Towards therapeutic disease control in inflammatory bowel diseases

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Chapter eight

Summarizing discussion

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This thesis focuses on various strategies towards disease control in IBD; new treatment targets, working mechanism of established therapies, side effects and future therapy. A better understanding of disease pathology and the working mechanism of IBD therapy, could help to find new treatment targets, disease markers and novel therapies. Also, this would allow the selection of patients for a certain therapy based on genotype or phenotype.
An overview of a selection strategy based on disease phenotype is provided in **chapter two**. It has been shown that patients with young age at onset of disease, presence of perianal disease, stricturing disease and initial need for corticosteroids have a high risk at developing a complicated disease course. In addition, CD typically evolves from an inflammatory disease to a fibrotic disease. At this stage, the disease is difficult to treat and response rates to various therapies, among which anti-TNF, are lower than in patients with recently diagnosed disease. It is suggested that patients with a high risk of developing complicated disease would particularly benefit from a "top-down" approach. Importantly, side effects need to be weighed against the benefits, and strong genetic or phenotypic factors in order to predict disease course, therapeutic response and risk of side effects are still lacking but would be highly valuable.

A new role for autophagy in CD pathogenesis: clinical implications

Since autophagy has been described as a degradation mechanism, [1, 2] many studies on the role of autophagy in CD pathogenesis focused on its role in the clearance of pathogens. [3-5] A defect in the autophagy pathway could lead to impaired clearance of intracellular organisms, and in that way contribute to uncontrolled microbial expansion and excessive immunity. Likely, these mechanisms contribute to disease pathogenesis, but the results described in **chapter three** provide new insight into the role of autophagy in the absence of pathogens. We found that autophagy is important in the breakdown of the immunological synapse, and in that way identified a role of autophagy in tolerance and immunity in general. Importantly, we also confirmed these findings in patients carrying the autophagy SNP, and these data may explain the aberrant immune response observed in these patients. The identification of a new role for autophagy in the pathogenesis of CD may provide new therapeutic targets in patients carrying this SNP. Interestingly, azathioprine has been described to suppress APC-T cell interactions, [6] and therefore patients carrying the autophagy risk allele may benefit from azathioprine therapy in particular. In addition, other autophagy inducing drugs may be effective in this specific patient group, such as rapamycin, which inhibits mammalian target of rapamycin (mTOR) and thereby induces autophagy. Indeed, two case reports show an impressive effect of this drug, [7, 8] but this has not been tested in patients with the risk allele specifically. In addition, the efficacy of this drug has not been evaluated in clinical trials. Yet, selecting patients based on genotype for a certain therapy might be a promising future strategy.

Relevance of Mϕ2 induction and future implications

In **chapter four** we describe the Fc region dependent induction of regulatory macrophages *in vitro* by anti-TNF antibodies and we further show their immunosuppressive features. Both the presence of monocytes and T cells was required in order to induce Mϕ2. Interestingly, this induction was only observed when mixed lymphocyte reaction cultures were treated with agents that have the ability to bind to mTNF and have an Fc region to interact with Fc receptors. Anti-TNF agents that do not bind to mTNF (etanercept, onercept) and anti-TNF agents that do not interact with Fc receptors (certolizumab, CDP571) did not induce Mϕ2 *in vitro*. These agents are also the ones that failed to show efficacy in clinical practice, and it is tempting to speculate that the induction of Mϕ2 is crucial in this process.
Mucosal healing is nowadays an important treatment goal, since it has been shown that mucosal healing is associated with less surgery, less hospitalization, less corticosteroid use and improved quality of life. [9-11] The factors that contribute to mucosal healing are not completely understood, but the role of regulatory macrophages (Mϕ2) in wound healing in general has been extensively studied before. [12, 13] Next to wound healing properties, Mϕ2 have immunosuppressive effects. They produce mainly anti-inflammatory cytokines, [14] have minor ability to respond to bacterial stimuli, [15] and inhibit proliferation of activated T cells. [16] Since IBD is thought to result from loss of tolerance towards the mucosal environment, the induction of this cell type might be of particular interest in restoring the balance and inducing mucosal healing. Indeed, decreased numbers of this cell type are found in the mucosa of IBD patients. [17] Since the successful introduction of anti-TNF agents during the late '90s in terms of mucosal healing and improved quality of life, [18-21] much research has focused on the mechanisms of action to elucidate factors involved in mucosal healing. In addition, the inefficacy of several anti-TNF agents in the clinic suggested that neutralization of TNFα was not the most important aspect.
In **chapter five**, we confirmed the induction of Mϕ2 *in vivo* and showed that patients responding to infliximab therapy have increased numbers of CD206+/CD68+ cells after 4 weeks of induction therapy compared to baseline. Since we used endoscopic and histologic healing as a definition of response, these data suggest that the induction of Mϕ2 may be important in the process of mucosal healing. Also, we demonstrated the wound healing capacity *in vitro*, which further suggests a critical role of this cell type in mucosal healing.
The induction of Mϕ2 by anti-TNFα antibodies provides a model which helps to elucidate several other features related to anti-TNF treatment. Not only may this hypothesis help to explain why certain anti-TNF agents have not succeeded in clinical practice, also the superiority of infliximab/azathioprine combination treatment observed in the clinic in terms of remission induction and maintenance and mucosal healing [22] may be explained. The results obtained with combination treatment in the clinic correlate properly with the results we observed *in vitro*. We found increased numbers of Mϕ2 upon combination treatment, and interestingly, these macrophages also displayed stronger immunosuppressive properties. In addition, this hypothesis may help to resolve several side effect issues, for instance the increased risk of tuberculosis which is observed with anti-TNF antibodies. [23, 24] Remarkably, this increased risk is only observed with the anti-TNF antibodies, and not with the soluble TNF receptor etanercept, both in human [25] and in mice. [26] It has been shown that mycobacteria like *M. tuberculosis* bind stronger to Mϕ2 than Mϕ1, [27] and that the mannose receptor

