Towards therapeutic disease control in inflammatory bowel diseases

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Chapter one

Introduction

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1 The immune system

The immune system protects the human body from disease. To accomplish this, many cell types are involved with different functions and tasks. When bacteria or viruses enter the body, the innate immune system first comes into play. [1] This system is non-specific, and provides an immediate inflammatory response which is characterized by redness, swelling, pain, heat and dysfunction. Once the innate immune system is activated, cells produce several cytokines and chemokines. In response to these secreted factors, other immune cells are recruited to the site of inflammation. The innate immune system then activates the adaptive immune system, consisting of highly specialized cells, B cells and T cells. [2] In the absence of antigen, T cells and B cells are naïve. They are activated by the innate immune system, when an antigen presenting cell (APC) presents an antigen to the B or T cell. Some of these activated B and T cells will become memory cells which provide long-term immune memory as these cells are able to respond to antigens without the help of the innate immune system. This will result in a more rapid and efficient response when the immune system encounters an antigen which has been recognized before.

1.1 The innate immune system

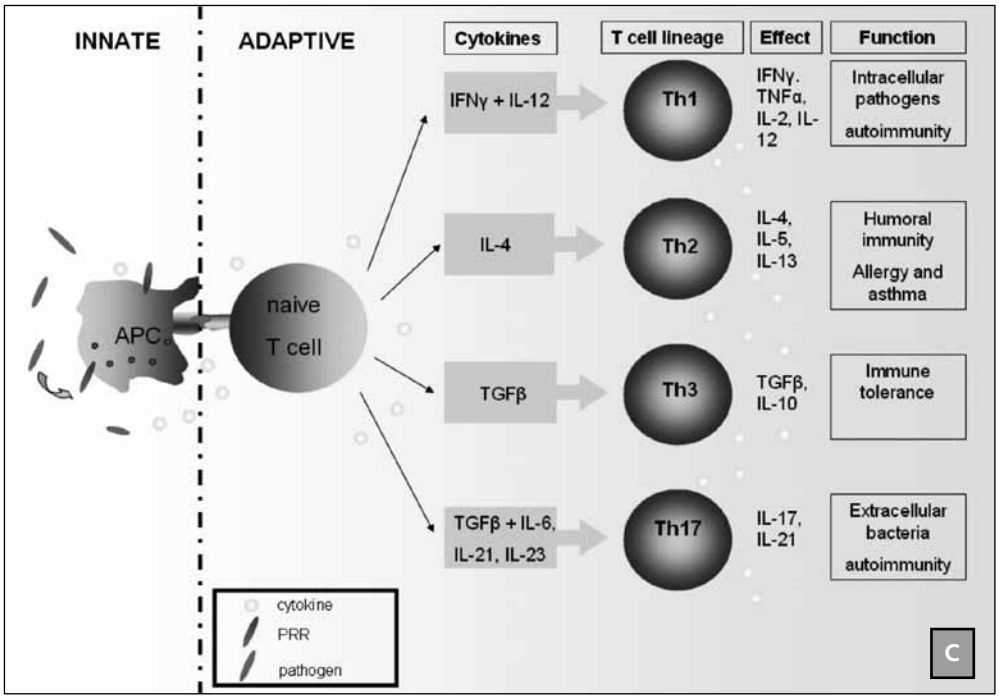
APCs are the key players of the innate immune system: they sense and process antigens, produce cytokines in response to pathogens, and activate the adaptive immune system. APCs express several pattern recognition receptor (PRR) molecules to sense pathogens in the environment. Two important types of PRRs are membrane-associated toll-like receptors (TLR) and cytosolic nucleotide-binding oligomerization domain (NOD proteins). The TLR family includes a family of 10 studied TLRs which all have different specificities for various pathogens. When lipopolyssaccharide (LPS, a cell component of Gram-negative bacteria and responsible for septic shock) binds to TLR4, the cell starts to produce several pro-inflammatory cytokines, among which tumor necrosis factor alpha (TNFα) and interleukin-1β (IL-1β). [3] Next, other cells are recruited to the site of inflammation and an immune reaction is initiated. NOD2 is located intracellular and recognizes molecules that contain muramyl dipeptide (MDP), which is present in several bacteria. [4]

Dendritic cells (DCs) are the most specialized APC and are usually one of the first cells that come into action once a bacterium or virus enters the body. When a DC senses a pathogen by ligation of a PRR, it phagocytoses ("eats") the potential harmful foreigner, processes it inside the cell, and presents pieces of the protein on the cell membrane loaded on MHCII molecules. [5, 6] After ligation of PRRs, co-stimulatory molecules like CD80, CD83 and CD86 are upregulated. The antigen presented on the MHCII molecule is then recognized by the T cells receptor complex (TCR) on the T cell, and this provides the first signal to initiate an immune response. [7] To achieve a full immune response, additional stimulation is often needed; the interaction of costimulatory molecules on DCs and T cells and the presence of various cytokines provide the second signal and finally determine the outcome of the immune response. [8, 9]

Like DCs, macrophages (Mϕ) are part of the innate immune system, derive from monocytes and function as APCs. They are present in many tissues and contribute to tissue homeostasis. [10, 11] Mϕ are a heterogenous population of cells and have different functions depending on their differentiation status and the type of cytokines present in their environment.

