Crohn's disease: Mucosal immunology and immune modulating therapy

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Summary, discussion and future perspectives

Crohn’s disease (CD) and ulcerative colitis (UC) are debilitating chronic diseases that affect millions of people worldwide, profoundly impacting patient quality of life and incurring large costs in terms of treatment and lost productivity. While current interventions ameliorate disease symptoms, they do not provide a cure, and there is significant unmet medical need. Successful discovery and development of more effective CD therapies depends on a better understanding of the underlying mechanisms of disease, including how pro-inflammatory cells proliferate unchecked, and how the body’s own mechanisms might be enlisted to control inflammation. The studies presented in this thesis were undertaken to provide a better understanding of immunological parameters operational in CD. Current therapies target cytokines produced by pro-inflammatory immune cells and thereby reduce intestinal inflammation and induce remission of disease. Nevertheless not all patients respond to these therapies. Furthermore, among those responding initially, many patients fail subsequent efficacy of maintenance therapy. Starting a treatment for remission induction therapy is a matter of trial and error, since mechanisms predisposing or regulating non-response have not been elucidated. Moreover, the assessment of therapy efficacy is hampered by the lack of objective markers. To identify biomarkers for therapy response, this thesis describes in depth analyses on patients that respond and patients who do not respond to remission induction therapy. By the identification of differential gene expression and cytokine profiles between responders and non-responders novel insights for biomarkers and underlying mechanisms are obtained. These studies provide a path to development of better diagnostics predicting response to therapy.

Part I: Innate lymphoid cells in inflammatory bowel disease

To improve therapeutic strategies, novel insights in the aetiology of CD are urgently needed. CD is considered an inflammatory autoimmune disease. Both the innate and adaptive immune systems are thought to contribute to the chronic intestinal inflammation. An involvement of myeloid cells is suggested by noted deficiencies in macrophages in inflamed tissues. Since cytokines such as IL-17, IL-22 and IFN-γ which are produced by T cells are elevated in CD inflamed intestine, a role of Th1 and Th17 cells has been postulated as well. Recent research has uncovered the existence of a class of lymphocytes called innate lymphoid cells (ILCs). ILC populations represent only a small fraction of the leukocytes in the circulation and tissues but by producing large amounts of cytokines they can shape a pro- or anti-inflammatory environment in specific tissues and contribute to several chronic inflammatory diseases. As explained in the introduction (chapter 1) ILCs consist of three groups that can be distinguished on the basis of cytokine production profiles and transcription factors they depend on for development and function. As studies in mouse models suggested a role of ILCs in IBD, we explored a possible role of these cells in the pathology of CD.
Chapter 2 describes the discovery of a novel subset of ILCs, referred to as ILC1 which are dedicated to the production of IFN-γ. These ILC1 are different from natural killer (NK) and at least a proportion of these cells differentiate from RORγt+ ILC3 under the influence of IL-12. In this process RORγt is downregulated whereas the transcription factor Tbet, which drives production of IFN-γ, is strongly upregulated. In the inflamed intestine of CD patients these pro-inflammatory ILC1 accumulate and the number of IL-22-producing ILC3 decreases. Experiments using Dextran sodium sulphate (DSS) induced inflammation in human immune system (HIS) mice revealed that the increase in proportion of ILC1 and decrease of ILC3 in the gut occurs as a consequence of the inflammation induced by DSS. Based on these observations we speculate that in CD patients, high levels of IL-12 (from e.g. myeloid cells) could skew ILCs towards an ILC1 subset producing mostly IFN-γ. In a chronic inflammatory environment ILC1 persist and thereby contribute to the pathology of chronic immune-mediated inflammatory diseases (IMIDs).

Although this study has provided valuable information that point to a role of ILC1 in CD, it should be noted that the CD patient’s resection specimens used in this study reflect end-stage disease, where no drug is anymore sufficient to suppress inflammation. Thereby the data extrapolated form these materials cannot be directly translated to newly onset inflammation in CD patients. Future studies on ILC subsets should comprise early onset IBD patients, or children, to accurately determine the contribution of ILC1 in CD onset. It is becoming increasingly clear that microbiota is one of the drivers of the inflammatory responses in the gut. However the underlying mechanisms are incompletely understood. In particular it needs yet to be determined whether and how the microbiota is responsible for induction of ILC1 responses. Currently we are only in the beginning of elucidating the roles of ILCs in human inflammatory diseases and of the factors responsible for changing a protective ILC subset towards a pathogenic phenotype.

