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Rheumatoid arthritis (RA) is a chronic inflammatory joint disease leading to destruction of cartilage and bone. The inflammation in the joints is mainly caused by inflammatory cytokines that are over-produced by various types of immune cells. Artritis is an autoimmune disease that is characterized by the presence of autoantibodies. These autoantibodies form immune complexes (IC) which are other important players in joint inflammation because they activate various immune cells by binding to Fcγ receptors (FcγR). Binding and activation of FcγRs initiates intracellular signaling that triggers activation and release of various inflammatory mediators. In this thesis we describe a variety of aspects of arthritis research that has been performed to get a better understanding of the underlying molecular and cellular disease mechanisms and to develop novel therapeutic strategies.

Interleukin 1 (IL-1) is one of the most important pro-inflammatory cytokine that plays role in the pathogenesis of arthritis so to keep IL-1 levels under control might be a good therapeutic strategy. In order to achieve this we used a method called antisense oligonucleotide (AON) mediated exon skipping (Chapter 3). This method allows skipping of an exon by interfering with pre-mRNA splicing. The AONs binds to the target regions on the exon and prevent recognition of this region by the splicing machinery and as a result, the target exon is removed together with the flanking introns. We applied this technique on the exon that encodes the transmembrane region of IL-1 receptor accessory protein (IL-1RAcP), which leads to formation of a novel soluble form of the protein. IL-1RAcP is an indispensable subunit of a functional IL-1R. This newly formed soluble IL-1RAcP protein will act as an inhibiting IL-1 scavenger. We have shown that this concept works in-vitro and in-vivo, and in addition we showed that the newly formed protein is functional. We have targeted liver hepatocytes with the IL-1RAcP specific AONs to increase production of the novel soluble IL-1RAcP protein in vivo. In order to achieve higher cell type specific delivery and uptake by hepatocytes, we conjugated AONs to lipid-based nanoparticles. The success of AON based RNA modulation therapies in the clinic, strongly depends on the efficacy of cell/tissue specific delivery of the oligonucleotides. Our experiments showed that AONs mediated exon-skipping can be efficiently used to redirect IL-1RAcP pre-mRNA splicing to produce a novel soluble

form of IL-1RAcP that can bind excess IL-1 to control the in vivo levels of this cytokine as a therapeutic strategy in inflammatory diseases.

Fcγ receptors are cell surface receptors, which bind to the constant regions (Fc) of immunoglobulins (Ig) of the IgG class. The inhibitory Fcγ receptor, FcγRIIb, is expressed on almost all immune cells such as macrophages, neutrophils and B cells and plays a role in the regulation of immune responses at different levels. FcγRIIb on myeloid cells, modulates the activation of these cells by immune complexes. On B cells, FcgRIIb is involved in a negative feedback mechanism that controls B cell activation, proliferation and antibody production. Therefore inactivation of FcγRIIb results in uncontrolled antibody production and increased myeloid cell activation, which results in increased susceptibility to arthritis in mice. However, it was not clear which FcgRIIb associated pathway plays a dominant role. In order to understand cell-type specific contribution of the inhibiting FcγRIIb to susceptibility to arthritis, we have performed extensive analysis with different knock-out mice lacking FcγRIIb either on their B-cells (CD19Cre), all myeloid cells (c/EBPαCre), DCs (CD11cCre) or on all cells (Chapter 4). We observed that only the mice that lack FcγRIIb on all cells developed significantly increased severity and incidence of arthritis compared to WT control mice. The mice lacking FcγRIIb on all myeloid cells had higher disease incidence. However, the mice lacking FcγRIIb on B-cells did not show increase in any disease parameter. This indicates that FcγRIIb on myeloid cells plays more crucial role in disease susceptibility compared to FcγRIIb on B cells. Although arthritis is characterized by the production of autoantibodies in our experiments not all mice with high autoantibody titers developed disease. Autoantibodies are essential but not sufficient to develop full-blown disease and some additional changes are required, like increased cytokine, chemokine production, activation of complement system, infections or other environmental conditions.
Absence of FcγRIIb lowers the threshold for the triggering of activating FcγR expressing effector cells by immune complexes so much less auto-antibodies are required to drive the chronic inflammation in arthritis explaining the increased susceptibility to arthritis of pan-myeloid FcgRIIb deficient mice.

Activation of the complement system also contributes to the pathological process in rheumatoid arthritis. It has also been shown that, mice are resistant to arthritis when

complement factors or their receptors are inactivated. CD55 is a negative regulator of the complement system but also the ligand for receptor CD97. The role of CD55/CD97 interaction in arthritis was investigated in Chapter 5. Although CD55 negatively regulates complement activation, mice lacking CD55 showed decreased susceptibility to collagen induced arthritis (CIA) and additionally blocking CD97–CD55 interactions by using a CD97 antibody ameliorated collagen induced arthritis (CIA). Mice lacking CD97 have lower incidence and severity of arthritis. These results support the idea that interference with binding of CD55 to CD97 might have an inhibiting effect on arthritis development. Therefore it is important to know the exact role of CD55 and CD97 or CD55/CD97 interaction in RA, which might be targets for therapeutic intervention.