



Universiteit
Leiden
The Netherlands

Matrix metalloproteinases in inflammatory bowel disease : expression, regulation and clinical relevance

Meijer, M.J.W.

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Chapter 1

Introduction

Inflammatory Bowel Disease - a major health problem

Inflammatory bowel disease (IBD), i.e., Crohn's disease (CD) and ulcerative colitis (UC), are characterized by an idiopathic, chronic and recurrent inflammation of the gastrointestinal tract. In CD, inflammation is segmental and transmural, often localized in (but not confined to) the ileocaecal area, while UC is limited to the mucosal lining of the colon, often starting in the rectum and extending in proximal direction through the years. In both CD and UC, inflammation may result in severe tissue damage, i.e., discontinuation of the epithelial border, ulcers, fissures, loss of circular folding, cobblestone appearance, fibrosis, stenosis and in CD also the formation of entero-entero, entero-viscero or entero-cutaneous fistulae. Abdominal pain, increased defecation frequency, bloody diarrhea, nausea, significant body weight loss and anemia related fatigue all contribute to the general malaise IBD patients often experience. Disease course may be complicated by osteoporosis, arthritis, ankylosing spondylitis, iritis, uveitis, erythema nodosum and primary sclerosing cholangitis (UC). Patients are at increased risk for developing colorectal carcinoma^{1,2} and recently, IBD was associated with a higher incidence of adverse pregnancy outcomes.³ First line of treatment consists of 5-ASA containing mesalazine/sulphasalazine or corticosteroid budesonide/prednisolone tablets, enema or suppository. In corticosteroid refractory patients disease modifying drugs azathioprine, cyclosporin, methotrexate or mycophenolate mofetil are administered. The therapeutic arsenal for CD patients has been expanded recently with biological agents specifically targeting TNF- α in the immune cascade. Chimeric (75% human/25% murine) infliximab and humanised adalimumab anti TNF- α antibodies have proven to be effective in the clinical setting for steroid dependent/refractory CD patients.⁴⁻⁶ Disadvantages include the large placebo effect requiring further optimization, host antibody response to infliximab and high medical costs.^{7,8} Biological agents against IL-12, adhesion molecules and IL-6 receptors are promising new candidates but are not expected to enter the market soon.^{9,10} Despite advances in treatment protocols, the natural history of the disease appears unmodified.^{11,12} Cumulative 10 year surgical resection probability rates vary from 25-60 % in CD and 25% in UC patients and these percentages have remained similar for 4 decades although surgical resection rates within the first year of

diagnosis might be decreasing.¹³⁻¹⁶ The socioeconomic burden remains high: in the United States total costs of IBD related healthcare are approximately 1 billion US dollar per year.¹⁷ When work-productivity losses due to chronic disability are taken into account, total costs of IBD in the USA were estimated \$ 5 billion in 2000.¹⁸ Therefore, much effort is spent in research aimed at identifying the epidemiology, etiology and pathogenic mechanisms underlying IBD.

Inflammatory Bowel Disease - epidemiology & etiology

Annual incidence rates of CD in white residents of Western countries vary between 4-9 per 100,000, whereas the incidence of UC appears somewhat higher (9-14 per 100,000), with concordant prevalence rates of 130-175 (CD) and 240-275 (UC) per 100,000 persons.^{16,19-22} Studies from Canada and New Zealand, however, reported higher incidence figures for CD compared to UC (14.6 versus 14.3 and 16.5 versus 7.6 per 100,000, respectively).^{23,24} In both CD and UC, distribution of age at onset of disease is skewed, with an incidence peak between 20-30 years. Incidence and prevalence rates have risen after the Second World War, reaching the current plateau in the 1970s, affecting more people in urban versus rural areas.^{25,26} People in white collar occupations appear more at risk compared to other groups in the population.²⁷ A north-south gradient has been postulated,²⁸ but in industrializing countries, incidence and prevalence of IBD are also increasing,²⁹ as are disease rates among Asian immigrants in Western countries.³⁰ Although disease concordance rates in monozygotic twins are high (CD: 60, UC: 20 %), pointing to a genetic influence, these sub maximum figures also suggest the involvement of one or several environmental factor(s) in the etiology of IBD.³¹ Smoking was established as such a factor, worsening the prognosis in CD³² and instead protective in UC.³³ Other studies have suggested an association of CD and/or UC with infectious agents such as *Mycobacterium avium* sp. *Paratuberculosis*,³⁴ invasive *Escherichia coli* strains^{35,36} and measles virus infection or vaccination.³⁷⁻³⁹ In case of *Helicobacter pylori*, a protective effect was suggested.⁴⁰ Married couples are at greater risk of sequentially contracting the disease after cohabitation.⁴¹ Persons born in the winter months were more at risk for IBD⁴² and onset of symptoms was also especially observed during this period, thus arguing for the