CD206 plays a role in the phagocytosis of *M. tuberculosis*. [28] Interestingly, we only found an induction of Mϕ2 only in the conditions treated with anti-TNF antibodies, and in addition we observed an upregulation of CD206 on these induced cells. The results we obtained *in vitro* fit properly with the observations *in vivo*, and insight into these mechanisms provides new opportunities to challenge this problem in the future.

The immunosuppressive effect of Mϕ2 is probably mediated by a soluble factor, as we observe strong dose-dependent inhibition of T cell proliferation when we culture T cells with Mϕ2-conditioned medium (data not shown). However, we did not find wound-healing effects of Mϕ2-conditioned medium (data not shown) and this might suggest that the immunosuppressive properties and wound healing properties of Mϕ2 are mediated by distinct factors. Therefore, the exact mechanisms that underlie Mϕ2-induced effects are yet to be defined, but likely both soluble factors and cell-cell interactions are involved in the various processes. IL-10 has been proposed as a responsible factor in Mϕ2-induced amelioration of colonic disease in mice, [17] but it is less likely that IL-10 is the key factor leading to the results we observed *in vitro*. In our hands, Mϕ2 mainly produce large amounts of IL-10 in response to LPS, but we observed strong immunosuppressive and wound healing properties in the absence this stimulus. Therefore, we think that IL-10 may contribute to the effects, but is unlikely to be the key mediator. Several other factors have been described in the literature, including the induction of Treg, [29] but this mechanism was not involved in our *in vitro* experiments.

We found that patients that do not respond to infliximab therapy have lower amounts of Mϕ2 at baseline, and it is possible that these patients have a defect in the differentiation or the recruitment of Mϕ2. Identifying factors involved in this defect and Mϕ2 differentiation in general would allow the selection of patients for a certain therapeutic strategy. A recent paper showed that UC patients with high IL13Rα2 expression at baseline, show poor response to infliximab therapy. [30] IL13Rα2 has been described to function as a decoy receptor, and thereby prevents proper IL-13 signaling. [31] Interestingly, IL-13 in combination with IL-4 is a known inducer of regulatory macrophages, [32, 33] and disturbed IL-13 signaling might therefore result in reduced induction of regulatory macrophages.

It would be of great value to identify the factors that are responsible for the induction and function of Mϕ2, since this would facilitate the identification of a marker that reflects disease severity and therapy efficacy. In addition, the identification of responsible factors would possibly provide new treatment targets.

The findings described in chapter 4 and 5 may have further implications. The idea that binding to Fc receptors is a critical step in the induction of Mϕ2 suggests that the generation of an anti-TNF antibody with stronger affinity to the Fc receptor might have enhanced efficacy. Indeed, antibodies with higher affinity to Fc receptors have been developed in other fields of medicine in order to augment efficacy, [34, 35] and this approach may be promising in the development of new therapies with greater efficacy for the treatment of IBD as well.