[12-14] Type 1 macrophages are typically induced in the presence of LPS or pro-inflammatory cytokines. In this setting, Mϕ are primed to become effector cells that are highly efficient in killing intracellular bacteria and in the production of pro-inflammatory cytokines. [15] In contrast to Mϕ1, type 2 macrophages (Mϕ2, regulatory macrophage or alternatively activated macrophage) have a more anti-inflammatory phenotype. They are induced by Th2 cytokines, glucocorticoids or immune complexes. Mϕ2 have several characteristics that are functionally different from Mϕ1: they are able to dampen immune responses by inhibiting T cell proliferation, production of anti-inflammatory cytokines and they contribute to wound healing. [16-18] In addition, Mϕ2 inhibit Th1 responses by skewing the immune response towards a Th2 response.

Figure 1 Innate and adaptive immunity.



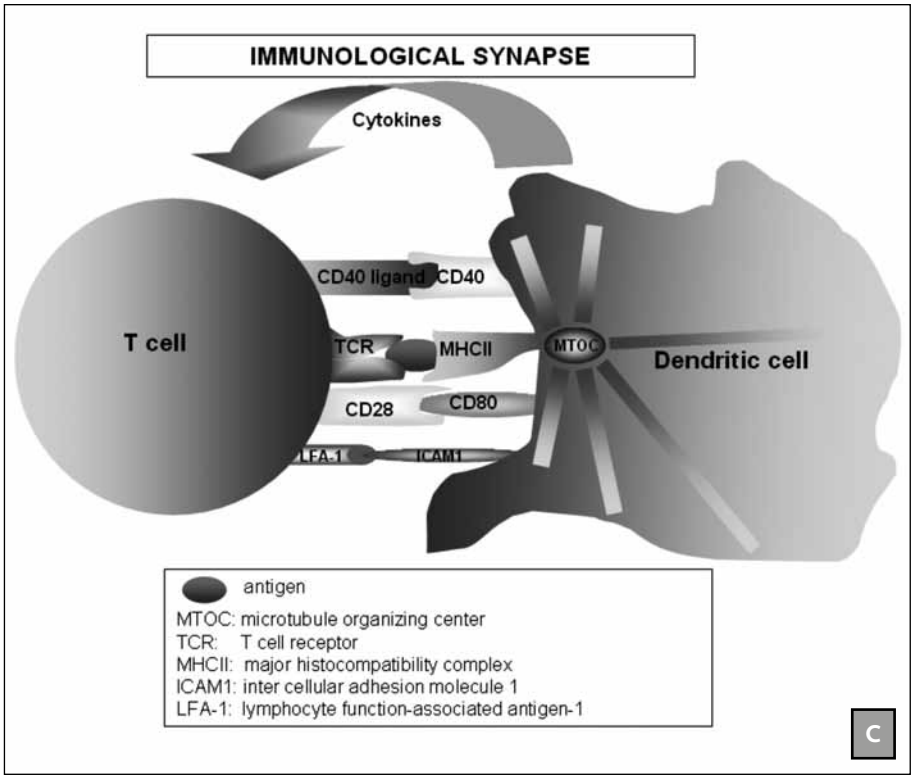
APC antigen presenting cell; IL interleukin; PRR pathogen recognition receptor

1.2 The adaptive immune system

The adaptive immune system consists of lymphocytes, i.e. B cells and T cells. B cells are involved in the production of Immunoglobulins (Ig, i.e. antibodies), whereas T cells differentiate into helper T cells that further support the ongoing immune response. After an APC has presented an antigen to the T cell, the T cell starts to proliferate, and differentiates into one of the known helper T cell lineages: Th1, Th2 or the more recently described Th3 [19] and Th17 (Figure 1). [20, 21] The direction of differentiation is determined by the inflammatory environment and the presence of other factors, like cytokines. A Th1 response is characterized by the production of interferon-γ (IFNγ), TNFα and interleukin-2 (IL-2).

These cytokines stimulate T cell proliferation and activate macrophages. The activated macrophages produce cytokines that further promote Th1 differentiation of T cells. During a Th2 response, IL-4, IL-10 and IL-13 are typically produced, which activate B cells to produce immunoglobulines (Ig) and inhibit Th1 responses. Cytokines produced by Th2 effector cells further augment the differentiation signal towards a Th2 response, thereby maintaining the Th2 response. When the cells differentiate into the Th3 lineage, the T cells start to produce large amounts of transforming growth factor bèta (TGFβ), a factor which is known to be involved in tolerance and for the differentiation of regulatory T cells (Tregs). [22] Tregs are cells with anti-inflammatory properties; they inhibit activation of the immune system and thereby maintain immune homeostasis. [23] A Th17 response is characterized by the massive production of interleukin-17 by T cells, which is induced by IL-23, or IL-6 in combination with TGFβ. [24, 25] IL-17 induces and promotes pro-inflammatory responses, [26] and triggers the production of pro-inflammatory cytokines like TNFα and IL-1β. Th1, Th2, Th3 and Th17 effector T cells all express CD4 on their membrane, making them CD4+ T cells. Another subset of T cells, CD8+ cells, plays an important role in the recognition and elimination of intracellular pathogens. [27] CD8+ T cells recognize antigens presented by MHCI molecules, and are able to kill infected cells by secreting perforin and enzymes. [28]

Figure 2 The immunological synape.