rationale of an infectious agent involved in the etiology of IBD,⁴³ although this remains to be confirmed.⁴⁴ Other studies have focused on an association with dietary food intake, such as breastfeeding, fast food, coca cola beverages and chocolate consumption.⁴⁵⁻⁴⁷ Use of oral contraceptives,^{48,49} menstrual cycle⁵⁰ and psychological distress⁵¹ have also been implicated, again with inconclusive or even contradictory results.⁵² Probably, the rise in incidence of IBD after the Second World War in Western and industrializing countries might be attributed to the introduction of better sanitary conditions, thus improving survival of susceptible individuals and/or shifting the development of the immune system towards hypersensitivity.⁵³⁻⁵⁵ It should be noted that incidence and prevalence of asthma and diabetes type I have also increased, pointing to a more general promoting effect of western lifestyle on auto-immune disease.⁵⁶

Several studies indicate genetic susceptibility in the etiology of IBD as well. For instance, IBD concordance rates are higher in monozygotic versus dizygotic twins,³¹ IBD affecting multiple family members is frequently seen,⁵⁷ and incidence rate of IBD appears to be associated with ethnicity and religion group, as demonstrated by the increased prevalence in white Caucasian subjects and Ashkenazi Jews compared to blacks, Asians and Hispanics.⁵⁸⁻⁶⁰ Early genome-wide association studies have identified (potential) IBD loci on chromosomes 1, 3, 4, 5, 6, 7, 10, 12, 14, 16, 19, 22 and X.⁶¹⁻⁶⁶ Subsequent fine-mapping has revealed the involvement of the nucleotide-binding oligomerisation protein 2 (NOD2)/caspase activation and recruitment domain 15 (CARD15) gene on chromosome 16q12 in CD susceptibility,^{67,68} which was confirmed in other studies.^{69,70} Interestingly, single nucleotide polymorphisms (SNPs) within this gene were also found to be associated with asthma,⁷¹ Blau syndrome,⁷² increased mortality following sepsis⁷³ and allogeneic stem cell transplantation.^{74,75} This landmark success and the increasing availability of high density SNP arrays led to a surge in genome wide research and to the identification of other loci strongly implicated in the pathogenesis of CD and/or UC, including the gene encoding a subunit for the IL-23 receptor (IL-23R) on chromosome 1p31,^{76,77} genes involved in autophagy/breakdown of intracellular pathogens (autophagy related 16-like 1 gene (ATG16L1) on 2q37 and immunity related p47 guanosine triphosphatase murine

orholog (IRGM) on 5q33,⁷⁸⁻⁸² a gene desert on 5p13 regulating expression of the prostaglandin receptor EP4 (PTGER4) (83), the nel-like 1 precursor (NELL1) gene on 11p15⁸⁴ and just recently 3p21.31, NKX2-3, CCNY (CD and UC) and PTPN2, HERC2 and STAT3 (UC).⁸⁵ Importantly, these studies were conducted in Caucasian populations and not replicated in Asian IBD patients, reinforcing the pivotal role of ethnicity in this matter.^{86,87} Other genes potentially involved in the susceptibility and/or phenotype of CD and/or UC are derived from candidate gene approach and include those encoding interleukin-1 receptor antagonist (IL-1RA), IL-2, IL-4, IL-10, IL-11, tumor necrosis factor- α (TNF- α), nuclear factor kappa B (NF κ B), Toll like receptors (TLR), discs large homolog 5 (DLG-5), mucins, organic cation transporter-1 and -2 (OCTN-1, -2), mannan binding lectin (MBL), multidrug resistance 1 protein (MDR) and pregnane X receptor (PXR).⁸⁸⁻¹⁰² Occasionally, conflicting data were generated, probably due to the relatively small sample size often used, especially in the early studies.¹⁰³⁻¹⁰⁶ Also, because of the nature of this approach, significant results might indicate the association of an adjacent predisposing gene in linkage disequilibrium with the examined gene, complicating this matter. However, it appears that, in cooperation with selected environmental stimuli, different sets of predisposing genes might give rise to essentially the same clinical disease manifestation, collectively called IBD. This is corroborated by the large number of mouse models, where targeting (i.e., IL-10) or overexpressing (i.e., TNF- α) of different genes leads to similar IBD like disease.¹⁰⁷⁻¹¹²

