Celiac disease : how complicated can it get?

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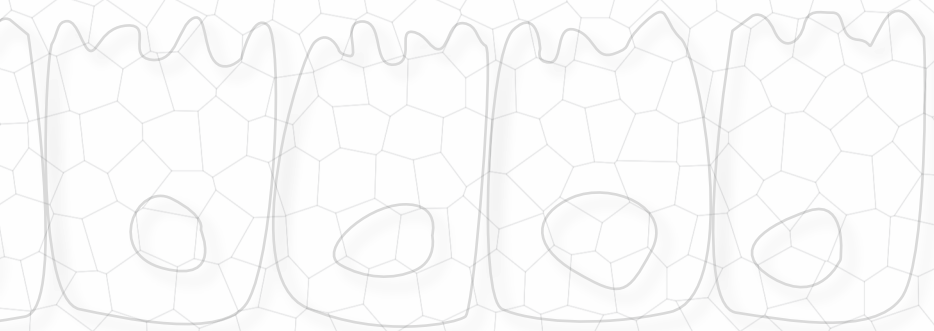

Chapter 6

General Discussion

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Although celiac disease (CD) is a relatively benign condition where elimination of gluten from the diet can reverse disease, a small percentage of adult-onset CD patients fail to recover on a gluten-free diet. These refractory celiac disease (RCD) patients have persisting villous atrophy and elevated levels of intraepithelial lymphocytes (IELs) in the small intestine. In a subset of these patients an aberrant monoclonal IEL population is present that lacks expression of the T cell receptor (TCR)-CD3 complex on the cell surface, but expresses CD3 intracellularly. This aberrant IEL population is now regarded as an intraepithelial lymphoma that can develop into overt lymphoma[1]. Therefore, this disease state -RCD type II (RCD II)- is considered an intermediate stage between CD and lymphoma. This thesis reports on novel insights on aberrant IELs that increased our knowledge on these (pre)malignant cells and that might aid in the development of future therapies for RCD II and RCD-associated lymphoma.

RCD cell lines: a model for aberrant IELs in RCD II and RCD-associated lymphoma

About a decade ago, aberrant IELs in RCD II were identified as the missing link between TCR^+ IELs in CD and the TCR^- lymphoma cells of enteropathy associated T cell lymphoma (EATL)[2;3] Although the aberrant IELs did not express the TCR-CD3 complex on the cell surface, CD3 was expressed intracellularly and clonal TCR-γ gene rearrangements were commonly found. One view is, therefore, that aberrant IELs are mature TCR^+ IELs that lost cell surface expression of the TCR-CD3 complex due to overstimulation[4].

Studies on aberrant IELs were mainly performed on IELs *in situ*, which limited the type of experiments that could be performed to investigate molecular events that are linked to malignant transformation. The isolation and propagation of aberrant IEL lines isolated from small intestinal biopsies of RCD II patients (Chapter 2) allowed us to perform more in-depth analysis of aberrant IELs. These RCD cell lines had the characteristic aberrant IEL phenotype: surface $TCR\text{-}CD3^-CD4^-CD8^-CD7^+CD103^+$, intracellular $CD3^+$. In addition, the RCD cell lines displayed monoclonal TCR-γ gene rearrangements identical to the predominant rearrangements found in the aberrant IELs of the patients from which the cell lines were derived. The proliferative response of the RCD cell lines to IL-15, which is highly upregulated in RCD II and RCD-associated lymphoma[5], further supported the notion that RCD cell lines could serve as a model for aberrant IELs (Chapter 2).

Aberrant IELs contribute to the propagation of tissue damage and inflammation in RCD II.

In active CD, the number of TCR^+ IELs is markedly increased. It has become clear that TCR^+ IELs can acquire an activating NK cell receptor repertoire, presumably under the influence of IL-15[6]. These NK cell receptors co-stimulate TCR-mediated lysis of epithelial cells[7]. Upon stimulation with IL-15, which can alter the NK cell receptor function, IELs can even display TCR-independent cytotoxicity[8]. It seems, therefore, likely that these TCR^+ IELs that have acquired an NK cell phenotype play a role in perpetuating tissue damage in RCD.