1.3 The immunological synapse

As mentioned before, cytokines present in the inflammatory environment and the interaction between the APC and the T cell together determine the outcome of the immune response. The immunological synapse (IS) is the initial site of interaction between the DC and the T cell. [29, 30]

It is a highly organized structure, with the MHCII-TCR complex in the center, and the co-stimulatory molecules in the periphery (Figure 2). Importantly, a mature synapse is formed only upon recognition of a foreign antigen. When this is the case and an immunological synapse is formed, the actin skeleton polarizes towards the synapse. [31] A proper formation of the IS is important for an efficient T cell response, as destabilization of the IS has been reported to result in decreased T cell signaling. [32] As a consequence, formation of the IS plays a crucial role in the activation of T cells, and thereby the outcome of the immune response.

1.4 The immune system and tolerance in the gastrointestinal tract

The immune system in the gastrointestinal tract has several unique features. In the gut, an enormous amount of microorganisms (more than 500 different bacteria species) [33] is present that constitutes the gut flora (commensals), and intestinal APCs are constantly exposed to these microorganisms and food antigens. An immunologic response to the gut flora or other harmless antigens like food antigens would result in the recruitment of immune cells, production of cytokines, and consequently in inflammation, tissue damage and dysfunction. Therefore it is very important that intestinal APCs do not respond to these antigens, a process called tolerance. [34] The physical barrier separating the commensals from the underlying tissues consists of a single cell layer of epithelial cells and is the first line of defense. The presence of a mucus layer, antibacterial molecules (defensins) and IgA further helps to protect the invasion of antigens. [35] Since APC are then the first cells to respond to an antigen, Mϕ and DCs are the key players of innate immunity and tolerance. [36] To this end, intestinal Mϕ lack several innate response receptors, do not produce pro-inflammatory cytokines in response to various inflammatory signals, [37] and produce large amounts of IL-10 but no IL-12 and IL-23, [38] giving them an Mϕ2-like appearance. [39] Loss of tolerance would lead to an immune reaction, and subsequently result in autoimmunity. In the absence of inflammation but in the presence of commensals, a close balance between effector T cells (i.e. Th1, Th2 or Th17 T cells) and regulatory T cells is maintained by a complex network of cytokines.

2 Inflammatory Bowel Diseases

2.1 Epidemiology, symptoms and diagnosis

Inflammatory bowel disease (IBD) refers to a chronic inflammation affecting the gastrointestinal tract. The highest incidence rates for both Crohn's disease (CD) and ulcerative colitis (UC) are reported in the western world. [40-42] However, the incidence in other parts of the world is increasing. In Europe and the US, incidence rates range from 1.5 to 20.3 per 100.000 person-years for UC, and from 0.7 to 14.6 for CD. The observation that IBD incidence is low in developing countries, suggests that environmental factors and diet play an important role in the pathogenesis.

Crohn's disease (CD) is characterized by deep ulcerations that can occur in the entire gastrointestinal tract, i.e. from the mouth to the anus. [43, 44] The disease may affect only one area in the gut, or several areas with healthy areas in between, so called "skip lesions". An early feature of CD is apthoid ulceration, followed by deep ulcers and fissures in the mucosa at a later stage, which makes up the typical cobblestone pattern. Fistulae and abscesses are often present in a later stage. The inflammation is transmural (affecting all layers of the bowel), and lymphoid hyperplasia, an increase in inflammatory cells and granulomatous lesions are often observed.
Ulcerative colitis (UC) on the other hand, only affects the colon. [43, 44] It can affect the rectum alone (proctitis), it may involve the sigmoid and descending colon (left-sided colitis), or it may involve the whole colon. Typically, the mucosa has a red appearance, bloods easily and is inflamed. The inflammation is restricted to the mucosa, and crypt abscesses and goblet cell depletion are common features.
Typically, a patient presents with abdominal pain, bloody stools, diarrhea and weight loss. Some patients may also have complaints of malaise, fever, nausea and vomiting. Also, CD can be complicated by anal or perianal disease.
The diagnosis IBD can usually be made based on clinical, radiographic and histologic data. [45] The distinct patterns of the two diseases often enable the final diagnosis and differentiation between UC and CD on histologic basis. However, this is not always possible, and sometimes the diagnosis interderminate inflammatory colitis is made. In both UC and CD, anemia is common, and erythrocyte sedimentation rate (ESR) is often raised. CRP has been described as a sensitive marker for CD; elevated CRP levels are detectable in 70 – 100% of the CD patients. On the other hand, only 50 – 60% of UC patients have an elevated CRP at diagnosis. [46, 47]

2.2 Pathogenesis of IBD

CD and UC are diseases of unknown etiology. The fact that higher incidence rates are reported in the Western world compared to developing countries, suggests that environmental factors and nutrition may play a role. However, different incidence rates might also result from differences in access to health care and thus lower incidence rates may be reported in developing countries. Next to environmental factors, genetic factors and defects in innate and adaptive immunity may contribute to inflammatory bowel diseases.