Inflammatory Bowel Disease - pathogenic mechanisms

A widely accepted hypothesis states that IBD is an exaggerated immune response towards commensal bacteria in genetically susceptible individuals.¹¹³ The recent rise in incidence of both forms of IBD might be attributed to increased exposure to so-called psychrotrophic bacteria by introduction of refrigerated food (cold chain hypothesis).¹¹⁴ The concept of commensal bacteria playing a pathogenic role is corroborated by the increased frequency of anti-*Escherichia coli* outer membrane porin C, anti-flagellin and autoreactive mycobacterial HSP65 antibodies in CD and/or UC.¹¹⁵⁻¹¹⁷ Flagellin specific T cells were able to cause fulminant colitis in an adoptive mouse transfer model.¹¹⁸ Moreover, murine models for IBD do not

develop enteritis given a pathogen-free environment, although this was just recently challenged by the development of chronic ileitis in germ-free SAMP1/YitFc mice, suggesting that bacteria exacerbate disease but are not required for induction.¹¹⁹ In CD patients, expression of anti-bacterial α -defensins HD5 and HD6 by ileal Paneth cells is reduced, especially in NOD-2 mutation carriers.¹²⁰ The levels of mucus forming proteins mucin3, 4, 5B and of sealing tight junction proteins claudin 5 and -8 are downregulated, whereas expression of pore-forming claudin 2 and rate of epithelial cell apoptosis are increased, resulting in impaired mucosal barrier function.^{121,122} After epithelial injury, in CD patients an abnormally low neutrophil accumulation was observed compared to healthy controls, suggesting an impaired innate immune response.¹²³ The impaired mucosal barrier and/or the impaired innate immunity might result in overexposure to commensal bacteria, initiating and/or propagating the uncontrolled adaptive immune response seen in IBD. Importantly, IBD T-lymphocytes and neutrophils demonstrate increased resistance to apoptosis, thus sustaining the immune response.¹²⁴⁻¹²⁷ Also, T cell regulatory (Treg) function might be insufficient to dampen the inflammatory reaction.¹²⁸ Both diseases are characterized by upregulation of pro-inflammatory cytokines (i.e., IL-1 α , IL-1 β , IL-2, IL-6, IL-8, IL-15, -16, -17, -32, TNF- α , IFN- γ), chemokines (MIP1 α , MIP1 β , MIP3 α , MCP1, MCP2, RANTES), neuropeptide substance P, growth factors (bFGF, VEGF, KGF), eicosanoid PGE2, corresponding receptors (TNF-R2, neurokinin-1/substance P receptor) and endothelial/leucocyte adhesion molecules (ICAM-1, selectins, LFA-1, α 4 β 7 integrin/MAdCAM) in the (inflamed) intestinal mucosa, not compensated for by anti-inflammatory cytokines, soluble receptors and/or receptor antagonists (i.e., IL-10, TGF- β 1, sTNFR, sIL1-RII, sgp130, IL-1RA).¹²⁹⁻¹⁵⁶ However, some important differences are observed in the cytokine profile between CD and UC (upregulation of IL-12, IL-23 versus IL-13, respectively), reflecting the Th1 versus Th2 nature of the corresponding disease.¹⁵⁷⁻¹⁶⁰ The cytokine expression in IBD is different in chronic versus early lesions, thus complicating this issue.^{161,162} The upregulation of cytokines, chemokines, neuropeptides, growth factors, receptors and adhesion molecules chronically activates resident mesenchymal, epithelial and immune cells and continuously attracts new leucocytes from the peripheral circulation. In the

battle against their unknown targets, these cells may damage the intestinal tissue in various ways. T cell activated neutrophils and macrophages release massive amounts of harmful reactive oxygen metabolites by NADPH-dependent oxidative burst, targeting membrane lipids, protein and DNA, thus disrupting cellular structure at the molecular level and promoting malignancy.¹⁶³⁻¹⁶⁶ An imbalanced anti-oxidant response in IBD patients may exacerbate disease.¹⁶⁷⁻¹⁶⁹ Cytotoxic CD8+ T cells release pore forming perforin, proteolytic granzymes and/or express Fas ligand, triggering apoptosis in epithelial cells and disrupting mucosal barrier function.¹⁷⁰⁻¹⁷² Cytotoxic perforin releasing CD4+ T cells were demonstrated in CD^{173,174} and activated complement in conjunction with IgG1 auto antibodies against tropomyosin isoform 5 may target epithelial cells in UC.^{175,176} Increased expression of tissue remodeling neutrophil elastase by neutrophils and of chymase and tryptase by mast cells is associated with IBD.¹⁷⁷⁻¹⁷⁹ Synthetic elastase and tryptase inhibitors were found beneficial in experimental colitis.^{180,181} Concurrent attenuated induction of serine anti-proteases might exacerbate disease.¹⁸² All activated cells also release specific members of the tissue remodeling Matrix Metalloproteinases (MMP), which are described below.

