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

This thesis concerns two main research objectives. In the first part we address the contribution of pro-angiogenic macrophage Tie2 signaling to the pathology of rheumatoid arthritis (RA). In the second part we studied the role of sustained T-cell Rap1 signaling in RA and multiple sclerosis (MS). The primary findings will be reviewed separately below.

Section I: The contribution of pro-angiogenic macrophage Tie2 signaling to pathology in rheumatoid arthritis

Rheumatoid arthritis is a chronic systemic immune-mediated inflammatory disease and is characterized by chronic tenderness and swelling of primarily the small joints of the hand and feet, together with an elevated acute phase response and seropositivity for rheumatoid factor and/or anti-citrullinated protein antibodies (ACPA). Due to chronic inflammation, the affected joints consist of a hypertrophic, highly vascularised layer with activated macrophages, lymphocytes, plasma cells and fibroblast-like synoviocytes (FLS). Macrophages play an important role in tissue homeostasis, phagocytosis of microorganisms and cell debris, antigen presentation, production of cytokines and other inflammatory mediators. The number of macrophages in the inflamed synovium correlates with clinical disease activity and significantly decreases after efficient therapy. Apart from the presence of inflammatory cells, angiogenesis, the formation of new blood vessels, is also crucial in the inflammatory process, providing sufficient nutrients and recruiting new cells into the synovium. Tie2 is a receptor for the angiogenic factors angiopoietin (Ang)-1 and Ang-2, which is important in later stages of vessel and lymphatic maturation, remodelling, and stabilization. Tie2-expressing monocytes (TEMs), a subset of monocytes representing 2-7% of the total human peripheral blood mononuclear cells, are essential for tumor neovascularisation, cancer progression and are actively recruited to solid tumors. Tie2 is also identified on RA and psoriatic arthritis (PsA) synovial endothelial cells, FLS and macrophages. However, we do not know where Tie2 is activated in the synovial tissue and to what extent Tie2 activation contributes to pathology in RA.

In chapter 2 we found that synovial macrophages are the primary targets for Tie2 activation, while only limited Tie2 activation was observed on endothelial cells and FLS. Moreover, we observed that Tie2 is functionally expressed on in vitro cultured macrophages. In these cells, Tie2 activation by Ang-1 or Ang-2 in combination with TNF enhanced several pro-inflammatory effects, such as IL-6 and macrophages inhibitory protein (MIP)-1α production, and suppression of thrombospondin (TSP)-2 production. Interestingly, neutralization of Ang-2 is sufficient to reduce pathology in murine CIA. Because we observed an important role for macrophage Tie2, we further extended our study to explore the relationship between macrophage polarization and Tie2 expression.
and function in detail. Macrophages can broadly be classified into classically activated (M1) or alternatively activated (M2) with more immunosuppressive and wound healing capacities. In order to facilitate the study of macrophage subsets in vivo, we aimed in chapter 3 to systematically validate phenotypic surface markers for the three main polarized macrophages subsets in humans. We validated CD80 as the most robust marker for MΦIFN-γ (M1 macrophage), CD200R for MΦIL-4, and CD163 and CD16 for MΦIL-10 (both M2 macrophages). In chapter 4 we observed that Tie2 expression is not restricted to pro-inflammatory M1 nor immunoregulatory M2 macrophages. When expressed and activated, macrophage Tie2 signaling can cooperate with TNF to promote pro-inflammatory and pro-chemotactic gene expression, independently of macrophage polarization conditions.

Section II: The role of Rap1 GTPase signaling in immune-mediated inflammatory diseases

Rap1 is a member of the Ras superfamily of small GTPases. These cytosolic enzymes are able to switch a signal transduction chain on and off and thereby couple extracellular stimuli to several cellular responses. T cell Rap1 is activated by signaling via the T cell receptor (TCR), chemokines and several adhesion proteins. Active Rap1 plays a dominant role in cell adhesion and cell junction formation by regulating the function of integrins and other adhesion molecules which are needed for T cell trafficking and adhesion to antigen-presenting cells. Moreover, under certain conditions, active Rap1 suppresses TCR-dependent ERK (extracellular signal-regulated kinase) activation and IL-2 production. A potential role for Rap1 in RA is suggested by the fact that Rap1 is inactivated in RA SF T cells. Rap1 inactivation by CD28 can be circumvented by CTLA4 ligation or interference with CD28 activation using soluble CTLA4 (Abatacept treatment), leading to clinical improvement in RA. Altogether, there is evidence that maintaining T cell Rap1 function might be an interesting therapeutic approach in immune-mediated inflammatory diseases (IMIDs).

