Matrix metalloproteinase inhibitors as therapy for inflammatory and vascular diseases

Jialiang Hu[*§], *Philippe E. Van den Steen*[*§], *Qing-Xiang A. Sang*[‡] *and Ghislain Opdenakker*[*]

Abstract | Matrix metalloproteinases (MMPs) have outgrown the field of extracellular-matrix biology and have progressed towards being important regulatory molecules in cancer and inflammation. This rise in status was accompanied by the development of various classes of inhibitors. Although clinical trials with synthetic inhibitors for the treatment of cancer were disappointing, recent data indicate that the use of selective inhibitors might lead to new therapies for acute and chronic inflammatory and vascular diseases. In this Review, we compare the major classes of MMP inhibitors and advocate that future drug discovery should be based on crucial insights into the differential roles of specific MMPs in pathophysiology obtained with animal models, including knockout studies.

Matrix metalloproteinases (MMPs) form a group of more than 20 zinc-dependent enzymes that are involved in the remodelling of several components of the extracellular matrix (ECM). They play a role in many physiological processes, such as embryo implantation, bone remodelling and organogenesis, and have additional roles in the reorganization of tissues during pathological conditions such as inflammation, wound healing and invasion of cancer cells[1,2].

MMPs enable normal cells to remodel the ECM, including basement membranes, for example, in bone remodelling during development or in immunosurveillance by leukocytes against infections[3]. This classical paradigm was further developed by tumour biologists who discovered that an increased expression of proteinases, including MMPs, is a marker of invasion and metastasis of cancer cells. At the time, the hope was to use MMP inhibitors (MMPIs) to halt the spreading of cancer cells. However, during clinical trials of metastatic cancer, severe side effects were observed, thus leading researchers to reappraise the use of MMPIs for the therapy of invasive cancer[4,5]. The positive effect of these cancer research and clinical studies is that the marker functions of MMPs have been refined in several ways. In this Review, we discuss important biological aspects of MMPs in view of the uses of MMPIs in inflammatory and vascular diseases. Important aspects that we discuss include the redundancy of MMPs, similarities and differences between cancer and inflammation, the functions of enzyme cascades in balance with natural inhibitors, and the distinction between acute and chronic inflammatory processes.

Redundancy, expression patterns and balances

MMPs are multidomain enzymes containing a zinc ion, which is coordinated by three histidine residues in their active site. Although all MMPs possess different primary structures, they are composed of shared modules, known as protein domains. This modular structure, illustrated in FIG. 1, already gives an insight into the functional redundancy of MMP subclasses. In particular, the hallmark of the MMP family is a catalytic domain that possesses a zinc-binding consensus sequence, a characteristic shared with other metalloproteinase families such as the ADAMs (a disintegrin and metalloproteinases) and the ADAMTSs (ADAMs with a thrombospondin motif) (BOX 1). Another signature of the MMP is its activation by the so-called cysteine switch[6,7]. When cells produce MMPs, most of the enzymes are secreted in a latent pro-form in which a cysteine sulphydryl group in the amino-terminal pro-domain interacts with the zinc ion and blocks the active site. Removal of the pro-peptide (about 10 kDa) from the active site, for example, by proteolysis, leads to activation of the enzymes.

Expression levels of MMPs depend on the biological context, for example, some constitutive or homeostatic *MMP* genes possess simple promoter enhancer regions with *cis*-acting elements for basal transcription and are

[*]*Rega Institute for Medical Research, Catholic University of Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium.*
[‡]*Department of Chemistry and Biochemistry, 262 DLC, Chemistry Research Building, Florida State University, Tallahassee, Florida 32306-4390, USA.*
[§]*These authors contributed equally to this work.*
Correspondence to G.O.
email: ghislain.opdenakker@rega.kuleuven.be
doi:10.1038/nrd2308