In addition to a role in tissue damage, TCR$^+$ IELs in active CD contribute to the pro-inflammatory milieu by the secretion of high levels of IFN-γ[9;10]. Analogous to IFN-γ secretion by gluten-specific CD4$^+$ T cells[11], IFN-γ secretion by TCR$^+$ IELs seems to be linked to gluten-intake as TCR$^+$ IELs in active CD patients and CD patients after gluten-challenge secreted higher levels of IFN-γ when compared to healthy controls or CD patients on a gluten-free diet[10]. Furthermore, TCR$^+$ IELs also secreted high amounts of IFN-γ after stimulation with IL-15[12;13]. Strikingly, none of the other T helper 1 (Th1) or T helper 2 (Th2) cytokines seemed to have a role as important as IFN-γ (and to a lesser extent IL-10) in the inflammatory response of active CD.

Much less is known about cytotoxicity and cytokine secretion of the aberrant TCR$^-$ IEL population in RCD II and the subsequent state of lymphoma. The availability of the RCD cell lines as a model for aberrant IELs allowed us to address these issues *in vitro*. In analogy with TCR$^+$ IELs in active CD, the TCR$^-$ RCD cell lines expressed multiple activating NK cell receptors (Chapter 3). In contrast to the TCR$^+$ IELs, however, epithelial-cell specific cytotoxicity was mainly mediated by DNAM-1[14] with only a minor role for other activating NK cell receptors (Chapter 3). We postulated that in a subset of RCD II patients the aberrant IELs acquire the ability to lyse epithelial cells via DNAM-1.

Analogous to TCR$^+$ IELs, the RCD cell lines secreted IFN-γ but none of the other Th1 cytokines upon stimulation with IL-15. This indicated that aberrant IELs can play a role in the propagation of the inflammatory response in RCD II (Chapter 4). Interestingly, simultaneous stimulation with an anti-CD30 antibody and IL-15 had a synergistic effect on IFN-γ secretion (Chapter 4). As CD30 is expressed on aberrant IELs in a subset of RCD II patients[15] and on virtually all RCD-associated lymphoma cells, combined stimulation of the CD30 receptor with IL-15 stimulation could occur *in vivo* and might contribute to the ongoing inflammation in the transition from RCD II into lymphoma.

Together, these results indicate that aberrant IELs could contribute to the ongoing tissue damage and cytokine secretion in RCD II and RCD-associated lymphoma.

Aberrant IELs possibly originate from a unique CD3$^-$CD7$^+$ lymphocyte population

Knowledge on the functional characteristics of aberrant IELs has thus greatly increased. The exact cellular origin of aberrant IELs, however, remains unclear. Following the hypothesis that aberrant IELs used to be TCR$^+$ IELs, we assessed the presence of the TCR-CD3 complex in our RCD cell lines. Intracellulary, all CD3-chains and ζ-chains were present and could form the CD3γε-, CD3δε- and ζζ-dimers (Chapter 2). The TCR-chains, however, were not always present (Chapter 2). Introduction of exogenous TCR-chains, however, restored surface expression and functionality (proliferation and IFN-γ production) in the RCD cell lines (Chapter 2 and unpublished). This indicates that the CD3-complex was functional in the RCD cell lines. Analysis of TCR-gene rearrangements with PCR and Southern blot revealed that clonal TCR-gene rearrangements could be found in the RCD cell lines. These TCR-gene rearrangements were, however, either incomplete, non-functional or out-of frame and can therefore not assemble into a functional TCR. Furthermore, one of the RCD cell lines only displayed germline configuration of the TCR genes (Chapter 5).

The RCD cell lines thus have characteristics of T cells, yet their phenotype does not match that of mature T cells. Phenotypic analysis of the RCD cell lines indicated that aberrant IELs have a unique phenotype: $CD45^{+}CD19^{-}CD14^{-}CD3^{-}CD7^{+}CD56^{-}CD34^{-}CD103^{+}CD127^{-}$ (Chapter 5). This phenotype is distinct from any known lymphocyte class. Furthermore, IELs with this phenotype were present in the small intestine of healthy adults, children and thymic cell suspensions (chapter 5). We hypothesized, therefore, that aberrant IELs do not derive from mature T cells but instead derive from cells in an early stage of extrathymic lymphocyte development.

Directions for further research

The different aspects of aberrant IELs described in this thesis help understand the events leading from uncomplicated CD to RCD II and gastrointestinal lymphoma. Many issues, however, remain to be answered.

