercept, golimumab, certolizumab). Other mechanisms of action of effective targeted therapies interfere with the T cell costimulatory molecules (abatacept) or B cells (rituximab). In the last 10 years it has become clear that early initiation of treatment is crucial to prevent tissue damage and complications. Rheumatologists now start treatment with conventional DMARDs at the earliest practical point to achieve a state of disease remission, and if this fails, biological treatment are considered according to emerging treatment algorithms. Early-goal directed treatment has improved disease outcome and long-term comorbidity dramatically.

IL-12 and IL-23 as therapeutic targets in RA

The IL-12 family of cytokines, including IL-12 and IL-23, are important mediators of immune-mediated inflammatory diseases, such as psoriasis, multiple sclerosis, Crohn’s disease, and RA. IL-12 and IL-23 are heterodimeric cytokines composed of the common 40 kDa subunit (p40) and a unique 35 kDa subunit (IL-12 p35) or 19 kDa subunit (IL-23 p19). The transmembrane receptor complexes for IL-12 and IL-23 consist of a common protein, IL-12RB1, and alternate subunits, IL-12RB2 or IL-23R, respectively. Both receptors which are expressed on activated T cells, natural killer cells, dendritic cells and macrophages interact with members of the Janus kinase and signal transducer and activator of transcription (JAK-STAT) pathway to mediate signal transduction to the cell nucleus. IL-12 primarily affects naïve T cells and initiates the early phase of inflammation by provoking a pro-inflammatory Th1 response, leading to the secretion of IFN-γ. On the other hand, IL-23 regulates the later phase of the Th1 response by inducing proliferation of memory CD4+ T cells, thereby maintaining the inflammatory process. Another role of IL-23, and not IL-12, is promoting the presentation of immunogenic peptides by antigen-presenting cells, implicating a causal relationship with autoimmune diseases. And finally, IL-23 induces proliferation of IL-17 producing T cells (Th17 cells). IL-17 is significantly elevated in RA synovium, plays a key role in the development of the murine collagen-induced arthritis model of RA and is a potent stimulator of osteoclastogenesis. Inhibition of IL-12 and IL-23 might have therapeutic applications because of their role in linking the innate and adaptive immune responses.

Experimental treatment targets in RA

Because a substantial proportion of RA patients does not respond to currently available treatments there is a growing interest in developing alternative mechanisms of action. It
has become clear that persistent activation of intracellular signalling pathways regulated by cell-cell contacts, several growth factors, chemokines and inflammatory cytokines present in RA synovial tissue, maintains the inflammatory cascade. Important roles for mitogen-activated protein (MAP) kinase and nuclear factor κB (NF-kB) signaling pathways in RA are well documented, and efforts to target Janus kinase (JAK) and spleen tyrosine kinase (Syk) have recently been successful. Another important signaling route is phosphatidylinositol 3-kinase (PI3K), an enzyme which upon activation of its downstream target protein kinase B (PKB) regulates crucial processes such as cell growth, differentiation, survival and proliferation. Evidence for deregulated PI3K signalling in RA is the overexpression of phosphorylated PKB in RA synovial tissue and FLS compared to cells derived from osteoarthritis patients. Enhanced phosphorylation of FoxO4, a downstream target of PKB, in synovial macrophages of RA patients has been observed as well. Furthermore, the expression of the negative regulator of PI3K activity, PTEN, is depressed in RA synovial tissue, whereas synovium of non-arthritis individuals shows clear staining.

Angiogenesis

Angiogenesis is a physiological process which consists of the outgrowth, sprouting or remodelling of blood vessels. It is essential for the early stages of growth and development in embryos, but also crucial later in life for regeneration, wound healing and female reproductive function. However, angiogenesis is also involved in the pathogenesis of different conditions like malignancies, proliferative diabetic retinopathy and chronic inflammatory diseases. In patients with RA, as well as in other IMIDs such as spondyloarthritids, increased vascularity and new blood vessel formation has been demonstrated, which further promotes synovial inflammation of the affected joints through the influx of nutrients and inflammatory cells. Due to hyperplasia of the synovial tissue, local areas of hypoxia are formed, and in combination with increased pro-inflammatory cytokines production (e.g., TNF and IL-1), these processes are a driving force of increased angiogenesis.