2.2.1 Genetics

IBD has a strong genetic component, since a positive family history for the disease is the largest independent risk factor. It has been reported that 2.2 – 16.2% of the CD patients have a first-degree relative with CD, and in 5.2 – 22% with IBD. For UC, this is 5.7 – 15.5% and 6.6 – 15.8% respectively. [48] Moreover, two studies performed in twins show a pooled estimated concordance in monozygotic twins of 37.3% for CD, and 10% for UC; [49, 50] pooled concordance in dizygotic twins shows 7% for CD and 3% for UC. This suggests that CD might have a stronger genetic component than UC.
However, the disease does not simply result from a single gene defect. Many studies investigating the contribution of genes have been performed, and several genes that are relevant in innate and adaptive immunity have been suggested to be involved in the pathogenesis of IBD. First, the involvement of the *CARD15/NOD2* gene in CD has been shown and con-

firmed in several studies. [51-53] Since CARD15/NOD2 is a known PRR, mutations in this gene might result in altered sensing of bacterial products. Indeed, several mechanisms linking NOD2 dysfunction to CD pathogenesis have been reported, among which enhanced production of IL-1β, [54] abnormalities in TLR2 mediated inflammation in intestinal mϕ [55] and altered NOD2 dependent expression of microbicidal α-defensins. [56, 57] Importantly, NOD2 knockout mice do not spontaneously develop colitis, indicating that defects in NOD2 only are not sufficient to induce inflammation. [58]

SNPs (single nucleotide polymorphisms) in the tumor necrosis factor superfamily 15 (TNFSF15) gene have also been reported in the pathogenesis of IBD. [59, 60] Briefly, TNFSF15 is a strong inducer of IFNγ production in T cells and is upregulated in CD4+/CD8+ T cells and macrophages in the lamina propria of CD patients. [59, 61] Also, a role for SNPs in the IL-23 receptor has been verified in several studies. [62-64] IL-23, together with IL-6 and TGFβ, drives the differentiation of naïve T cells towards a Th17 response, thereby initiating an immune response. In addition, other genes in the IL-23 pathway have been implicated in the pathogenesis of IBD, including IL-12B (encodes the p40 subunit of IL23 and IL12) and signals transducer and activator of transcription 3 (STAT3), further suggesting a prominent role for this pathway. [65-67]

Many other genes have been identified that may play a role in IBD pathogenesis, but a full overview of these genetic defects is beyond the scope of this chapter.

2.2.2 Defects in the immune system in inflammatory bowel diseases

The underlying defect possibly lies in the loss of tolerance towards the mucosal flora, and several defects in innate and adaptive immunity have been reported that may play a role in the development of IBD.

It has been shown that lamina propria mononuclear cells (LPMNCs) from UC and CD patients spontaneously produce large amounts of pro-inflammatory cytokines, thereby triggering an immune response. [68-70] In a mouse model of colitis, increased responsiveness to bacterial stimuli has been reported, [71] resulting in aberrant immunity. This suggests that Mϕ from IBD patients display a more Mϕ1 phenotype, while the ability of intestinal Mϕ to secrete pro-inflammatory cytokines is normally (and preferably) low compared to Mϕ1. Indeed, lower amounts of Mϕ2 were found in mucosal biopsies from active lesions in CD patients compared to non-affected colon of the same patient, and compared to healthy controls. [72] In addition, DCs from both UC and CD patients show higher expression of TLR2 and TLR4 compared to healthy individuals, [73] making them hyperresponsive to bacterial antigens. Colonic macrophages from IBD patients have increased expression of the co-stimulatory molecules CD80 and CD86 [74] resulting in an increased ability to activate T cells. As a consequence of these defects, a Th1 or possibly Th17 response is induced.

Leaks in the epithelial barrier, which is the first line of defense, also have been reported in IBD patients. [75] As a result, pathogens cross the epithelial layer more easily. Interestingly, this defect seems to precede the development of CD in individuals with familial risk, [76] suggesting a causal role for this defect. In addition, overgrowth of mucosa-associated *Escheria coli* has been observed in CD patients; adherent-invasive *E. coli* (AIEC) are found in 36.4% of the CD patients with ileal involvement. Although *E. coli* is considered a commensal, some strains acquire virulence factors. AIEC bind to the CEACAM6 receptor, which is overexpressed in ileal mucosa of CD patients, leading to abnormal colonization. [77] Next, they

invade the intestinal barrier, infect and replicate within mucosal macrophages [78] and induce production of TNFα.
Furthermore, defects in the T cell compartment might contribute to the induction and persistence of IBD. It has been shown that IBD patients have increased numbers of activated T cells in the circulation, and that activated T cells from CD patients are more resistant to apoptosis, [79] a mechanism involved in programmed cell death which takes place after T cell activation and thereby contributes to homeostasis. As a result, the balance between effector T cells and regulatory T cells is disturbed in IBD patients, followed by uncontrolled inflammation.