Although the introduction of anti-TNF agents was an important development in the treatment of IBD, about 30% do not respond to this therapy, and about 30% lose response after a certain period of time. Because side effects and occasionally limited efficacy are still obstacles in the treatment of IBD, there is need for new therapies with less toxicity and higher efficacy. Mesenchymals stem cells (MSCs) are multipotential nonhematopoetic progenitor cells that can be isolated from various tissues, including the bone marrow. Like Mϕ2, MSCs

have immunomodulatory [36, 37] and wound healing [38-40] properties. Several mechanisms have been suggested that may be involved in these processes, including cell-cell dependent mechanisms as well as soluble factors. Interestingly, it has been described that MSCs are able to induce Mϕ2. [41-43] Because of their wound healing and immunomodulatory properties, these cells are of particular interest in various fields of medicine, including in the treatment of IBD. In **chapter six** we described the treatment of CD patients with autologous MSC in a Phase I trial. Importantly, MSCs from CD patients showed the same characteristics and immunomodulatory properties as MSCs from healthy controls. No serious side effects were reported during the study period, and a clinical response was observed in 3 patients; 3 patients needed surgery. Since all patients had severe refractory disease, it is complicated to speculate on efficacy. MSCs seem to have high potential to serve as an efficacious cell based therapy in IBD, and currently numerous studies are underway to further evaluate this.

Side effects and risk profiles: current therapy and future implications

As mentioned before, the drugs used for the treatment of IBD are not without risk. Therefore, a better understanding of the mechanism of action of IBD drugs enables the identification of factors leading to side effects. In addition, a careful evaluation of side effects and defining specific risk groups is important to limit side effects as much as possible. Cancer, and especially lymphoma, is a long-standing concern in patients treated with immunosuppressive agents.

In **chapter seven** we aimed to assess the risk of lymphoma in IBD patients and to find specific patient groups at risk. Also, we investigated whether we could establish an association between azathioprine use and lymphoma. In this retrospective, nation-wide study, we observed 44 lymphomas in a cohort of approximately 18000 IBD patients. Compared to lymphoma risk in the general population, the risk of lymphoma in IBD patients was not increased in this study. However, a clear increased risk was observed in patients in the age groups 35 – 39 and 45 – 49 and a significant correlation was found between thiopurine use and the development of EBV^+ lymphoma. None of the 44 cases used anti-TNF agents, but it is risky to draw any conclusions from that observation since this study was not designed to specifically study lymphoma risk in anti-TNF treated patients. Our data are in line with other population-based studies, [44-47] but in contrast to data from a meta-analysis [48] and a recent prospective study. [49] Discrepancies in outcome may result from differences in patient recruitment, study design and a different lymphoma incidence in the general population. The latter determines the calculated expected number and in that way the relative risk. Nevertheless, it has been estimated that only a ten-fold increased risk would outweigh the benefit of thiopurine treatment, [50] so although a slightly increased risk is found in some studies, this would still have limited clinical implications. On the other hand, since it appears that particularly patients ≤ 50 years have an increased risk, and patients with young onset of disease more often require intensive therapy, watchfulness is warranted in this vulnerable group. It is yet unknown why younger patients might have an increased risk, and which factors may be involved. In addition, it remains to be determined to what extent disease severity and disease duration contribute to lymphoma development, or whether this simply results from immunosuppression in general, or azathioprine in particular. Other

aspects like medication dose, therapy duration, and disease-related factors like disease location and age at onset of disease also may play a role. Although our study involved approximately 18000 IBD patients in the Netherlands, the relative small number of 44 lymphomas does not provide the power which is needed to calculate the contribution of these specific factors. This would further allow the stratification of patients for a certain therapy, especially with regard to side effects.
In summary, the studies described in this thesis provide new insight into our current knowledge of IBD therapy. Increasing knowledge of the working mechanism of anti-TNF agents, safety profiles, cell-based therapy and the identification of new possible drug targets, could eventually lead to personalized treatment with higher efficacy and less toxicity.