Matrix metalloproteinases - classification

Based on the catalytic group at the active center, five classes of proteases are recognized, i.e., serine-, threonine-, cysteine-, aspartic- and metallo-proteases, divided into clans and families based on protein folding and sequence similarity, see also <http://merops.sanger.ac.uk/>.¹⁸³ Clan MA of the metalloproteases is divided into subclans MA (M) and MA(E). Proteases designated to subclan M all contain a conserved methionine residue to the carboxy side of the active center, thus forming a characteristic loop or “Met turn” in the protein secondary structure, providing the base of the active cleft and are therefore called metzincins.¹⁸⁴ The metzincins are currently categorized into 12 families, each split into a variable number of subfamilies. Subfamily M12B contains the ADAMs (A Disintegrin And Metalloproteinase) and ADAMTSs (A Disintegrin-like And Metalloproteinase with Thrombospondin type 1 motifS).^{185,186} It includes TACE (TNF- α Converting Enzyme, ADAM-17), which is important in releasing membrane-bound TNF- α and

TNF-R from the cell membrane¹⁸⁷ and ADAMTS-4 and -5, which cleave aggrecan in cartilage and might contribute to the structural damage seen in human arthritis.¹⁸⁸⁻¹⁹⁰ Subfamily M10A, also called the matrixins, contains the matrix metalloproteinases (MMP). The human genome currently comprises 23 different MMPs, according to substrate specificity and protein structure subdivided into the collagenases, gelatinases, stromelysins, matrilysins, membrane-type (MT) MMPs, and a rest group (Table 1, page 24-26).

Matrix metalloproteinases - structure

All MMPs consist of a pre-, pro- and catalytic domain and, apart from MMP-7, -23 and -26, also contain a hinge region of varying length connecting a hemopexin domain (Table 1).^{191,192} All MMPs except MMP-23 have a conserved sequence around cysteine in the propeptide (PRCGXPD), which is also found in the ADAMs and ADAMTSs. The cysteine in this motif maintains the latency of the MMP. Limited proteolysis of the pro-peptide, treatment with chaotropic agents or organomercurials (APMA: *p*-amino-phenyl-mercuric acetate is widely used in *in vitro* experiments) disrupts the cysteine-zinc bond and activates the enzyme.¹⁹³ A furin recognition motif (RX(R/K)R) is present on the carboxy terminus of the cysteine switch motif in several MMPs, allowing intracellular activation by furin-like pro-protein convertases in the Golgi apparatus. Recently, furin mediated inactivation of MMP-2 was observed and it appears other MMPs previously not recognized as furin substrates may also be targets.¹⁹⁴ The catalytic domain contains a conserved active site sequence: **HEXXHXXGXXH** with three histidine residues depicted in bold binding the zinc atom. The conserved sequence is shared with all other members of the metzincin group. MMP molecules contain additional non-catalytic zinc and calcium ions, which are involved in stabilizing the tertiary structure of the enzyme.^{195,196} In the catalytic domain of MMP-2 and -9 three fibronectin type II repeats are inserted which bind gelatin and collagen thus facilitating the breakdown of these substrates. The flexible O-glycosylated proline-rich linker of MMP-9 is exceptionally large and facilitates binding of the enzyme to TIMP and cargo transporters.¹⁹⁷ The hemopexin domain co-determines substrate specificity and affinity. It binds Tissue Inhibitor of Metalloproteinases (TIMP, natural inhibitors of

MMPs) and docking molecules on cell surface membranes, for instance integrins and CD44. The hemopexin domain is also involved in MMP di-/oligomerization and CD97 mediated MMP uptake and internal degradation.¹⁹⁸ The MT-MMPs are connected to the cell by a short hydrophobic transmembrane segment or alternatively are glycosylphosphatidylinositol (GPI) anchored, focusing the cell's proteolytic capacity. However, cleavage at the stem region by other MMPs or ADAMs may release an active ectodomain.^{199,200} The cysteine array and immunoglobulin-like domain in MMP-23 are of unknown function. The structural organization of the MMPs is also observed in the ADAMs and ADAMTSs, but these enzymes lack the hemopexin domain and possess other C-terminal extensions instead.¹⁸⁶