2.2.3 Autophagy
Another interesting but rather unexpected discovery is the contribution of the *autophagy-related 16-like 1* (ATG16L1) and *immunity-related GTPase family M* (IRGM) genes to CD pathogenesis, [80-82] two genes that are known to be involved in a process called autophagy (referring to the Greek work "autophagos", i.e. "self-eating"). Autophagy was originally described as a cell survival mechanism. When a cell experiences nutrient depletion, autophagy is induced in order to remove damaged organelles. [83] Upon induction of autophagy, a membrane is formed, creating an autophagosome which surrounds the cellular contents and next fuses with lysosomes. [84] More recently, it became clear that autophagy also plays a crucial role in the clearance of intracellular bacteria, [85] and in the delivery of cytoplasmic antigens to MHCII molecules for antigen presentation to T cells. [86] In an experimental ATG16L1 knockdown system, cells showed defective autophagy in response to nutrient depletion and infection, demonstrating the importance of ATG16L1 in the autophagy process. [87] Since ATG16L1 and IRGM have been confirmed in several Genome Wide Association Studies (GWAS), and given the role of autophagy in general and in immunity, it is likely that autophagy plays an important role in the development of CD. Several mechanisms have been suggested that link defective autophagy to CD. In a DSS colitis model, mice lacking ATG16L1 in hematopoetic cells showed increased production of the pro-inflammatory cytokine IL-1β. [88] Furthermore, abnormalities in paneth cells (cells specialized in the secretion of granule contents that contain antimicrobial contents) have been reported in ATG16L1 knockout mice and in CD patients carrying the risk allele. [89]
In summary, environmental, genetic and immunologic defects all contribute to the development of IBD. Likely, the presence of a combination of these factors leads to a loss of response towards the mucosal flora, resulting in inflammation in the gut.

3 Treatment

The main treatment goals in IBD are improving quality of life, reducing hospitalization, surgery and steroid dependency, improving mucosal healing and maintaining clinical remission to control the disease while minimizing side effects. Mucosal healing can lead to significantly higher steroid-free remission rates and less relapses, [90] and is therefore an important goal to achieve in the treatment of CD patients. Induction therapy is concentrated on quickly reducing signs and symptoms of acute inflammation. However, the underlying disease cause remains unchanged and therefore maintenance therapy is often needed to

prevent relapses. Relapsing disease frequently leads to surgical interventions and hospitalization and for that reason maintaining remission is of great importance. Response to treatment is defined as a decrease in Crohn's Disease Activity Index (CDAI) of 70 points (70-points response) or 100 (100-points-response) after four weeks from baseline in non-fistulizing disease. Here, response is defined as a decrease of 70 points unless stated otherwise. In fistulizing disease, response is achieved when a decrease of at least 50% in the number of draining fistulas after ten weeks is observed. Remission is defined as a CDAI score below 150. [91, 92].

The final goal is to understand the course of disease and to finally alter the course towards a less aggressive phenotype. Several therapeutics are available with different effects, side effects and efficacy profiles to achieve the above described goals.

3.1 5-ASA

5-Aminosalicylic acid (5-ASA, mesalazine), a derivate of salicylic acid, is a non-steroidal anti-inflammatory drug (NSAID).

Whereas induction with 5-ASA therapy for patients with mild-to-moderate CD seems effective, [93] it is known that this agent is not effective in inducing remission. [94] In addition, patients with ileal disease do not benefit from 5-ASA and side effects occur in about one-third of the patients. [95] The clinical significance of the CDAI reduction obtained with 5-ASA is controversial, and therefore, 5-ASA has limited value in severe disease and in maintenance in CD patients.

On the other hand, 5-ASA is important in the treatment of UC; the efficacy of 5-ASA in severe UC has been shown in several systematic reviews and meta-analyses. [96, 97]

3.2 Steroids

Glucocorticoids are steroid hormones that bind to the glucocorticoid receptor and activate or suppress certain target genes. This then results in decreased production of pro-inflammatory cytokines and inhibition of T cell proliferation. [98, 99]

Although budesonide is more effective than placebo in inducing remission in acute active CD, [100] it is not effective in maintaining remission. [101] Also, systemic corticosteroids are very effective in inducing remission in the first place, [102] but do not induce long-term remission, [103, 104] mucosal healing [105] and do not reduce the risk for surgery. [106]

Similar to CD, corticosteroids are effective in the induction of remission, [107] and are important in acute severe UC, [108] but are not useful in maintenance therapy. [109] Furthermore, corticosteroids are known to have serious side effects like diabetes mellitus, [110] osteoporosis, depression, hypertension, and as a result these agents are associated with increased morbidity and mortality. Therefore, long-term corticosteroid use is discouraged.