In chapter 5, we determined whether maintenance of T cell Rap1 signalling limits inflammation and joint destruction in an experimental model of RA (collagen-induced arthritis) by using transgenic mice expressing the hypermorphic mutant of Rap1a (RapV12). These RapV12 T-cells express RapV12 levels, which are equivalent to endogenous Rap1. We show that, compared to wild type (WT) mice, disease incidence, inflammation, and cartilage and bone damage were significantly reduced in RapV12 mice. During the onset of arthritis we observed a very limited TNF production in RapV12-transgenic T cells, whereas other cytokines such as IL-2, IFN-γ, IL-18 and IL-10 were not affected. A second explanation for the protective effect is a failure to upregulate the costimulatory proteins, ICOS and CD40L, in T cells of RapV12 transgenic mice. Together with our observation of a reduced anti-collagen antibody production in vivo, this points to a defect in the generation of follicular T cell help needed to promote B cell immunoglobulin class
switching. To determine whether the protective effect of RapV12 is specific for CIA or is a more general effect of immune-mediated inflammatory diseases (IMID), we assessed in chapter 6 the effect of T-cell RapV12 mutant expression in myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE), an experimental model for MS. The advantage of this experimental setup is that by using 2D2 single transgenic and 2D2RapV12 double transgenic mice, 85% of the total T cell pools are autoreactive T cells, which can be tracked and studied by their TCRs, which are specific for the immunodominant epitope MOG. In line with the clinical data in CIA, we observed that RapV12 attenuates EAE, although we only observed a protective effect for survival, not for disease incidence and severity. This modest clinical effect can not be explained by changes in qualitative responses (because auto-reactive T cell proliferation, cytokine production and survival are enhanced in mice expressing RapV12), but interestingly, the MOG-autoreactive T cell pool was reduced by approximately 30% in 2D2RapV12 animals. Because a similar reduced effect on autoreactive tetramer positive cells was also observed in RapV12 transgenic mice, this suggests that Rap1 signaling affects central tolerance. Possibly, sustained Rap1 function promotes negative selection of autoreactive T cells by strengthening thymocyte-APC interactions.

In chapter 7 we describe the results of a phase IIa, randomized, double-blind, placebo-controlled clinical trial with 29 patients active RA patients who were treated with either apilimod mesylate or placebo, in combination with methotrexate (3:1 ratio of apilimod-to placebo-treated patients). Apilimod is an orally administered small molecule, which inhibits IL-12/23 production. Treatment with apilimod did not induce robust clinical improvement. And consistent with the clinical results, apilimod did not have an effect on the expression of CD68+ synovial macrophages, which previously was shown to be a sensitive biomarker for clinical response. Although we and others demonstrate that IL-12/IL-23 inhibition with the small molecule apilimod does not provide a robust clinical effect, targeting IL-12/IL-23 with monoclonal antibodies, has been shown to provide significant clinical benefit in immune-mediate inflammatory disease such as Crohn’s disease and psoriasis.

To conclude, this thesis provides evidence that the proangiogenic Tie2 receptor is activated and functionally expressed on synovial macrophages and various polarized macrophages subsets. Activation of Tie2 by its ligands Ang-1/Ang-2 enhances pro-inflammatory gene and protein expression while Ang-2 blockade in murine CIA significantly reduces disease incidence and pathology. In future studies, it will be interesting to determine if Ang-1 and Ang-2 similarly cooperate with immunosuppressive agonist to regulate macrophage activation. Given the plasticity of macrophage function in complex immune-mediated inflammatory diseases, it will be of interest to determine if blocking macrophage Tie2 signaling may be a potential therapeutic target beneficial in diseases in which macrophages play a pivotal role, such as RA, other forms of arthritis,
psoriasis, Crohn’s disease, and multiple sclerosis. Concerning our second research objective, we demonstrate that the biological effects of sustained Rap1 activation are diverse, not restricted to a specific T cell subpopulation, and are of potential therapeutic interest in the treatment of IMIDs. However, the precise molecular mechanism of how Rap1 function modulates immunological processes is unknown and remains to be determined. Since disruption of CD28 costimulation, by Abatacept treatment, has proven clinical efficacy in the treatment of RA, identification of other T cell costimulatory proteins that regulate Rap1 activity may aid in the development of therapeutic strategies to suppress or augment T cell responses in IMIDs.