Reference List

1. Berry DL, Baehrecke EH. Growth arrest and autophagy are required for salivary gland cell degradation in Drosophila. Cell 2007;131:1137-1148.
2. Klionsky DJ, Emr SD. Autophagy as a regulated pathway of cellular degradation. Science 2000;290:1717-1721.
3. Birmingham CL, Smith AC, Bakowski MA et al. Autophagy controls Salmonella infection in response to damage to the Salmonella-containing vacuole. J Biol Chem 2006;281:11374-11383.
4. Perrin AJ, Jiang X, Birmingham CL et al. Recognition of bacteria in the cytosol of Mammalian cells by the ubiquitin system. Curr Biol 2004;14:806-811.
5. Gutierrez MG, Master SS, Singh SB et al. Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. Cell 2004;119:753-766.
6. Poppe D, Tiede I, Fritz G et al. Azathioprine suppresses ezrin-radixin-moesin-dependent T cell-APC conjugation through inhibition of Vav guanosine exchange activity on Rac proteins. J Immunol 2006;176:640-651.
7. Massey DC, Bredin F, Parkes M. Use of sirolimus (rapamycin) to treat refractory Crohn's disease. Gut 2008;57:1294-1296.
8. Dumortier J, Lapalus MG, Guillaud O et al. Everolimus for refractory Crohn's disease: a case report. Inflamm Bowel Dis 2008;14:874-877.
9. Schnitzler F, Fidder H, Ferrante M et al. Mucosal healing predicts long-term outcome of maintenance therapy with infliximab in Crohn's disease. Inflamm Bowel Dis 2009;15(9):1295-301.
10. Baert F, Moortgat L, Van Assche G et al. Mucosal Healing Predicts Sustained Clinical Remission in Patients with Early-Stage Crohn's Disease. Gastroenterology 2010;138(2):463-8.
11. Froslie KF, Jahnsen J, Moum BA et al. Mucosal healing in inflammatory bowel disease: results from a Norwegian population-based cohort. Gastroenterology 2007;133:412-422.
12. Daley JM, Brancato SK, Thomay AA et al. The phenotype of murine wound macrophages. J Leukoc Biol 2010;87:59-67.
13. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol 2008;8:958-969.
14. Kamada N, Hisamatsu T, Okamoto S et al. Abnormally differentiated subsets of intestinal macrophage play a key role in Th1-dominant chronic colitis through excess production of IL-12 and IL-23 in response to bacteria. J Immunol 2005;175:6900-6908.

15. Smythies LE, Sellers M, Clements RH et al. Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity. J Clin Invest 2005;115:66-75.
16. Schebesch C, Kodelja V, Muller C et al. Alternatively activated macrophages actively inhibit proliferation of peripheral blood lymphocytes and CD4+ T cells in vitro. Immunology 1997;92:478-486.
17. Hunter MM, Wang A, Parhar KS et al. In Vitro-Derived Alternatively Activated Macrophages Reduce Colonic Inflammation in Mice. Gastroenterology 2010;138(4):1395-405.
18. Targan SR, Hanauer SB, van Deventer SJH et al. The Crohn's Dc. A Short-Term Study of Chimeric Monoclonal Antibody cA2 to Tumor Necrosis Factor {alpha} for Crohn's Disease. N Engl J Med 1997;337:1029-1036.
19. van Dullemen HM, van Deventer SJ, Hommes DW et al. Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). Gastroenterology 1995;109:129-135.
20. D'Haens G, Van Deventer S, Van Hogezand R et al. Endoscopic and histological healing with infliximab anti-tumor necrosis factor antibodies in Crohn's disease: A European multicenter trial. Gastroenterology 1999;116:1029-1034.
21. Baert FJ, D'Haens GR, Peeters M et al. Tumor necrosis factor alpha antibody (infliximab) therapy profoundly down-regulates the inflammation in Crohn's ileocolitis. Gastroenterology 1999;116:22-28.
22. Colombel JF, Sandborn WJ, Reinisch W et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. N Engl J Med 2010;362:1383-1395.
23. Keane J, Gershon S, Wise RP et al. Tuberculosis Associated with Infliximab, a Tumor Necrosis Factor {alpha}-Neutralizing Agent. N Engl J Med 2001;345:1098-1104.
24. Denis B, Lefort A, Flipo RM, et al. Long-term follow-up of patients with tuberculosis as a complication of tumour necrosis factor (TNF)-alpha antagonist therapy: safe re-initiation of TNF-alpha blockers after appropriate anti-tuberculous treatment. Clin Microbiol Infect 2008;14:183-186.
25. Tubach F, Salmon D, Ravaud P et al. Risk of tuberculosis is higher with anti-tumor necrosis factor monoclonal antibody therapy than with soluble tumor necrosis factor receptor therapy: The three-year prospective French Research Axed on Tolerance of Biotherapies registry. Arthritis Rheum 2009;60:1884-1894.
26. Plessner HL, Lin PL, Kohno T et al. Neutralization of tumor necrosis factor (TNF) by antibody but not TNF receptor fusion molecule exacerbates chronic murine tuberculosis. J Infect Dis 2007;195:1643-1650.
27. Verreck FA, de BT, Langenberg DM et al. Human IL-23-producing type 1 macrophages promote but IL-10-producing type 2 macrophages subvert immunity to (myco)bacteria. Proc Natl Acad Sci U S A 2004;101:4560-4565.
28. Kang PB, Azad AK, Torrelles JB et al. The human macrophage mannose receptor directs Mycobacterium tuberculosis lipoarabinomannan-mediated phagosome biogenesis. J Exp Med 2005;202:987-999.
29. Savage ND, de BT, Walburg KV et al. Human anti-inflammatory macrophages induce Foxp3+ GITR+ CD25+ regulatory T cells, which suppress via membrane-bound TGFbeta-1. J Immunol 2008;181:2220-2226.
30. Arijs I, Li K, Toedter G et al. Mucosal gene signatures to predict response to infliximab in patients with ulcerative colitis. Gut 2009;58:1612-1619.