3.3 Thiopurines

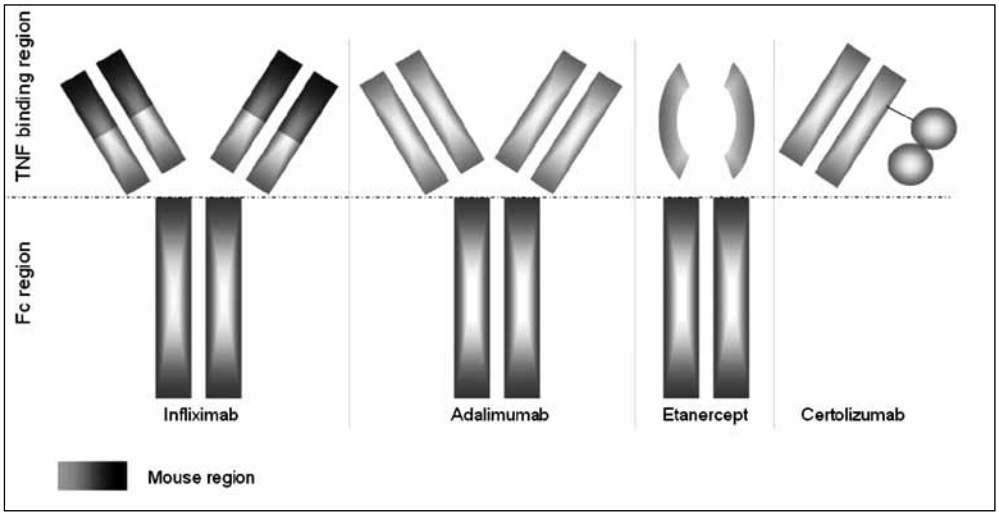
Azahioprine (AZA) and 6-mercaptopurine (6-MP) have been widely used in the treatment of IBD. AZA is a pro-drug which is converted to 6-MP, which is then metabolized to 6-thioguanine (6-TG). This acts as a DNA synthesis inhibitor, and thereby inhibits proliferation of cells, especially lymphocytes. In addition, it has been shown that azathioprine inhibits T cell proliferation by inhibiting APC-T cell conjugation [111] and it induces apoptosis in T cells by modulating Rac1 function. [112]

Thiopurines play an important role in controlling CD and have long term efficacy, [113, 114] but more than half of the patients still depend on corticosteroids. In UC, azathioprine is effective in inducing clinical and endoscopic remission, and is a drug of first choice in patients who are steroid dependent. [115] For maintenance therapy, the evidence for thiopurine in UC is weaker than in CD.
Unfortunately, thiopurine therapy has been associated with myelotoxicity, hepatotoxicity [116] and lymphoma. [117]

3.4 Anti-TNF: structure and mechanism of action

Elevated levels of TNFα are detectable in serum and the intestine of IBD patients, [118, 119] and therefore, blocking this cytokine would potentially alleviate the disease. The introduction of anti-TNF agents during the late '90s has proven to be an effective instrument to achieve the above described treatment goals. Anti-TNF agents induce mucosal healing, reduce steroid dependency, reduce the risk for surgery and hospitalization and improve the patient's quality of life. [91, 120, 121] In addition, healing of endoscopic lesions [121, 122] and reduction of chronic inflammatory infiltrates [122, 123] was achieved.

Figure 3 Different anti-TNF agents.



Anti-TNF agents are designed to neutralize soluble TNF-α, [124] an essential Th_1 cytokine produced by monocytes and T cells. [125] Several anti-TNF agents are available nowadays, and all of them have different structures and properties (Figure 3). Infliximab (Remicade®), is a chimaeric monoclonal antibody, and the first anti-TNF antibody on the market for CD. Adalimumab (Humira®), is a completely humanized anti-TNF antibody, and was designed in the hope to reduce immunogenicity. Adalimumab and infliximab are quite similar in structure; both have an Fc region and a Fab region. Certolizumab (Cimzia®) is different from infliximab and adalimumab, since it does not contain an Fc region and thus can not interact with Fc receptors. Whereas certolizumab was approved by the FDA for the treatment of moderate-to-severe CD, it has not been approved by the EMEA. Etanercept (Enbrel®), a

soluble TNF receptor fusion protein, is an effective drug for the treatment of rheumatoid arthritis and also efficiently neutralizes TNF-α. [124, 126, 127] Surprisingly, etanercept is not beneficial in CD, [128] implicating that neutralizing soluble TNF-α is not the only mechanism of action of anti-TNF agents responsible for their efficacy in CD. One of the differences between infliximab and etanercept is that infliximab induces apoptosis in lamina propria T cells, [129] but etanercept does not. [130] Infliximab, but not etanercept, binds to membrane bound TNF-α (mTNF), which can be cleaved by TNF-α-converting enzyme (TACE) to generate soluble TNF-α. Upon binding to mTNF, infliximab induces antibody dependent cytotoxicity (ADCC) and cell lysis. [131] Mitoma *et al.* described reverse signaling through mTNF induced by infliximab and adalimumab (but not etanercept), leading to cell cycle arrest in Jurkat T cells. [132, 133] Certolizumab does not induce apoptosis, but, like infliximab and adalimumab, inhibits LPS-induced IL-1β production by monocytes. [124] In addition, infliximab induces apoptosis in monocytes from CD patients with active disease. [134] Furthermore, infliximab reduces VCAM-1 (vascular cell adhesion molecule-1) and CD40 expression on mucosal endothelium, thereby disrupting the CD40-CD40L dependent interaction between T cells and endothelium. [135] Infliximab also acts on wound healing: infliximab increases tissue inhibitor of metalloproteinases-1 (TIMP-1) production and reduces matrix metalloproteinase (MMP) activity, and enhances myofibroblast migration *in vitro*. [136]