Matrix metalloproteinases - expression

The expression of MMPs is tightly controlled at the transcriptional, translational and/or secretory level. Pro-inflammatory cytokines, chemokines, growth factors and oxidants including IL-1 α , IL-1 β , IL-6, IL-12, TNF- α , bFGF, MCP-1 and manganese superoxide dismutase (Mn-SOD) generated H₂O₂ upregulate the expression of several members of the MMP family including but not limited to MMP-1, -2, -3, -9, -12 and/or -13 in a variety of cells encompassing fibroblasts, enterocytes, T cells, chondrocytes, osteoblasts, endothelium and/or macrophages, sometimes in a synergistic manner.²⁰¹⁻²¹¹ Anti-inflammatory (TGF- β), pleiotropic (IFN- γ) cytokines and steroid sex hormones (estradiol) may downregulate MMP, whereas other members of the MMP family (i.e., MMP-2) display a rather constitutive expression rate in distinct cell types.²¹²⁻²¹⁵ Importantly, results for a given combination of cytokine and MMP are not only dependent on cell type but also at what time point in their development and under what (experimental) conditions these cells are studied. Expression is also regulated by cell contact with the surrounding matrix, neighboring cells and pathogens. For instance: collagen I is able to induce MMP-1 in migrating keratinocytes thus promoting its own degradation and ligation of the fibronectin receptor α 4/5 β 1, CD40 and TLR-2 may induce MMP-9.²¹⁶⁻²²⁰ The extrinsic signals are relayed to the MMP promoter by one or more intracellular signaling pathways, including NF κ B, SMAD, STAT, MAPK kinase pathways and

are integrated at cis-acting elements in the promoter, resulting in altered mRNA transcription rate.²²¹ Promoter activity is also dependent on DNA and histone methylation, acetylation and/or phosphorylation status.^{222,223} Also, single nucleotide polymorphisms may result in the loss or gain of suppressor/enhancer DNA elements, affecting mRNA transcription. For instance, the replacement of cytosine with thymidine at -1306 in the MMP-2 promoter disrupts an Sp1 binding site, resulting in significantly decreased promoter activity.²²⁴ A single guanine insertion at -1607 of the MMP-1 promoter creates an Ets binding site, elevating the transcriptional level of MMP-1.²²⁵ The 3'UTR may contain ARE elements binding Hu and KH type splicing regulatory protein (KSFP) proteins increasing or decreasing mRNA stability, respectively, a mechanism shared with TNF- α and other pro-inflammatory cytokines.²²⁶ The 3'UTR of MMP-9 mRNA is involved in binding cytoplasmic nucleolin, promoting transport to polyribosomes and enhancing protein translation efficiency.²²⁷ Neutrophils and eosinophils store MMP-8, -9 and/or MT6-MMP in secretory granules which are released upon stimulation with pro-inflammatory cytokines such as IL-8 and TNF- α .²²⁸⁻²³⁰ MT-1 MMP is stored in trans-golgi network/endosomes and may be expressed on the cell surface within minutes following Concanavalin A treatment of HT1080 cells.²³¹ Vesicular trafficking from the Golgi apparatus to the plasma membrane is dependent on actin and tubulin polymerization and can be suppressed by exposure to hypoxia, with concomitant drastic reduction of MMP secretion in monocytes.²³²

Matrix metalloproteinases - activation, inhibition and degradation

Except for furin-like pro-protein convertase-activated MT-MMPs and MMP-11, all other MMPs are secreted as inactive zymogens. Limited proteolysis by plasmin, thrombin, trypsin and other proteinases removes part of the propeptide region, inducing a conformational change in the MMP molecule disrupting the bond between the protective cysteine and catalytic zinc residue. Autocatalysis subsequently removes the entire propeptide region after which the enzyme becomes fully active.²³³ The cysteine switch dogma is challenged by observations

of mutant MMP-3 with the cysteine replaced by serine or histidine. These molecules retained latency and could be activated with APMA, results not consistent with cysteine as primary regulator of MMP latency.²³⁴ The final MMP activity is dependent on the activating proteinase, i.e., MMP-3-activated MMP-1 displays a higher conversion rate of collagen substrate compared to plasmin-activated MMP-1.²³⁵ Contact with substrate or even non-functional protease may induce conformational change and activation of MMP without loss of the pro-peptide.^{236,237} Oxidative activation and inactivation may play an important role during inflammation.²³⁸ MMP-2 can be activated as described above, but the activation pathway in a complex with MT-MMPs and TIMP-2 is believed to be the most important physiologically.²³⁸ MMP stability can be enhanced by MMP- binding proteins. For instance, neutrophil-derived lipocalin protects MMP-9 and may worsen prognosis in breast and gastric cancer.^{239,240} MMP activity is inhibited by the endogenous inhibitors TIMPs, but glycosylation status co-determines affinity for TIMP and activity of the MMP.²⁴¹ General antiproteinases such as α 2-macroglobulin also inhibit MMPs and the resulting inhibitor-MMP complex is subsequently removed from the circulation by scavenger receptors on macrophages. MMPs undergo further autocatalysis, inactivating themselves. Uptake of soluble MMPs by cells is mediated by the LRP receptor followed by degradation in lysosomal vesicles.¹⁹⁸ MT-MMPs are internalized by dynamin-dependent endocytosis in clathrin-coated pits.^{242,243}