Tie2 receptor and angiopoietins

While members of the vascular endothelial growth factor (VEGF) family are required for the initial steps of vasculogenesis, angiopoietins are more important in later stages of vessel and lymphatic maturation, remodeling, and stabilization. The receptors for angiopoietins are the tyrosine kinase receptors Tie1 and Tie2 (also known as Tek). The ligands of Tie2 receptor are the angiopoietins (Ang) 1-4, of which Ang-1 and Ang-2 are best characterized. The exact molecular mechanisms of how Tie2 signaling elicits downstream effects are not known. There is evidence that upon binding of Ang1 in endothelial cells, the Tie2 receptor dimerizes, thereby allowing activation of the kinase domain which leads to autophosphorylation of specific tyrosine residues. These
residues serve as docking sites for tyrosine phosphatase SHP2 and the adaptor protein GRB2, which in turn activate several downstream signalling pathways such as PI3K/ PKB, MAPK/ERK and JAK/STAT. The Tie1 receptor, most probably acts as a negative regulator of Tie2 by forming heterodimers with Tie2. Dislacement of Tie1 by Ang-1 allows Tie2 to dimerize and become activated. In endothelial cells, activation of Tie1 occurs via ligation of phorbol esters, VEGF and inflammatory cytokines, such as TNF, resulting in endoproteolytic cleavage of the ectodomain and subsequently releasing the ligand-binding domain of the receptor. This soluble Tie1 is able to modulate Tie2 activation. Silencing of Tie1 by RNA interference or shedding of the Tie1 extracellular domain, enhances Ang-1 dependent Tie2 activation. On the other hand, Tie1 can also activate Tie2 receptor via trans-phosphorylation, which occurs upon VEGF-induced Tie1 cleavage.

The importance of Tie receptors and angiopoietins has been studied both in vitro and in vivo. In animal models, genetic deletion of Tie2 and Ang-1 resulted in severe defects of vascular remodelling and angiogenesis, leading to embryonic lethality. In diabetic mice, treatment with recombinant Ang-1 protein enhances wound healing associated with increased angiogenesis, lymphangiogenesis and blood flow. In vitro, Ang-1 also has anti-permeability and anti-inflammatory functions in endothelial cells. In an LPS-induced septic shock model, mice treated with an adeno viral construct encoding Ang-1 display an enhanced survival rate accompanied by an improvement in the hemodynamic function and a lower expression of inflammatory adhesion molecules. Ang-2, on the other hand, has a dual role both as a pro-angiogenic agonist and as a natural antagonist for Ang-1 function, depending on the experimental and cellular context. Evidence supporting the latter is that Ang-2 transgenic overexpression leads to a similar developmental phenotype to that observed in Ang-1 or Tie2 deficient mice. Moreover, in certain conditions, especially when VEGF-A levels are reduced, Ang-2 is associated with vascular regression instead of vascular remodelling. Although Ang-2 binds Tie2, it usually does not lead to autophosphorylation of Tie2 in endothelial cells, unless it is used at high concentrations or with prolonged exposure time. The proangiogenic role of Ang-2 has been elucidated in mice overexpressing Ang-2 in colon cancer cells, ultimately leading to enhanced angiogenesis and tumor growth compared to controls. Similarly, therapeutic inhibition of Ang-2 suppresses angiogenesis and growth of tumors.