3.5 Anti-TNF therapy efficacy in clinical trials

The efficacy and safety of anti-TNF therapy has been widely evaluated in clinical studies Targan *et al.* reported response rates at week 4 of overall 65% in patients treated with infliximab vs. 17% in the placebo group. [91] In the ACCENT I trial, 58% responded to infliximab at week 2. [137] In another study, much higher response rates were observed in patients naïve to immunomodulators and biologics with short duration of disease. [138] Response rates of anti-TNF-naïve patients treated with adalimumab were assessed in the CLASSIC-I trial. After 4 weeks, 54% (adalimumab 40/20 mg) to 59% (adalimumab 80/40 and 160/80) showed a clinical response. [92] Clinical response rates of patients receiving certolizumab pegol were evaluated in the PRECISE-1 trial and were 44% at week 4 [139]. The 100-points response at week 6 (primary end point) after full induction therapy was 35%. Both response rates did not reach statistical significance. Patients who received anti-TNF therapy within the previous three months, or had a hypersensitivity or lack of response to a first anti-TNF dose were excluded. Infliximab, adalimumab, as well as certolizumab appeared safe.

Altogether, around one-third of patients treated with infliximab, 45% of patients treated with adalimumab and 56% of patients receiving certolizumab fail to show a clinical response at week 4 or week 6 after full induction therapy. Because there are no head-to-head trials, it is complex to directly compare the results of different studies. It is not known whether these primary non-responders represent a specific group of patients. Patients who do not show a response after a first infusion of infliximab, also fail to show response after subsequent infusions, [91, 140] suggesting that lack of response is stable over time. In general, although many clinical trials have shown the efficacy and safety of anti-TNF therapy, there is a relatively large group of patients displaying lack of response after 4 weeks (primary non-responders). In addition to lack of response, a considerable group of patients lose response following an initial response after several months of treatment. Loss of response is generally defined as a history of initial response and lack of improvement or worsening of symptoms, including:

increased stool frequency, fever, rectal bleeding, daily abdominal pain and recurring drainage from a previously non-draining fistula. [141] Furthermore, a significant number of patients become intolerant to anti-TNF, which is characterized by acute (during or within 24 h post treatment) or delayed (occurring 24 h – 15 days post treatment) infusion reactions.
Lack and loss of response to anti-TNF therapy is an obstacle in the treatment of CD. Predictive factors for lack and loss of response are needed to select patients for a certain approach. This may improve treatment, reduce side-effects and reduce morbidity. Many studies have been done to identify factors for lack and loss of response. Genetic, clinical and demographic factors have been described to play a role in lack of response. FcγRIIIa, [142] TACE [143] and LTA [140] might be possible genetic factors, and young age, [144, 145] luminal CD, [145] short duration of disease [146] and concurrent immusuppression [145, 147-150] possible clinical factors. Patients with lack of response can switch to another anti-TNF, or to a biologic with another mechanism of action. Though controversial, the formation of antibodies against antibodies has been associated with loss of response. The use of concurrent immunosuppressive drugs may reduce the formation of antibodies against antibodies. Dose intensification in patients with low drug through levels or switching to another anti-TNF might be a good option in patients with loss of response.
Also, side effects might complicate anti-TNF treatment. Anti-TNF use is associated with an approximately 21-fold increased risk of tuberculosis (TB) without appropriate safety measures. [151] The TB incidence has been reported to decrease with 78% when suitable safety measures where undertaken. Most cases are presented during the first three months of treatment and have an atypical presentation, which makes the diagnosis more complicating. [152] For that reason, international guidelines advise to assess the risk of TB before starting treatment with an anti-TNF agent, including an X-ray, tuberculin skin testing (depending on national guidelines) and careful evaluation of the TB history. [153] Latent TB may be suspected in case of a positive initial tuberculin skin test and when the patient has recently been exposed to the disease. Physicians should be aware of the possibility of false-negative skin tests, especially when patients are immunocompromized.
Next to reactivation of *mycobacterium tuberculosis*, the use of immunosuppressive agents is associated with opportunistic infections. The risk of opportunistic infections in anti-TNF treated patients is estimated between 0.3 and 0.9%, [154] and an increased risk is observed in patients treated with concommittant immunosuppressives. [155] Indeed, in a large meta-analysis of 21 placebo-controlled trials including 5356 anti-TNF treated patients, the increased risk of opportunistic infections was likely due to disease severity and prednisone use, instead of merely anti-TNF. [156] In line with this observation, no increased risk was found in infections and mortality in 734 anti-TNF treated patients compared to controls, with a median follow-up of 58 months. [149]
Finally, the development of malignanies, and especially lymphomas, is a major concern. Whereas some studies do not show an increased risk, [157-160] other studies do find a moderately elevated risk, especially in patients on thiopurine therapy. [117, 161, 162] Lethal hepatosplenic T cell lymphoma has been reported in young patients on azathioprine/infliximab combination therapy, [163-166] and therefore long-term combination therapy in younger patients is not recommended. Still, the absolute risk appears to be low and should be weighed against the beneficial effects of immunomodulator therapy. In addition, differences in study design and patient recruitment complicate the interpretation of these data. Furthermore, in the

meta-analysis including 21 placebo-controlled trials, [156] no increased risk of malignancy was observed. These data were supported by another study [149] including 734 anti-TNF treated patients. However, long-term safety data are not available yet and therefore awareness of (serious) side effects is warranted.