Matrix metalloproteinases - substrate specificity

A whole array of structural matrix proteins including, but not limited to, collagen I-XI, proteoglycans, elastin, laminin, vitronectin, tenascin, entactin and fibronectin can be cleaved by one or more members of the MMP family. In addition, non-structural proteins such as cytokines, growth hormones and binding proteins (for instance: IL-8, TGF- β and IGFBP-3) are cleaved as well (Table 1).²⁴⁴ MMPs may also act intracellularly, targeting myosin light chain and troponin in cardiac myocytes.^{245,246} Although overlapping, every MMP is characterized by its own substrate specificity, determined by the size and shape of the substrate-binding pocket. For instance, the gelatinases preferentially cleave collagen IV and gelatin,

whereas MMP-1 and -8 preferentially convert collagen I and III. Importantly, most substrate specificities have been determined *in vitro* and remain to be confirmed *in vivo*. In addition, MMPs may act using a non-proteolytic mechanism. For example, binding of TIMP-2 to MMP-14 upregulates cell migration and proliferation by activation of ERK1/-2, a process mediated by the cytoplasmic tail of MMP-14 and not dependent on extracellular proteolytic activity.²⁴⁷

Tissue inhibitors of metalloproteinases

The four different TIMPs currently known in humans inhibit activated MMPs by forming non-covalent 1:1 stoichiometric complexes that are resistant to heat-denaturation and proteolytic degradation. TIMP-1 and TIMP-3 also inhibit members of the ADAM and/or ADAMTS family.²⁴⁸ TIMPs also bind to the proform of MMP-2 and MMP-9, thus regulating the activation process of these MMP members. Different TIMPs have different MMP binding specificities, for instance, TIMP-1 binds preferentially (pro-) MMP-9 but not MMP-2 while TIMP-2 binds (pro-) MMP-2 and not MMP-9. TIMPs are expressed by a variety of cell types including fibroblasts, enterocytes and leucocytes. Expression may be regulated by several cytokines, growth factors, hormones, etc., or is constitutive instead, dependent on TIMP and cell type studied, similar to the regulation of MMP expression. Of note, cytokines (i.e., TGF- β) that repress MMP expression, may enhance levels of TIMP and collagen, promoting a fibrotic phenotype.²⁴⁹ Expression is also dependent on DNA-methylation and histone-acetylation status.²⁵⁰ Hypomethylation of the TIMP-1 promoter may result in TIMP-1 expression from the otherwise inactive X chromosome in females, resulting in an overall increase of TIMP-1 levels.^{251,252} Conversely, hypermethylation of a TIMP-2 CpG island upstream of the transcription start site is associated with diminished TIMP-2 expression in cervical carcinoma.²⁵³ TIMP-1 and -2 were originally identified as erythroid potentiating factors and it now appears TIMPs are more generally involved in cell growth and/or apoptosis. The TIMP effect may be anti-apoptotic through ligation of the CD63/integrin- β 1 complex but also pro-apoptotic via inhibition of MMP-mediated degradation of cell death receptor.²⁵⁴ Several mutations in the TIMP-3 gene introducing an extra cysteine-residue and promoting dimerization, are associated with Sorsby's fundus dystrophy

and probably other degenerative retinopathies.²⁵⁵

Matrix metalloproteinases and tissue inhibitors of metalloproteinases - expression in IBD

MMPs are involved in normal physiological processes where matrix turnover is important, such as wound healing, embryogenesis, angiogenesis, etc. They are also implicated in several disease pathologies such as arthritis, dental disease and cancer metastasis. In IBD, high levels of proinflammatory cytokines in inflamed ulcerated tissue are associated with aberrant expression of MMPs and also TIMPs, but the balance between MMPs and TIMPs appears shifted to a more proteolytic phenotype.²⁵⁶⁻²⁶² The increased MMP/TIMP ratio in IBD may result in excessive tissue breakdown and facilitate leucocyte extravasation and migration, although MMP-specific substrate cleavages in IBD mucosa have not been detected so far.²⁶³ Excessive expression of MMPs may also enhance fibroblast trans migration, promoting fibrosis and stenosis, especially in CD.²⁶⁴⁻²⁶⁶ Alternatively, the MMP over TIMP ratio may not be sufficiently enhanced to compensate for the increased collagen production by IBD fibroblasts, again resulting in fibrosis.²⁶⁷ Targeting TIMP-1 with non-functional MMP-9 mutants inhibited liver fibrogenesis, in favor of the second hypothesis.²⁶⁸ MMPs may also generate new epitopes by cleaving substrates, thus perpetuating the immune response.²⁶⁹ In several IBD models, administration of synthetic MMP inhibitors improved disease course and DSS-induced colitis was attenuated significantly in MMP-9 deficient mice.²⁷⁰⁻²⁷² However, ablation of MMP-2 was observed to aggravate experimental colitis, demonstrating protective capacities of MMPs in IBD as well.²⁷³

Outline of the studies described in this thesis

Inflammatory bowel disease is of major concern in industrialized countries. Health and economical costs have prompted the initiation of studies aimed at revealing the epidemiology, etiology and pathogenesis of IBD. Both CD and UC are characterized by excessive tissue breakdown during inflammation. The matrix metalloproteinases are important in normal physiological and pathological tissue remodeling and repair processes, including IBD. A short overview of IBD and MMPs is given in **chapter 1**.