Tie2 signaling in inflammatory arthritis

Ang-1, Ang-2 and Tie2 are detected in the synovial tissue of patients with RA and psoriatic arthritis (PsA) and are closely associated with disease activity and joint destruction. Supporting this clinical association, treatment of endothelial cells with Ang-1 or Ang-2 stimulates MMP-9 production. Under normal conditions, Ang-1 is produced by many different, mainly non-endothelial cells while the primary source of...
Ang-2 is endothelial cells. Tie2 and Ang-1 expression are increased in human RA synovium and TNF upregulates Tie2 in endothelial cells and Ang-1 in synoviocytes. In chronic inflamed synovial tissue, Ang-2 is predominantly and highly expressed in rheumatoid FLS (70 to 120 fold increase compared to normal FLS). Moreover, successful treatment of inflammatory arthritis is associated with decreases in systemic and local expression of not only VEGF, but also Ang-1, Ang-2, adhesion molecules and endothelial markers, suggesting that targeting angiogenic processes has therapeutical potential in the treatment of established arthritis. In line with this, induction of apoptosis in newly formed blood vessels suppresses synovial inflammation in the murine collagen-induced arthritis (CIA) model. Of importance, blockade of Tie2 signaling is sufficient in vivo to prevent angiogenesis and joint destruction in this model. Interestingly, conditional overexpression of Tie2 in mice in a tetracycline-controlled manner results in a reversible psoriasis-like phenotype. Whether Ang-1 and Ang-2 make independent contributions to the pathology of RA and have potential therapeutical effects is as yet unclear.

As Tie2 expression was thought to be primarily restricted to endothelial cells and hematopoietic stem cells, the observation of a specific subset of Tie2-expressing monocytes (TEMs) in human peripheral blood and bone marrow, was an important finding. Whereas only 1-2% of leukocytes express Tie2, a substantial fraction of circulating monocytes express Tie2 (app. 20%). TEMs infiltrate and appear to play a critical role in the establishment and growth of solid murine and human tumors. Moreover, the selective elimination of TEMs impairs angiogenesis in mouse tumors and induced substantial tumor regression. Although a role for TEMs in immune-mediated inflammatory diseases has not been examined, Tie2 expression by synovial macrophages in RA has been previously observed.

Macrophages

Macrophages are myeloid cells, originating from the bone marrow, which differentiate from circulating monocytes. Macrophages are present in almost all tissues, for instance in the bone as osteoclasts, in the alveoli of the lungs, in the central nervous system (as microglial cells), in connective tissue (as histiocytes), and in the liver (as Kupffer cells). Under physiological conditions, macrophages play an important role in tissue homeostasis, phagocytosing apoptic cells to prevent tissue damage. They also phagocytose pathogens, and can initiate the activation of the adaptive immune system in their role as antigen-presenting cells, stimulating lymphocytes to respond to pathogens. Macrophages sense danger via specific receptors, the so called ‘pattern recognition receptors’ (PRR). When these receptors encounter bacterial lipopolysaccarides (LPS) or other pathogen-associated molecular patterns (PAMPs), an intracelular signaling cascade is initiated which leads to activation of macrophages.
Introduction

Chapter 1

Macrophage polarization

Macrophages are a very heterogenous population and are able to change their physiology in response to their environment, thereby displaying an enormous plasticity. As proposed by Mosser and Edwards\textsuperscript{109}, macrophages can be classified based upon their three main functions: host defense, wound healing and immune regulation. The classically activated macrophages involved in host defense (also known as m1 macrophages) arise \textit{in vitro} in response to pro-inflammatory cytokines, such as interferon-y (IFN\textgamma), while the latter two (also known as m2 macrophages) arise in response to the anti-inflammatory or immune regulatory cytokines IL-4 and IL-10, generating wound healing and regulatory macrophages, respectively\textsuperscript{107;110;111}. Granulocyte macrophage stimulating factor (GM-CSF) gives rise to macrophage populations that tend to produce higher levels of pro-inflammatory cytokines (e.g. TNF-a, IL-23) and lower levels of immune regulatory cytokines (e.g., IL-10) than macrophage colony stimulating factor (M-CSF)\textsuperscript{112;113}. GM-CSF-differentiated macrophages, known as classically activated macrophages, share many properties with m1 macrophages, while alternatively activated macrophages differentiated in M-CSF express a more anti-inflammatory cytokine repertoire shared by m2 macrophages\textsuperscript{114}.