3.6 Other therapies

There is not much data on the efficacy of methotrexate in UC, but the only randomized placebo-controlled trial did not show any benefit. [167] In contrast, methotrexate efficacy has been shown CD patients. [168] and at the present time, methotrexate therapy is used in patients with active or relapsing CD who are refractory or intolerant to thiopurine therapy or anti-TNF agents. [169] Other immunosuppressives, like ciclosporin or tacrolimus, may be of benefit in patients with severe UC who are intolerant to i.v. corticosteroids. [109] Ciclosporin is of limited value in CD, [45] but data are lacking on the efficacy of tacrolimus in CD.

3.7 Future treatment

Unfortunately, the medication used to control CD is not without risk. Although substantial progression has been made with regard to treatment, there is still no cure for IBD. In addition, side effects like lymphoma, *Mycobacterium tuberculosis* and opportunistic infections further complicate treatment. Moreover, lack and loss of response to anti-TNF therapy are problems in daily practice that are even now unsolved, and surgery is then often the only option left.

Our understanding of the mechanism of action and side effects of several therapies is still incomplete. In an ideal situation, it would be possible to select patients based on genotype or disease phenotype, age or other yet undefined factors for a certain therapeutic strategy. To accomplish this, a better understanding of the pathogenesis of CD and the complex mechanism of action of anti-TNF therapy is warranted in order to tailor therapy. In that way, it is possible to reduce side effects, surgery and chronic use of corticosteroids. In addition, therapy risks need to be re-assessed at any given time point, and if necessary, the therapeutic approach should be re-adjusted. Finally, we have to continue exploring new therapies with less side effects and high efficacy profiles. One interesting development in the treatment of inflammatory disorders, is the administration of mesenchymal stem cells (MSC). MSC are cells with immunosuppressive properties [170, 171] and have been studied in various fields of medicine. Administration of MSCs to patients with severe steroid-refractory graft-versus-host disease (GvDH), including GvDH of the gut, has been shown to be effective. [172, 173] This may be a promising strategy in the treatment of CD.

4 Scope of the thesis

In *chapter 2*, we give an overview of current treatment strategies for CD, especially the top-down approach. Since CD typically progresses from an inflammatory to a fibrotic phenotype, it may be beneficial to interfere in an early disease stage in patients with high risk at developing complicated disease.

In *chapter 3*, we demonstrate the role of autophagy in DCs in regulation of the immunological synapse and CD pathogenesis. We show that decreased levels of autophagy lead to

hyperstabilization of the immunological synapse. This results in increased interaction duration between DCs and T cells and increased T cell activation and IL-17 production. Also, we demonstrate that autophagosomes contain components of the synaps, suggesting that autophagy might be involved in the synaptic breakdown, and thereby plays a role in controlling T cell responses. In addition, we found the same results in patients carrying the ATG16L1 risk allele, indicating a novel role for autophagy in CD pathogenesis by modulating adaptive immune responses.
In *chapter 4*, we further dissect the mechanism of action of anti-TNF agents *in vitro*. We describe the Fc-receptor dependent induction of Mϕ2 upon infliximab therapy and their immunosuppressive phenotype. Since loss of tolerance and hyperresponsiveness contribute to IBD, the induction of Mϕ2 by infliximab might restore the dysbalance.
The induction of Mϕ2 *in vivo* is shown in *chapter 5*, and a significant relation between response to infliximab and induction of Mϕ2 is described. Also, we show the wound healing capacity of infliximab-induced macrophages, further supporting their role in mucosal healing. Furthermore, we show an enhanced induction of Mϕ2 upon infliximab/azathioprine combination treatment, and that Mϕ2 induced by combination treatment have a stronger immunosuppressive phenotype. This might explain the superiority of infliximab/azathioprine combination treatment observed in patients.
In *chapter 6* a Phase I study investigating the safety and feasibility of MSC therapy in steroid-refractory CD patients is described. We show the immunosuppressive properties of MSCs *in vitro* and that MSC therapy in CD patients is safe and feasible. Importantly, no serious side effects were reported during the study period. The efficacy of MSC therapy in CD patients should be further assessed in Phase II/III trials.
In *chapter 7* we further examine the safety profile of common IBD drugs in relation to lymphoma development. In a cohort of approximately 18000 patients, no increased risk was found compared to the general population, but a clear association was observed between thiopurine therapy and EBV positive lymphoma, especially in younger patients. These data give more insight in the risks in specific patient groups.

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