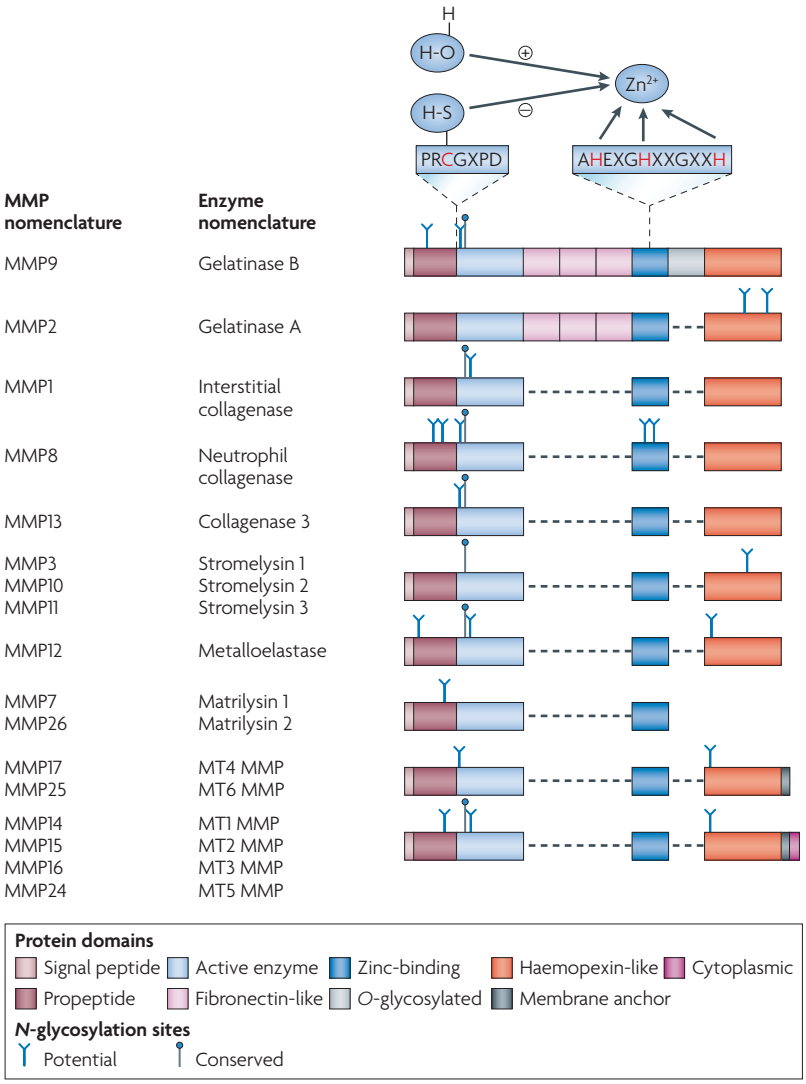


Figure 1 | **Domain structures of MMPs.** Matrix metalloproteinases (MMPs) are multidomain enzymes that have a pro-domain, an active domain, a zinc-binding domain and a haemopexin domain (except MMP7 and MMP26). Additionally, membrane-type MMPs (MT-MMPs) contain a membrane anchor with certain MT-MMPs also possessing a cytoplasmic domain at the carboxyl terminus. Gelatinases contain a gelatin-binding domain with three fibronectin-like repeats. In particular, MMP9 also contains a serine-, threonine- and proline-rich *O*-glycosylated domain. *N*-glycosylation sites, one of which is conserved among most MMPs, are indicated with a Y symbol. Part of the pro-peptide, which contains the chelating cysteine, and part of the zinc-binding domain with three histidines are indicated in one-letter code for the amino acids at the top of the figure.

switched on in most cells under steady-state conditions. Other MMPs have complex promoter regions. The expression of these MMPs is regulated by various agonists. The biological milieu will determine the levels of expression of these inducible or inflammatory MMPs. Interesting examples of this dichotomy are the constitutive MMP2 and the inducible MMP9. Several other redundant enzymes exist with similar catalytic functions in ECM biology. The three classical collagenases, interstitial collagenase (MMP1), neutrophil collagenase (MMP8) and collagenase 3 (MMP13), all cleave a specific scissile bond in the triple-helical collagens at one specific site.

Tissue inhibitors of metalloproteinases
Tissue inhibitors of metalloproteinases (TIMPs) are natural inhibitors of matrix metalloproteinases (MMPs). Four are present in humans. This redundancy illustrates the importance of the fine-tuning needed for MMP inhibitors. Some TIMPs possess other functions than regulation of extracellular matrix turnover.

This redundancy, also observed for the stromelysins, ensures that the biological processes of ECM remodelling can take place under various conditions by different cell types, and so if one enzyme is inactivated then the host can still survive.