The role of macrophages in rheumatoid arthritis

Macrophages play a crucial role in the pathogenesis of rheumatoid arthritis. They contribute considerably to inflammation and joint destruction in early and later stages of the disease. As prominent producers of several pro-inflammatory cytokines, chemokines, growth factors, bone degrading proteins, and pro-angiogenic mediators, macrophages make up around 30-40\% of the cellular content in the inflamed synovium\textsuperscript{4;115}. They function as local and systemic amplifiers of disease severity by cell recruitment into the synovial tissue and activate lymphocytes and FLS by creating an inflammatory cytokine milieu or by cell-cell contact. Importantly, the degree of macrophage synovial infiltration correlates with joint pain and the general inflammation status of the patient\textsuperscript{4}, as well as with the radiological progression of joint damage\textsuperscript{116}. The decrease in numbers of synovial macrophages, especially among the CD68-positive sublining macrophages, is a biomarker of response in clinical trials of patients with RA\textsuperscript{117}, independently of the treatment type\textsuperscript{118;119}.

T-lymphocytes in immune-mediate inflammatory diseases

One consequence of macrophage activation in RA synovial tissue is the recruitment of T lymphocytes and their subsequent activation. Several chronic inflammatory diseases, including RA and multiple sclerosis (MS) are classically considered as T cell mediated autoimmune diseases because of their genetic association with the expression of specific major histocompatibility complex (MHC) class II molecules\textsuperscript{120}. Shared epitopes present on HLA-DR1 and HLA-DR4 alleles of RA patients are thought to present arthritogenic
peptides to lymphocytes which contribute to pathogenesis\textsuperscript{121} and disease severity\textsuperscript{122}. Another mechanism of action for the development of autoimmunity is the presence of autoreactive T cells which recognize auto-antigens as foreign substances. In the thymus, thymocytes that are capable of strongly binding with “self” peptides presented by MHC, will be deleted (‘negative selection’), allowing for tolerance of self by the immune system. However, when some of these T cells escape this process autoreactive T helper (Th) T cells will arise which will be able to stimulate B cells, leading to auto-antibody production. As already mentioned earlier, another T cell subset known to play an important role in RA are the pro-inflammatory IL-17 producing Th17 cells, which potently activate many cell populations in the synovial tissue. A final T-cell subpopulation with unique properties and capable of downregulating or suppressing functions of other cells, are regulatory T cells\textsuperscript{123}. Regulatory T cells, characterized as CD4+CD25+FoxP3+ cells, are actively able to regulate the responsiveness of autoreactive T cells that escape negative selection \textit{in vivo} and prevent the development of experimentally induced autoimmune diseases\textsuperscript{124-126}.

In the synovium, T cells can activate neighbouring macrophages via cell-cell contact, which in turn are responsible for TNF, IL-1\textbeta and MMP production\textsuperscript{127-129}. Interaction of T cells with FLS induces IL-6, IL-8 and MMP-1 production\textsuperscript{130;131}. However, no significant synovial tissue T cell cytokine production has been observed in situ\textsuperscript{132;133} or in freshly isolated synovial fluid T cells from RA patients\textsuperscript{134}. The lack of T cell cytokine production or proliferation in synovial T cells has led to the idea that TCR signaling is repressed and that synovial T cells contribute to pathology by TCR-independent mechanisms\textsuperscript{120}. Although synovial T cells express markers of recent activation such as CD69 and HLA-DR and are charaterised as highly differentiated CD45RO+ T cells\textsuperscript{135;136}, there is also evidence that a chronic inflammatory milieu alters the function of activated T cells. Chronic TNF exposure resulted in suboptimal expression of the IL-2R alpha chain\textsuperscript{137} and downregulation of TCRzeta chain expression\textsuperscript{138} which causes an impaired TCR/CD3 assembly and potential hyporesponsiveness of the T-cell\textsuperscript{139}. T-cells require two stimulatory signals to get fully activated: first, the antigen-specific TCR-MHC engagement and second, a costimulatory signal by the interaction of CD28 on T cells to either CD80 or CD86 on antigen-presenting cells. Ligation of Cytotoxic T-lymphocyte-associated antigen-4 (CTLA4), the high-avidity receptor for both CD80 and CD86\textsuperscript{140;141}, prevents the delivery of the second costimulatory signal that is required for optimal T cell activation. Abatacept, CTLA4Ig treatment has been developed as a novel therapeutic approach and significantly improves signs and symptoms in RA patients\textsuperscript{142}.

