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ENGLISH SUMMARY

Summary

Rheumatoid arthritis (RA) is a chronic autoimmune disease for which no cure is yet available. At the moment therapies achieving clinical success are not effective in all patients, and occurrence of undesirable side effects may lead to the discontinuation of treatment. Therefore, there is still a need to develop novel therapies which may result in clinical benefit in patients who do not respond well to current therapies. In this context, investigating cellular mechanisms that are altered in RA may contribute to the development of these new therapeutic strategies.

Small GTPases are important mediators of the immune system. When a cell receives an external trigger, small GTPases are transiently activated and transmit signals that result in a cellular response. Abnormal GTPase function may thus alter proper cellular function. In this thesis we analyzed the contributions of representative small GTPases to inflammation and joint destruction in cells from RA patients and animal models of arthritis.

Previous studies in RA synovial T cells have shown several defects downstream of the T cell receptor (TCR) such as constitutive inactivation of the small GTPase Rap1, chronic downregulation of TCRzeta expression and impaired phosphorylation of TCR downstream proteins. While many of those reports described T cell responses in RA to be defective, we demonstrate in **chapter 2** that T cells isolated from RA synovial fluid are fully functional and capable of initiating proliferation and cytokine production upon TCR triggering. We show that previous studies concluding that there were defects in RA synovial T cells were compromised by increased spontaneous apoptosis of these cells upon removal from the joint. We show that there are no defects in RA synovial T cell TCR signaling. Furthermore, we found that altered ratios of pro-apoptotic Noxa and anti-apoptotic Mcl-1 expression were associated with the increased susceptibility of these cells to apoptosis. These findings suggested two important conclusions. First, the previously noted defects in TCR pathway components, such as Rap1, might contribute to disease in RA. Second, since only low levels of T cell cytokines are found in the joints of established RA, our findings suggest that in the effector phase of disease, T cells might contribute to inflammation by TCR-independent mechanisms.

T cells derived from the synovial fluid of RA patients have a block in Rap1 activa-

tion. **In chapter 3** we evaluated whether constitutive T cell Rap1 activation would be able to suppress disease in an animal model of RA. Using transgenic (Tg) mice expressing constitutively active Rap1 we performed collagen-induced arthritis (CIA) experiments and observed that Tg mice were significantly protected from arthritis development and severity. We found that Tg T cells with constitutive active Rap1 had defective production of the pro-inflammatory cytokine TNF-α. Additionally, Tg T cells were not capable of providing proper help for B cell activation and production of pathogenic anti-collagen antibodies. This was associated with diminished up-regulation of ICOS and CD40L T cell co-stimulatory molecules. The results from this chapter show that enhancement of T cell Rap1 activation might be an attractive strategy for therapy in RA.

The small GTPase Rap1 is inactivated by GTPase-activating proteins (GAPs). In **chapter 4** we show that the five Rap1GAPs are differentially expressed in resting and TCR/CD28 -activated T cells. We demonstrate that Spa-1 upregulation occurs after TCR/CD28 specific signals and is PI3-kinase and NF-κB dependent. The data in this chapter suggests that each Rap1GAP protein may differentially contribute to T cell activation. We propose that modulation of distinct Rap1GAPs may allow the specific regulation of Rap1 activation in diseases such as RA, where a block in Rap1 is observed.

Small GTPases are activated by guanine nucleotide exchange factors (GEFs). Changes in the expression patterns of these activators might therefore result in altered cellular responses. In **chapter 5** we explored the expression levels of Ras guanine nucleotide-releasing factor (RasGRF) 1, a specific activator of the GTPase H-Ras, in RA synovial tissue (ST) and fibroblast-like synoviocytes (FLS). We found RasGRF1 expression to be significantly increased in RA ST and to co-localize with matrix metalloproteinase (MMP)-1 and MMP-3 expression. In vitro modulation of RasGRF1 expression in RA FLS was able to regulate MMP-3 production. These findings indicate that modulation of GEF activation or expression levels might be of interest in protecting against joint destruction in RA.

The small GTPase Rac1 regulates many of the cellular processes required for the perpetuation of inflammation and joint destruction in RA. In **chapter 6** we treated animals with Rac1 inhibitory peptide and analyzed effects of this peptide on arthritis development. Treated animals displayed less swelling of the paws and reduced circulating levels of anti-collagen antibodies. We found, however, no amelioration in

bone damage. These results suggest that while Rac1 inhibitory peptide may be used to reduce auto-antibody production, additional studies targeting Rac2 as well, for instance, might achieve greater therapeutic efficacy.

In conclusion, the studies provided in this thesis show that modulation of small GTPase function regulates cellular activation and inflammation in RA and animal models of the disease. Small GTPases are therefore emerging targets in RA and further studies should explore the development of novel small GTPase modulators and evaluate their potential in the treatment of patients with RA.