First, most patients do well on a gluten-free diet but some individuals develop RCD II and lymphoma. It has been suggested that HLA-DQ2 gene dose is involved in the risk to develop complicated CD as RCD II patients and gastrointestinal lymphoma patients were more often HLA-DQ2.5 homozygous[16]. Genome-wide association studies (GWAS) on uncomplicated CD patients have identified several risk variants on non-HLA-loci for development of CD[17]. The presence of a higher number of additional non-HLA risk alleles is directly correlated with an increase in the risk to develop CD[18]. An interesting issue is whether these genes also influence the risk to develop complicated forms of CD. A GWAS on RCD I, RCDII and RCD-associated lymphoma patients with uncomplicated CD patients and healthy individuals as controls would be a way to uncover the role of non-HLA-genes on the development of complicated CD. Screening of CD patients on potential risk alleles might then lead to earlier intervention. Recent GWAS have indicated that to identify risk variants with small effect sizes the amount of samples should be increased to obtain significant results. As only 2-5% of adult-onset CD patients develop complicated CD, even pooling of sample collections from multiple European countries would not obtain a sufficient sample-size for such a study.

Second, cells with a TCR-CD3-negative phenotype are present in the intestine of healthy adults and children (Chapter 5). These cells could be an immature lymphocyte type that represents the physiological counterpart of the aberrant IELs in RCD II. To test this hypothesis, future studies are needed to determine whether these cells can differentiate into mature T cells and/or NK cells. Hematopoietic precursor cells from fetal liver and postnatal thymus were shown to differentiate into T cells on OP9 cells expressing Notch ligand Delta-like-1(DL1)[19;20]. In analogy, cells with the $CD45^{+}CD19^{-}CD14^{-}CD3^{-}CD7^{+}CD56^{-}CD34^{-}CD103^{+}CD127^{-}$ phenotype could be isolated from the small intestine of a healthy individual and cultured in the presence of stromal cell lines OP9 or OP9-delta-like-1[19]. Such co-culturing could indicate whether the cell population identified in Chapter 5 can indeed differentiate into a mature lymphocyte type. Alternatively, the presence of essential transcription factors Notch1 and GATA3 could indicate in which stage of development these lymphocytes are[21]. Microarray analysis might inform whether these genes are differentially expressed when compared to mature T and NK cells. In addition, in mice the transcription factor E4BP4[22] has been appointed as a critical factor in NK cell development. It remains to be determined if this factor is also important in human NK cell development.

Third, a subset of RCD II patients acquires the ability to lyse epithelial cells via DNAM-1 (Chapter 3). It remains unclear, why DNAM-1 that normally acts as a co-receptor, can now act autonomously. Recently, the inhibitory receptor TIGIT was identified that can be co-expressed with DNAM-1 on T cells and NK cells and competes for binding to the ligands CD155 and CD112[23;24]. TIGIT was shown to have a 100-fold higher affinity for CD155 than DNAM-1 suggesting that, when co-expressed, TIGIT has the dominant function and can regulate the function of DNAM-1[23]. Dysregulation of the balance of expression of TIGIT and/or DNAM-1 might enable DNAM-1 to induce cytotoxicity autonomously. Determining expression of TIGIT and DNAM-1 in different stages of CD and in healthy controls could be a way to address this issue.

Future therapeutic possibilities for RCD II and RCD-associated lymphoma

RCD II and RCD-associated lymphoma are severe complications of CD with 5-year survival rates of 44-58% and <20% respectively[25]. RCD II is very resistant to therapy and transition into lymphoma cannot be prevented. At present, only Cladribine therapy[26] or autologous stem cell therapy[27] might have an effect on reducing the amount of (pre) malignant aberrant IELs.

Blocking of IL-15 has been suggested as a therapy as this cytokine influences many aspects of aberrant IELs including apoptosis[1], proliferation (Chapter 2), cytotoxicity (Chapter 3) and cytokine secretion (Chapter 4). Alternatively, we suggest that blocking of DNAM-1 and CD30 may be interesting as these molecules seem to be linked to malignant transformation of the aberrant IELs into lymphoma cells. Monoclonal antibody therapy against these two receptors might have immediate effects on cytotoxicity and cytokine production, thus limiting tissue destruction and inflammation.

In conclusion, the research described in this thesis improved our understanding of the pathogenesis of complicated CD and gave us suggestions on possibilities for future therapy.

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