In addition to regulation by activation processes and gene expression, the activities of MMPs are also controlled by the four natural tissue inhibitors of metalloproteinases (TIMPs). This implies that the biological processes involving MMPs are always dependent on balances between proteinases and natural inhibitors. In conclusion, treatment with MMPIs will cause a distortion of these natural balances and so the targeting of MMPs is a challenging exercise in selectivity.

Comparison of inflammation with cancer

In terms of molecular events and cell behaviour, many similarities exist between cancer and inflammation. For instance, leukocytes on their way to a site of inflammation and metastasizing cancer cells use similar enzymes to cross endothelial basement membranes from or into the blood or lymph circulation. At another level, signalling molecules, such as cytokines and chemokines, regulate not only cell proliferation but also the local protease load — the balance between proteinases and natural inhibitors — in both inflammation and cancer. Such examples of cellular and molecular similarities form the subject of specific in-depth reviews[2,4,5,8,9].

However, one essential difference between cancer and inflammation is genetics. Whereas we assume that the genetic constitution of host cells during inflammation is normal and the biological processes follow the expected directions of cell regulation, in cancer this is completely different, or even unpredictable. Environmental factors might have a role in cancer by inducing genetic alterations and so cancer cells are genetically unstable. At the level of gene expression, recent transcriptome analyses using microarrays have demonstrated that MMP9 expression is better associated with inflammatory diseases than with neoplastic diseases[10]. In retrospect, this knowledge partially explains the failure of MMPIs in cancer therapy. It would have been simpler if one had started to use MMPIs in acute inflammatory diseases that are not confounded by genetic heterogeneities and instabilities. Nevertheless, tumour biologists have gathered considerable knowledge about the roles and mechanisms of action of MMPs in cancer.

A second difference between cancer and inflammation relates to chemokine biology. The idea that cancer cells produce chemotactic factors for host leukocytes and therefore activate the white blood cells for release of MMPs, which in turn assist in the invasive phenotype, has been coined the countercurrent principle of invasion[11,12]. Recent experimental studies have shown several aspects of how this system might operate[13,14]. These data are not only biologically relevant but also point to the fact that the use of MMPIs in cancer therapy must be seen as interference not only with the tumour but also with normal inflammatory cell functions, which possibly results in adverse effects. Indeed, because of the genetic instability of tumour cells, agonists that influence

Box 1 | **From MMPs to ADAMs/ADAMTSs and back**

- ADAMs (a disintegrin and metalloproteinases) are multidomain metalloproteinases with additional disintegrin domains that are anchored in cell membranes. Tumour-necrosis factor (TNF)-converting enzyme (TACE; also known as ADAM17) is an example of an ADAM[137].
- ADAMTSs (ADAMs with a thrombospondin motif) additionally possess one or several thrombospondin motifs and other protein domains. They are secreted as they lack a membrane anchor. Typical examples are the aggrecanases 1, 2, 3 (ADAMTS4, ADAMTS5 and ADAMTS1)[138].
- ADAMs and ADAMTSs have similar active sites as matrix metalloproteinases (MMPs) and, hence, are also inhibited by broad-spectrum MMP inhibitors.

the balance between proteinases and inhibitors, or the enzymes and inhibitors themselves, might become out of control. This will complicate normal host cell functions and also therapeutic intervention. We therefore advocate that research on MMPIs in inflammatory diseases be conducted first, and to gain better insights in the already complex immunobiology of inflammation before using this information in cancer treatment.