The role of T cell in animal models of arthritis and multiple sclerosis

The CIA model is a commonly used animal model beacuse it shares immunological and pathological similarities to human RA\textsuperscript{143-145}. Arthritis is induced in a mouse or
rat by immunization with type II collagen in adjuvant. Susceptibility to CIA is strongly associated with MHC class II genes, which suggests the involvement of CD4+ T cells in the pathogenesis, similar to human RA. The development of arthritis is caused by a robust T- and B-cell response to type II collagen leading to synovial hyperplasia, cellular infiltration and ultimately to articular cartilage degradation and bone destruction. Another important feature that CIA and RA have in common is the expression of TNF and IL-1β in the inflamed joints and the finding that blockade of these cytokines reduces clinical and histological severity of the disease146.

Experimental autoimmune encephalomyelitis (EAE) is an inflammatory autoimmune demyelinating disease of the central nervous system (CNS); the animals develop a disease process that closely resembles human multiple sclerosis (MS). The mice are injected with the whole or parts of proteins that make up myelin, which is the insulating sheath that surrounds nerve cells (neurons). One of these peptides is myelin oligodendrocyte glycoprotein (MOG) which is injected in a 2D2 (MOG-specific) transgenic mouse model147 whereby MOG-specific auto-reactive T cells can be tracked because of their expression of a specific T-cell receptor. While in CIA B-cells play an important role in the pathogenesis through the induction of anti-collagen antibodies148, EAE is mediated by pathogenic autoreactive T cells without a major contribution of autoantibodies, supported by the role of pathogenic T cells in transfer experiments149. Similar to EAE, in CIA effector CD4+ T cells produce pro-inflammatory cytokines148. The role of Th17 cells in both models is crucial as both CIA and EAE disease development was markedly suppressed in IL-17-/- mice45;150. The role of Tregs in EAE has been controversial: a few studies describe the potential of Treg therapy in reducing disease severity151 although others describe that myelin-specific Tregs accumulate in the CNS but fail to control auto-immune inflammation152. In CIA, adoptive transfer of regulatory T cells slowed disease progression and interestingly Tregs could be found in the inflamed synovium soon after transfer, indicating local disease regulation153. To conclude, there are some similarities in the pathobiology of both disease models, but the main difference is that EAE is mainly a T-cell-driven disease while in CIA both B- and T-cells play important roles in the pathobiology.

Regulation of T cell activation by the small Ras GTPases

Small GTPases are intracellular molecular switches, turned on when bound to guanosine-triphosphate (GTP), and turned off when bound to guanosine-di-phosphate (GDP). In response to extracellular stimuli, guanine nucleotide exchange factors (GEFs) remove GDP, allowing GTP to bind to the GTPase. GTPase activating proteins (GAPs) catalyze conversion of GTP back into GDP, abrogating GTPase signalling154. The Ras superfamily of small GTPases consists of a group of more than 150 structurally related proteins which regulate multiple downstream signaling pathways thereby regulating cellular proliferation, survival, cytokine expression, trafficking and retention155. This large
superfamily can be further divided into at least six families: Ras, Rho, Ran, Rab, Rheb, and ARF, of which Ras is the prototypical member\textsuperscript{156}. In RA synovial tissue, abundant expression of Ras proteins can be found, predominantly in intimal lining layer cells at the site of bone erosions\textsuperscript{157}. The best-characterized signaling pathways activated by Ras family GTPases are the Raf/Mek/Erk pathway, the PI3K pathway, NF-\textit{kB} and the RapGDS signaling pathway. Of these Ras-effector pathways, MAP kinases and PI3-kinases are prominently activated in RA synovial tissue compared to disease controls\textsuperscript{49,158,158}. Overexpression of an adenoviral dominant-negative Ras mutant in FLS reduced proliferation, ERK activation and IL-1 induced IL-6 production\textsuperscript{159}. Moreover, inhibition of Ras family function \textit{in vivo} has been shown to be protective in experimental arthritis models\textsuperscript{159,160}.