Several studies have documented on the altered expression of MMPs in IBD tissue. **Chapter 2** reports on the expression of MMP-2 and MMP-9 in IBD inflamed versus non-inflamed IBD and control intestinal mucosa. MMP levels were measured by enzyme linked immunosorbent assay (ELISA), zymography, activity assay and reverse transcription polymerase (RT-PCR) assay. The cellular localization of MMP expression was determined by immunohistochemistry.

Infliximab is administered to steroid refractory CD patients and targets TNF- α , disrupting proinflammatory communication and promoting apoptosis in leucocytes via reverse signaling. The effect of infliximab on MMP-1, -2, -3, -9 and TIMP-1, -2 protein expression is described in **chapter 3**. Intestinal explants were cultured *ex vivo* with/without (w/wo) infliximab and relative expression of MMP, TIMP, TNF- α was measured by ELISA, activity assay and/or RT-PCR. In addition, explants were cultured w/wo pokeweed mitogen (PWM), to study the expression profiles under inflammatory conditions.

Chapter 4 describes the MMP-2 and MMP-9 serological and mucosal expression profile after the administration of infliximab to CD patients with fistulizing or active disease. Whole blood cultures w/wo infliximab and/or lipopolysaccharide (LPS) were performed to study *in vitro* the contribution of TNF- α in the regulation of MMP-2 and MMP-9 mRNA and protein expression.

In **chapter 5** expression of MMP-1, -2, -3, -9 and TIMP-1, -2 as measured by ELISA and/or activity assays in a large collection of IBD and control intestinal mucosa and related to CD phenotype is reported. The net MMP activity was compared to MMP over TIMP ratio and correlated with myeloperoxidase (MPO) content.

Single nucleotide polymorphisms (SNP) in genes may affect mRNA transcription, stability and/or protein function, thus enhancing disease susceptibility and/or phenotype. **Chapter 6** documents the distribution of (functional) SNPs in the genes encoding MMP-1, -2, -3 and -9, TIMP-1, -2 and TNF- α in a large cohort of IBD patients versus control subjects. Results were correlated with protein expression and clinical course, i.e., development of fistulae, stenotic complications and organ involvement.

After surgical resection, CD patients often experience recurrence of disease. Numerous studies have attempted to identify causal factors and smoking has been established as a bad prognostic factor. In **chapter 7** the clinical course of a large cohort of fully documented CD patients was related to MMP and TIMP genotypes and protein levels in surgically resected intestinal mucosa. In addition, several clinical and demographic variables such as smoking habits, sex and age at resection were retrospectively collected and related to the clinical outcome as well. The different studies are finally compiled as a summarizing discussion in **chapter 8**.

Table 1 (next pages). MMPs and TIMPs, adapted from references.^{191,192,244,274}

Pre = prepeptide signal sequence, pro = propeptide, catalytic = catalytic domain, Zn = catalytic zinc, F = fibronectin type II repeat, Fu = furin pro-protein convertase cleavage site, TM = transmembrane region, cyt = cytoplasmic tail, GPI= glycosylphosphatidylinositol anchor, CA = cysteine array, Ig = immunoglobulin, V= vitronectin insert. Note: substrate specificities were determined *in vitro* and remain to be confirmed *in vivo*. MMPs may digest other substrates not mentioned in this overview. *Cellular expression is dependent on stimulation by cytokines, extracellular matrix, DNA-methylation and acetylation, oncogenic transformation, etc., and expression should not be viewed as limited to those cells or tissues mentioned. References indicated between brackets.

Table 1.

