Transcriptional regulation of the human interleukin-12 receptor beta2 gene
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SUMMARY
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In this thesis, studies are described on the regulation of the human IL-12Rβ2 gene. IL-12 plays a key role in Th1 development and IFNγ production. IL-12 responsiveness depends on the expression of the high-affinity IL-12R consisting of a β1 subunit and a β2 subunit. The IL-12Rβ2 chain is selectively expressed on Th1 cells, rendering Th2 cells unresponsive to IL-12. IL-12 and IL-4 both act in opposite directions on Th cell polarization and are involved in the regulation of IL-12Rβ2 expression in a positive and negative fashion, respectively, as summarized in Chapter 1.

In Chapter 2, we describe the intron-exon organization of the human IL-12Rβ2 gene. The coding region was found to consist of 15 exons and 14 introns and all intron-exon boundaries are consistent with the consensus sequence for splice junctions (5'GT/AG3'). The IL-12Rβ2 chain is a member of the class I family of cytokine receptors, characterized by conserved features. In Chapter 2 we show that the intron-exon organization of the IL-12Rβ2 was remarkably well conserved between another class I receptor family member G-CSFR, suggesting an evolutionary link. In addition we describe an alternatively spliced mRNA, expressed in Th cells, encoding a putative, truncated protein, lacking all signaling potential. No protein expression or functional regulation of this alternatively spliced mRNA has been found yet.

In Chapter 3 we describe the analysis the 0.6 kb proximal promoter fragment by transfection of serial deletion-reporter constructs in Jurkat cells. The -151 through +54 promoter fragment was shown to contain the minimal promoter. The proximal promoter did not contain a typical TATA box, and transcription was driven by Sp-1 family members instead. Furthermore we have shown the presence of a negative regulatory NFAT element at -206, which was found to specifically bind NFATc2. Our data gave no indication for differential NFATc2 activity in Th1 and Th2 cells. Instead, NFATc2 may play a role in the general low expression rate or the kinetics of IL-12Rβ2 expression, in particular in the shut-down of expression at later time points after TCR triggering.

In Chapter 4 the question was raised to what extent the loss of the IL-12Rβ2 chain in Th2 cells has bearing on the stability of the human Th2 phenotype. It was shown that restimulation of fully polarized Th2 cells in the presence of IL-12 primes for a shift towards Th0/Th1 cells. These cells were shown to express the IL-12Rβ2 chain again, exhibiting STAT4 activation, IFNγ production and showed reversed GATA3 and T-bet expression levels. The IL-12 induced phenotypic shift was proven to be stable. Identical results were obtained with cells from atopic patients. These
findings suggest that IL-12-promoting immunotherapy can be beneficial for Th2-mediated immune disorders, targeting both naive and memory effector T cells.

In the case of low levels of IL-12 and the presence of IL-4, naive T cells will polarize into Th2 cells involving STAT6 and transcription factor GATA3. Experiments with ectopically expressed GATA3 in mouse T cells suggest a role for this Th2-specific transcription factor in the suppression of IL-12Rβ2 expression. Inspection of the DNA sequence of the 1.2 kb proximal promoter region of the human IL-12Rβ2 gene revealed the presence of four GATA consensus sites. In Chapter 5 we describe that we unexpectedly found no evidence for a direct effect of GATA3 on the activity of the IL-12Rβ2 promoter through any of the sites. Similarly, ectopic expressed GATA3 had no effect on the intrinsic IL-12Rβ2 expression in Jurkat cells. This data suggests that GATA3 has no direct effect on the IL-12Rβ2 transcription.

In the last experimental chapter, Chapter 6, we analyzed the 1.2 kb 5' regulatory region of the human IL-12Rβ2 gene in a small cohort of allergic asthma patients (n = 40) by DHPLC, to identify polymorphic sites. Polymorphic changes may alter the structure of transcription factor binding sites within gene promoters and may affect transcription. We found five novel single nucleotide polymorphisms (SNP's) in the proximal promoter region. -465A/G was of particular interest, because promoter activity was increased with almost 200 % in case of the -465G allele, and it disrupted a GATA binding site. DNA binding activity of the -465A GATA site was stronger in Th2 extracts as compared to Th1 extracts, in line with higher GATA3 expression levels in Th2 cells. We found an incidence of 80 % (over 20 % for the G allele) in the asthmatic cohort, but no changed incidence for the GATA site-containing -465A allele in healthy controls. Still this polymorphic GATA site may affect transcriptional activity of the human IL-12Rβ2 gene under Th2 polarizing conditions.