The contribution of bacterial colonization is briefly touched upon in the next chapter. The presence of different ILC subsets during fetal development is of interest since no bacterial colonization has occurred before birth. We can conclude that ILC1 co-exist with the presence of bacteria in the intestine since no ILC1 were present before birth. Chapter 3 describes characteristics and development of IL-17 producing ILCs that lack expression of the natural cytotoxicity receptor (NCR), NKp44, and IL-22 producing NKp44+ ILCs. NKp44+ILCs, do not depend on bacterial colonization since these cells arise in the fetal intestine during the first trimester. The proportion of those cells increase upon gestational age indicating that development of this subset in the gut is programmed. Confirming the potential homeostatic role of IL-22, the intestine of healthy human individuals contains mostly IL-22-producing NCR-ILC3. The NCR-ILC3 cells are also present in fetal gut but their proportions decrease over time. This population includes lymphoid tissue inducer cells (LTi cells) which are mainly present in fetal developing lymph nodes. After birth and through adulthood, NCR-ILC3 persist in peripheral non-inflamed lymph nodes as resting cells. In the inflamed CD intestine increased numbers of NCR-ILC3 have been described, which were thought to mediate inflammation by the production of IL-17. On the contrary, drugs blocking IL-17 worsened inflammation in CD patients. The exact role of NCR-ILC3 in the human gut remains to be pinpointed, but
these cells may be involved in generation of isolated lymphoid follicles as they do in mouse gut. In chronic inflammation in mice, and possibly in CD patients, organized tertiary lymphoid structures have been identified which are absent in healthy intestine. These structures, also referred to as ‘tertiary immune systems’, are not unique for CD but are associated with other IMIDs such as rheumatoid arthritis and multiple sclerosis. Thereby, novel insights in innate immune cells in CD will contribute to a better understanding and hopefully therapeutic target development for more IMIDs.

Part II: Therapeutic efficacy in Crohn’s disease patients

The aim of chapter 4 was to screen patients during induction therapy and to determine potential biomarkers that support the treating physician’s assessment on response to therapy. In the course of this research, it became clear that only the treating physician’s opinion on active disease did not match results obtained with colonoscopy or MRI. Poor correlation was found between subjective disease activity scores, CD activity index (CDAI) and Inflammatory Bowel Disease Questionnaire (IBDQ) and the CD Endoscopic Index of Severity (CDEIS). It was realized that almost a third of patients would previously have been treated with expensive and/or potentially harmful therapies while they did not have active CD. Why these latter patients did have symptoms mimicking active CD is not known but is probably due to irritable bowel syndrome (IBS) complaints which represent a major component of IBD symptoms. These patients do not need anti-TNF drugs, but rather counselling to change their behaviour in food intake, stress and environmental factors of which many are still unknown. Based on the many patients that were false positively judged to be active by clinical assessment, it is strongly recommended to ascertain intestinal inflammation by endoscopy or MRI before the start of remission induction. Moreover, to assess therapeutic efficacy, a second endoscopy should be contemplated to ascertain response. Hopefully in the future, this burdensome investigation can be replaced by the measurements of objective biomarkers for therapy efficacy in peripheral blood. The next chapter elaborates on the pursuit for these biomarkers.

Chapter 4 describes several candidate biomarker profiles distinguishing non-responders from responders to remission induction therapy. At very early time points after the start of remission induction therapy differential profiles in gene expression can be identified. Genes expressed as early as 3 days after the start of therapy could be followed throughout induction therapy and retrieved after 7 and 14 days. Most genes identified had not been associated with IBD before except for two genes, fillagrin-2 (FLG2) and calcyclin (S100A6) that turned out to be family members of calprotectin, a faecal marker for active IBD that was validated recently. At the time this study was designed, calprotectin had not been validated and therefore we cannot correlate faecal calprotectin levels with those of other members of this family. Nevertheless, this is the first study showing peripheral blood gene expression time series in CD revealing the possibility to identify biomarkers in peripheral blood of CD patients. Presumably, these early
differences in gene expression between responders and non-responders reflect changes in patient’s metabolic state or general inflammatory state rather than healing of the intestinal mucosa. For example, ANK1, MICAL2, FHL2 and IGF2BP2 expression levels decrease quickly after 3 days in non-responders and remain their expression in the following weeks. Other genes show different profiles, including FLG2 and CNOT4, which are most differentially expressed after 7 days of therapy and restore after 14 days of therapy. The preliminary data on diagnostic accuracy of the identified genes seems promising and should be validated in an independent cohort. These biomarkers could be measured before and after one week of therapy revealing a risk for patients to fail induction therapy when expression levels decrease in patients, whereas patients seem likely to respond when expression levels increase after one week. Possibly the combination of genes could provide more diagnostic accuracy however this needs to be investigated in future studies. The main strength of this study is the consecutive blood samples that were drawn from each patient. Individual fold changes in gene expression were calculated from repeated measurements after 3, 7, 14 and 56 days in comparison with baseline expression. By differentiating responders from non-responders at consecutive time points during therapy, these data are likely more robust than single observations per patient. Moreover, the false discovery rate is limited by consecutive sampling. Patients were extensively phenotyped to determine response or non-response, however the primary endpoint of individual CDEIS improvement after 8 weeks of therapy is not infallible. The best timing to determine response has been debated, where some suggest to assess response about a month later. Furthermore, one might argue the value of mucosal healing versus patient’s subjective interpretation of complaints. We should integrate these parameters to avoid the difference between patient’s concern to feel better and doctor’s intention to improve healing of the mucosa.