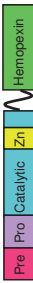
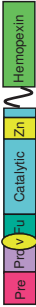
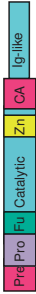
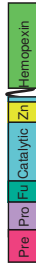
Subgroup	MMP	Trivial name	Chromo- somal location	Domain Organization	Subgroup substrate specificity	Individual MMP substrate specificity	Cellular source*
Collagenases	MMP-1	Interstitial collagenase; collagenase 1	11q22-q23		Collagen I, II, III, VII, X, gelatin, entactin, aggrecan, tenascin, perlecan	IGFBP-2,3 Pro-MMP-1,2; pro-TNF- α , α 1-antichymotrypsin, α 1-proteinase inhibitor	Fibroblasts, keratinocytes, macrophages ^{273,277}
	MMP-8	Neutrophil collagenase; collagenase 2	11q21-q22			Pro-MMP-8, α 1-proteinase inhibitor	Neutrophils, fibroblasts ^{278,279}
	MMP-13	Collagenase 3	11q22.3			Pro-MMP-9, 13; α 1-antichymotrypsin	Chondrocytes, osteoblasts ^{280,281}
Gelatinases	MMP-2	Gelatinase A	16q13		Gelatin, elastin, fibronectin, collagen I/IV/V/VII/X/XI, laminin, aggrecan, vitronectin	Decorin, pro-TGF- β 2, pro-IL-1 β , MCP-3, IGFBP-3/5, pro-TNF- α , FGF-R1, pro-MMP-1, 2, 13	Fibroblasts, vascular smooth muscle cells, T lymphocytes ^{282,285}
	MMP-9	Gelatinase B	20q11.2-q13.1			Pro-TGF- β 2, pro-IL-1 β , IL-8, cell surface bound IL-2Ra, plasminogen, α 1-proteinase inhibitor, pro-TNF- α	Neutrophils, monocytes/ macrophages, Fibroblasts ²⁸⁶⁻²⁸⁸
Stromelysins	MMP-3	Stromelysin 1	11q23		Proteoglycans, laminin, fibronectin, gelatin, collagen III/IV/V/IX/XI, fibrin/fibrinogen, entactin, tenascin, vitronectin	Perlecan, decorin, pro-HB-EGF, pro-IL-1 β , plasminogen, E-cadherin, IGFBP-3, α 1-antichymotrypsin, α 1-proteinase inhibitor, pro-MMP-1, 3, 7, 8, 9, 13, pro-TNF- α	Fibroblasts, chondrocytes, vascular smooth muscle cells, keratinocytes, macrophages and endothelial cells ^{289,294}
	MMP-10	Stromelysin 2	11q22.3-q23			Pro-MMP-1, 8, 10	Keratinocytes, monocytes, fibroblasts ²⁹⁴⁻²⁹⁶

Table 1, continued.

Subgroup	MMP	Trivial name	Chromo- somal location	Domain Organization	Subgroup substrate specificity	Individual MMP substrate specificity	Cellular source *
Matrilysins	MMP-7	Matrilysin 1	11q21- q22		Proteoglycans, laminin, fibronectin, gelatin, collagen III, IV, V, IX, X, XI, fibrin/fibrinogen, entactin, tenascin, vitronectin, pro- α -defensin, decorin, cell surface bound FasL, β 4-integrin, E-cadherin, plasminogen, pro-TNF- α , pro-MMP-2, 7	Epithelial cells ^{237,238}	
	MMP-26	Matrilysin 2	11p15				
	MMP-11	Stromelysin 3	22q11.2				
	MMP-14	MT1-MMP	14q11- q12				
Membrane type MMPs -trans- membrane type	MMP-15	MT2-MMP	15q13- q21		Gelatin, fibronectin, vitronectin, collagen, aggrecan	Endothelial cells, hepatic stellate cells, fibroblasts ³⁰³⁻³⁰⁵	
	MMP-16	MT3-MMP	8q21				Central Nervous system microglia ³⁰⁶
	MMP-24	MT5-MMP	20q11.2				

Table 1, continued.

Subgroup	MMP	Trivial name	Chromo- somal location	Domain Organization	Subgroup substrate specificity	Individual MMP substrate specificity	Cellular source*
Membrane type MMPs -GPI anchored	MMP-17	MT4-MMP	12q24.3			Gelatin, pro-MMP-2	Monocytes, B-lymphocytes ²⁹⁵
	MMP-25	MT6-MMP	16p13.3			Gelatin, collagen IV, fibrin, fibronectin, laminin-1, pro-MMP-2	Monocytes, neutrophils ^{228,295}
	MMP-12	Macrophage elastase	11q22.2- q22.3			Elastin, fibronectin, fibrin, fibrinogen, laminin, proteoglycan, plasminogen	Macrophages ³⁰⁸
	MMP-19	-	12q14			Gelatin, tenascin, fibronectin, collagen IV, laminin, entactin, fibrin/fibrinogen, aggrecan, COMP	Keratinocytes, brain microglia ^{308,310}
Others	MMP-20	Enamelysin	11q22.3			Amelogenin, aggrecan, COMP	Tooth enamel forming ameloblasts (epithelial origin) ³¹¹
	MMP-21	-	?			Gelatin	Keratinocytes ³¹²
	MMP-23	CA-MMP	1p36.3			Gelatin	Reproductive organs ³¹³
	MMP-27	-	11q24				B-lymphocytes ²⁹⁵
	MMP-28	Epilysin	17q21.1				Keratinocytes, intestine, T-lymphocytes ^{257,285,314}

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