Experimental colitis and translation to human inflammatory bowel disease

Zwiers, A.

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AIMS AND OUTLINE OF THE STUDY

Inflammatory Bowel Diseases consist of two clinically recognized disease entities: Crohn's disease and Ulcerative Colitis of which the exact etiology is still largely unknown. There is a general consensus however that it concerns a deranged and fierce reaction of the mucosal immune system against common, non-pathogenic elements of the gut flora.

From epidemiological, family, and especially twin studies it is clear that a strong genetic component is involved in the development of the disease. These studies also made it clear that this genetic component is a complex one, in which several genes distributed on different chromosomes are involved.

A way to dissect such complex genetic interactions into the constituting elements is through the use of animal models. Using inbred mice the genetic heterogeneity is reduced and also the etiology is similar which reduces phenotypic heterogeneity.

In the studies underlying this thesis we made use of the trinitrobenzene sulfonic acid (TNBS) inducible colitis model in mice. In previous studies we found that resistance to or susceptibility for TNBS colitis between two mouse strains, the SJL/J and the resistant C57Bl/6 strain, resided on two loci on mouse chromosomes 9 and 11. **The aim of this study was to further dissect the genetic susceptibility to disease in this model and to translate these findings to human IBD.** Two strategies were applied to gain further insight in the genes and the genetic mechanisms that are responsible for the association with these loci: a candidate gene approach and a micro-array based approach.

The locus on chromosome 11 contains the IL-12B gene which codes for a subunit that is part of two key pro inflammatory cytokines in IBD, IL-12 and IL-23. IL-12 had previously been identified as a central mediator for both TNBS-induced colitis as well as human Crohn's disease since both diseases can be cured by antibodies directed against IL-12. Based on these considerations the IL-12B gene was a logical candidate gene for the association with this locus. We therefore applied a candidate gene approach to investigate possible molecular mechanisms at work which could explain these differences between strains and subsequently investigated whether these mechanisms were also relevant for the human situation.

In **chapter 2** we investigated whether polymorphisms present in the IL-12B gene of the SJL/J mice are responsible for the enhanced IL-12 synthesis seen in the SJL/J strain and in this way contributes to enhanced susceptibility.

Subsequently we investigated in **chapter 3** whether a polymorphism found in the human IL-12B gene is associated with susceptibility to Crohns disease. This was done by measuring the IL-12 secretion of monocytes and relating these levels to the presence or absence of the polymorphism.

In **chapter 4** we searched for additional, unknown, polymorphisms in the IL-12B gene which could be related to the high or low secretor phenotype that was found in the study described in chapter 3.

To identify possible candidate genes in the loci implicated in the genetic study we applied micro-array technology. The expression of a substantial number of genes is controlled by interaction with regulatory sequences in the proximity of a gene (so called cis-acting elements), and a change in such an element can lead to an altered expression of this gene. Micro-array technology is a tool of choice to identify such altered expression.

In **chapter 5** we first used micro-array data from the TNBS model in a bio-informatics approach to search for similarities and dissimilarities with human IBD by comparing them with data derived from human array studies. With this overall picture of differential expression in the TNBS colitis model, we focused in **chapter 6** on the genes located in the chromosomal regions associated with TNBS induced colitis. This way we could identify a plausible candidate gene, the tight junction molecule claudin-18. We subsequently analyzed differences in gene expression of claudin-18 between susceptible and resistant strains and evaluated possible involvement of this gene in human UC patients.

Finally in **chapter 7** the results are discussed in relation to current data from the literature.