Rap1 proteins

The related protein Rap1 (Ras-proximate-1), which shares approximately 50\% sequence homology to Ras, regulates multiple cellular functions via several coordinated signaling pathways. Upon activation \textit{in vitro} Rap1 mediates integrin activation, integrin-dependent chemotaxis, cadherin-mediated adhesion, and cell-cell junction formation\textsuperscript{161-163}. Rap1 was identified as antagonist of Ras-mediated signaling\textsuperscript{164}, because constitutively active Rap1 resulted in downregulation of ERK activation via binding to and inhibition of Raf1\textsuperscript{165,167}. More data supporting an immunosuppressive role for Rap1 comes from the notion that Rap1 suppresses Ras-dependent reactive oxygen species (ROS) production. ROS are proposed to act as second messengers in T cell activation\textsuperscript{168} and it has been proposed that chronic oxidative stress leads to an enhanced inflammatory response in T lymphocytes\textsuperscript{169}. Rap1 is also involved in regulating T cell responses to costimulatory signals. Rap1 is transiently activated upon TCR triggering and further enhanced by CTLA-4 engagement, whereas CD28 ligation blocks Rap1 activation\textsuperscript{170-172}. Furthermore, CTLA4 ligation activates Rap1 to antagonize ERK-dependent T-cell function\textsuperscript{171}. Experiments with knockout and transgenic mice have increased the knowledge about Rap1 function \textit{in vivo}. In T cells of mice, transgenically expressing activated Rap1, an enhanced integrin function is observed\textsuperscript{173}. Also, suppression of effector and memory T cell function, parallels the promotion of CD4+CD103+ regulatory T cell generation and function\textsuperscript{174}. Moreover, T cells of RapV12 transgenic mice, expressing constitutive active Rap1 in the T cell compartment, have increased adhesion which enhances T-cell function in conditions where TCR-MHC interactions are of low affinity\textsuperscript{173}. In contrast, inactivation of T cell Rap1, in mice transgenically overexpressing RapGAP1 results in age-dependent accumulation of hyperresponsive and activated memory T cells in lymphatic tissue\textsuperscript{171}. To conclude, Rap1 plays an important role in the qualitative responses of T cells \textit{in vivo}. 

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Outline of this thesis

In the first part of this thesis we studied the pathogenic role of the pro-angiogenic macrophage Tie2 pathway in RA. In chapter 2 we examined the targets of Ang signaling in RA synovial tissue, and the functional effects of Tie2 signaling on human peripheral-blood derived macrophages. We also studied the effects of Ang-2 neutralisation in murine CIA. As we identified synovial CD68+ and CD163+ macrophages as important targets of Tie2 signaling and we found that human macrophages functionally express Tie2 we extended our research question to other macrophage subsets. To address this research question, in chapter 3 we first aimed to systemically validate phenotypic surface markers for the three main polarized macrophages subsets in humans. In chapter 4 we studied the potential of macrophage Tie2 signaling in these three polarized macrophage subsets.

In the second part of this thesis we examined the role of Rap GTPase signalling in T-lymphocytes in immune-mediated diseases. In chapter 5 we explored the consequences of a constitutive active form of Rap1 within the T-cell lineage and assessed susceptibility and severity of arthritis. In chapter 6 we examined the qualitative and quantitative effects of sustained Rap1 function in the autoreactive T cell population of a MOG-induced transgenic mice model for EAE. And finally, in chapter 7 we assessed the safety, tolerability, pharmacokinetics and efficacy of an interleukin-12/interleukin-23 inhibitor in patients with active RA.
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Introduction