31. Rahaman SO, Sharma P, Harbor P et al. IL-13R(alpha)2, a decoy receptor for IL-13 acts as an inhibitor of IL-4-dependent signal transduction in glioblastoma cells. Cancer Res 2002;62:1103-1109.
32. Hart PH, Bonder CS, Balogh J et al. Differential responses of human monocytes and macrophages to IL-4 and IL-13. J Leukoc Biol 1999;66:575-578.
33. Gordon S. Alternative activation of macrophages. Nat Rev Immunol 2003;3:23-35.
34. Zalevsky J, Leung IW, Karki S et al. The impact of Fc engineering on an anti-CD19 antibody: increased Fcgamma receptor affinity enhances B-cell clearing in nonhuman primates. Blood 2009;113:3735-3743.
35. Horton HM, Bernett MJ, Pong E et al. Potent in vitro and in vivo activity of an Fc-engineered anti-CD19 monoclonal antibody against lymphoma and leukemia. Cancer Res 2008;68:8049-8057.
36. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 2005;105:1815-1822.
37. Krampera M, Glennie S, Dyson J et al. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. Blood 2003;101:3722-3729.
38. Stappenbeck TS, Miyoshi H. The role of stromal stem cells in tissue regeneration and wound repair. Science 2009;324:1666-1669.
39. Chen L, Tredget EE, Liu C et al. Analysis of allogenicity of mesenchymal stem cells in engraftment and wound healing in mice. PLoS One 2009;4:e7119.
40. Chen L, Tredget EE, Wu PY et al. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. PLoS One 2008;3:e1886.
41. Kim J, Hematti P. Mesenchymal stem cell-educated macrophages: a novel type of alternatively activated macrophages. Exp Hematol 2009;37:1445-1453.
42. Maggini J, Mirkin G, Bognanni I et al. Mouse bone marrow-derived mesenchymal stromal cells turn activated macrophages into a regulatory-like profile. PLoS One 2010;5:e9252.
43. Zhang QZ, Su WR, Shi SH et al. Human gingiva-derived mesenchymal stem cells elicit polarization of m2 macrophages and enhance cutaneous wound healing. Stem Cells 2010;28:1856-1868.
44. Lewis JD, Bilker WB, Brensinger C et al. Inflammatory bowel disease is not associated with an increased risk of lymphoma. Gastroenterology 2001;121:1080-1087.
45. Askling J, Brandt L, Lapidus A et al. Risk of haematopoietic cancer in patients with inflammatory bowel disease. Gut 2005;54:617-622.
46. Persson PG, Karlen P, Bernell O et al. Crohn's disease and cancer: a population-based cohort study. Gastroenterology 1994;107:1675-1679.
47. Loftus EV, Jr., Tremaine WJ, Habermann TM et al. Risk of lymphoma in inflammatory bowel disease. Am J Gastroenterol 2000;95:2308-2312.
48. Kandiel A, Fraser AG, Korelitz BI et al. Increased risk of lymphoma among inflammatory bowel disease patients treated with azathioprine and 6-mercaptopurine. Gut 2005;54:1121-1125.
49. Beaugerie L, Brousse N, Bouvier AM et al. Lymphoproliferative disorders in patients receiving thiopurines for inflammatory bowel disease: a prospective observational cohort study. The Lancet 2009;374:1617-1625.
50. Lewis JD, Schwartz JS, Lichtenstein GR. Azathioprine for maintenance of remission in Crohn's disease: benefits outweigh the risk of lymphoma. Gastroenterology 2000;118:1018-1024.