The difficulties to determine therapeutic response in daily clinical practice are further discussed in chapter 5, 6 and 7. In daily clinical practice physicians experience an extensive variability in symptoms and endoscopic lesions. Clinical trials that elaborate on therapy efficacy only represent a subset of patients, treated in tertiary referral centres, and inclusion based on strict criteria limiting the variety of disease activity and co-morbidity. On the other hand, retrospective studies are restricted in the ability to classify response in retrospect, since data originate from medical charts depending on the documentation of symptoms, laboratory values and endoscopic assessment by the treating physician. Nevertheless, to counsel a diverse population of CD patients and improve treatment strategies, retrospective data on therapy efficacy are essential to reflect daily clinical practice.

**Chapter 5** described a cohort of CD patients, and their treatment with adalimumab (anti-TNF) in a daily clinical practice setting. Eighteen hospitals in North-Holland, a province of the Netherlands, participated which resulting in a population-based cohort of 438 CD patients. Primary non-response, as described in the previous chapter, occurred in 7.5% of patients. After 1 year 83% of patients showed response defined as ongoing therapy, followed by 74% after 2 years, and 62% after 3 years. Steroid-free remission at the end of follow-up was only observed in one third of patients. This low number might have been influenced by the limited duration of adalimumab therapy (median follow-up of 2 years) in this cohort. Patients with
observed intestinal strictures were at risk to fail induction therapy (OR 3.73; \( P = 0.04 \)). Some of these strictures will probably have been fibrotic rather than inflammatory and therefore these should have been resected instead of treated with medication. However, this distinction is often not clearcut at imaging before the start of therapy. Furthermore, increased levels of CRP predicted higher rates of initial response (OR 0.31; \( P < 0.01 \)). It is likely that this association reflects the difficulty in the assessment of disease activity in CD patients. When endoscopy is not performed in patients lacking inflammatory markers, such as CRP, one might treat IBS-like symptoms in stead of intestinal inflammation. Since these patients did not have active inflammation to start with, response rates will be low and efficacy will be based on placebo response. These findings stress the importance for imaging before the start of remission induction therapy to avoid anti-TNF treatment of IBS rather than IBD symptoms. During maintenance therapy, the additive effect of thiopurines has been accepted for infliximab but this has been controversial for adalimumab. Yet, in this study concomitant thiopurines in the first 6 months of adalimumab treatment decreased the risk to fail maintenance therapy (HR 0.69; \( P = 0.05 \)). Recent meta-analyses showed no beneficial effect for thiopurines next to adalimumab. However, most trials and retrospective studies include patients who are more severely affected and are therefore referred from regional hospitals to tertiary referral centres. The present cohort is population based, comprising all CD patients treated with adalimumab in North-Holland, and thereby reflect real-life practice more accurately. Future prospective studies, randomizing thiopurines next to adalimumab both in tertiary referral centres and in regional hospitals, are needed to elucidate this matter. Different from a decade ago, when infliximab was investigated, many patients treated with adalimumab previously received infliximab (mostly with concomitant thiopurines) and thereby TNF-driven pathways, and response, may have been altered. In this cohort, previous infliximab therapy did not affect response to adalimumab, however dose escalation was more often deemed necessary (\( P < 0.01 \)). Careful consideration of patient’s individual risks to fail induction therapy is important, based on e.g. the onset of disease at young age, ileal involvement or peri-anal fistula’s, and previous failure of therapy. Some subgroups may benefit from more aggressive treatment strategies, including concomitant thiopurines next to adalimumab, whereas others should be protected from over-treatment with potentially harmful drugs.

In Chapter 6 a cohort study was performed to assess long-term safety and efficacy of infliximab (anti-TNF) therapy for CD patients. In total, 469 patients were included from two tertiary referral centres. In 1993, infliximab was administered for the first time to a young CD patient in our hospital and has since then been administered with different strategies. Since (long-term) side-effects were not fully known, the first infliximab infusions were administered only when complaints of active CD appeared, also called episodic treatment. Currently, all patients receive infliximab maintenance therapy according to a regular scheme since adverse events are thereby reduced and efficacy is improved. Nevertheless, CD patients are affected for life thus prospective safety registries are urgently needed to assure long-term safety. The present cohort lists the reported adverse events during and in the first years following infliximab therapy. Nine patients (1.9%; 0.39/100 patient years) died at a median age of 48 years due to malignancies, myocardial infarction, a bowel perforation and bilateral pneumonia. Most malignancies occurred at a median age of 45 years and were located at the gastrointestinal
site, followed by haematological and respiratory malignancies. Half of the malignancies were diagnosed during infliximab treatment after a median of 11 (IQR 4-23) infusions. All malignancies were estimated to be possibly related to infliximab. Nevertheless, IBD itself also gives rise to an increased risk for gastrointestinal malignancies based on chronic inflammation of the intestine. And so do other immunosuppressive drugs like thiopurines, where patients have been previously treated with. Thus the additive effects of infliximab on development of malignancies in IBD are unambiguous to investigate. Still, these risks should be taken into account when starting anti-TNF treatment in each individual patient.

In the recent decade of infliximab treatment 15% of this cohort showed primary non-response. After 5 years about 55% of all patients showed sustained benefit with infliximab therapy. CD is a chronic disease and therefore 5-year data are of interest but more long-term data remain essential. Half of CD patients lost their response to infliximab therapy after 5 years. To improve maintenance therapy, a few years ago concomitant immunosuppressants were shown to ameliorate the outcome of maintenance therapy. This change in treatment strategy will bias future studies focussing on 10 or even 20 year efficacy of infliximab, thus patients should be stratified for concomitant immunosuppressants and moment in history that infliximab was administered.

No prospective trial has compared head-to-head the efficacy of adalimumab and infliximab for CD. When we compare this cohort with the adalimumab cohort described in the previous chapter, both therapeutics result in similar response rates. Notwithstanding, adalimumab did not result in a significant decrease in surgical interventions whereas infliximab did ($P < 0.02$). This may be due to the limited time of follow-up in the adalimumab cohort, and the high rates of surgeries before the start of adalimumab.

Since the introduction of biologicals, many patients started infliximab, switched or had preferences for adalimumab and failed both therapies. However, was this really failure of therapy or rather inefficient administration, e.g. lack of concomitant thiopurines next to infliximab? In other words, what can we do for highly refractory CD patients? To address this question we performed an in depth analysis on treatment outcome in patients who switched from infliximab to adalimumab and returned to infliximab. Chapter 7 reports on the clinical outcome of a second infliximab treatment after earlier discontinuation and previous switch to an alternative anti-TNF agent. Twenty-nine CD patients with sequential infliximab and adalimumab treatment and a restart of infliximab were retrieved from the cohort investigated in chapter 6. An intensified dosing schedule (>5mg/kg or <8 weekly) was started in 45% of patients and 79% of patients were re-started on a similar infliximab schedule as before. During the second infliximab treatment course, dosing was further intensified in 38% of patients. After 18 months of infliximab retreatment 62% of patients continued their second infliximab course. After a median of 7 months, 24% of patients stopped infliximab due to loss of response, intolerance (10%) or non-compliance (3%). Use of an induction schedule or concomitant immunomodulators was not associated with treatment benefit. Important to note is that not all patients failed infliximab at the time of first discontinuation. About 24% of patients were in clinical remission and 10% preferred a subcutaneous route of drug administration. Still, sustained benefit and clinical remission rates after 18 months were similar. The previous
chapter showed that temporary discontinuation of infliximab, without adalimumab in between, also did not result in more loss of response. So far “drug-holidays” were discouraged since more loss of efficacy was observed next to increased adverse events (e.g. infusion reactions) after “drug-holidays”. Indeed, in the previous chapter, 20% of patients ceased their second episode of infliximab therapy due to adverse effects, which was more often than first-episode users. What mechanisms cause these adverse events are of major interest since selective blocking of antibody formation or other causes of infusion reactions would provide new possibilities in re-treatment of highly refractory patients. Recent reports showed that infliximab re-treatment is even a viable option after “drug-free-years” in people with limited therapeutic options. Nevertheless, switching from one anti-TNF to another should be discouraged since recent prospective analyses showed that after a complete response to infliximab, direct elective switching to adalimumab is associated with loss of efficacy and loss of tolerance after one year. Still, for many CD patients there are no alternatives than surgery, due to the lack of therapeutic drugs. For these highly refractory patients, when failing adalimumab, after prior infliximab, returning to infliximab should not be precluded since this could be a valuable strategy. In summary, these results stress the importance of development of better diagnostics and novel therapeutic drugs.