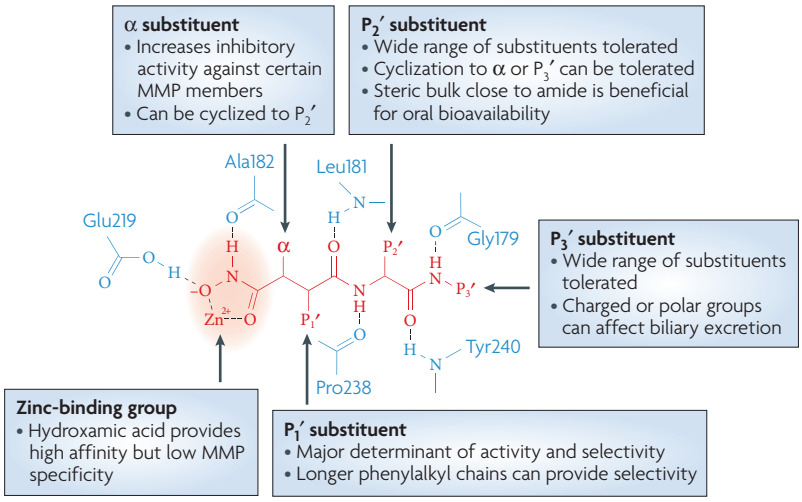


Figure 2 | **Schematic representation of the interaction of a peptide inhibitor with an MMP active site.** The numbering of amino acids follows that for matrix metalloproteinase 1 (MMP1). The P_1', P_2' and P_3' amino acids (red) located on the right-hand side of the cleavage site are also referred to as primed residues and mimic parts of the peptide substrate. The blue residues and the zinc are from the enzyme and form the subsites for the recognition of natural substrate cleavage sites[32,140]. The requirements for a small molecule to be an effective MMP inhibitor (MMPI) are summarized as zinc-binding group (ZBG) and one or more non-covalent interactions between the inhibitor and the enzyme backbone, such as hydrogen bonds or van der Waals interactions. Typical ZBGs include carboxylic acid, hydroxamic acid and suphydryl groups. By comparing different ZBGs without changes to the rest of the inhibitor structure, the question is: which ZBG is optimal? In terms of MMP inhibition, the order of affinity for zinc is as follows: hydroxamate > sulphydryl > phosphinate > carboxylate. Based on different ZBGs and the backbone structures, MMPIs can be classified into a few broad classes (TABLE 1). For the different ZBGs that have been explored, the hydroxamate function provides the best potency, followed by reverse hydroxamates. Thiols are typically 20-fold to 50-fold less potent and carboxylates are 100-fold to 2000-fold less potent[141]. Certain large substituents at P_1' lead to more potent inhibitors for several MMPs, including MMP9. In general, small effects on potency are observed by substitution at P_2' and P_3', although particular substituents have been found to provide useful levels of selectivity for specific MMPs.

Enzyme networks and balances with inhibitors

It is clear that the biological context of inflammation or vascular injury is complex in its topology and regulation. This is true for secreted MMPs and also for the membrane-type MMPs (MT-MMPs), which are directly anchored to cell membranes, or for MMP receptors that might provide ways for pericellular proteolysis or negative feedback by MMP internalization and degradation. Furthermore, MMPs interplay with the fibrinolytic cascade in that fibrinolysin or activated plasminogen (plasmin) can activate pro-MMPs through many circuitries[8,15]. This implies that MMPIs might block specific arms of this network of interactions. In addition, the effects will be dependent on the biological context of inflammation, vascular injury or tumour dissemination.

Inflammation and MMPs

During an inflammatory response, leukocyte traffic through tissue barriers, including basement membranes, is only possible if these cells are equipped with enzymes that can remodel the ECM[16–18]. MMPs are therefore crucial effector molecules of inflammatory cells[19]. However, MMPs can also modify cytokines and chemokines[20–23]. MMPs can act as switches or as delicate tuners in acute and chronic inflammation, during autoimmune diseases, when triggered in vascular diseases and in the regenerative phase after inflammation. Thus, MMP biology is important in the initiation, execution and resolution phases of acute and chronic inflammatory and ischaemic processes and consequently, MMPIs might interfere with these. These functions of MMPs will be addressed later in the section on various animal models of human diseases, in which one or more of these phases predominate and in which MMPs contribute to the pathology. Endotoxin shock, myocardial infarction, stroke and acute flares of multiple sclerosis or rheumatoid arthritis are examples of acute inflammatory reactions, whereas atherosclerosis and the slowly progressive events in autoimmune diseases carry all the characteristics of chronic inflammation. It will be evident that specific MMPs are involved in different kinds and phases of these diseases. Whether highly specific or broad-spectrum MMPIs are useful will depend on the clinical situation.

Inhibition of MMPs with small molecules

Both macromolecular inhibitors (natural TIMPs and monoclonal antibodies) and small molecules (synthetic and natural products) have been considered as potential therapies for diseases in which excess MMP activity has been implicated[24]. However, technical difficulties with the biotechnological production of macromolecular proteins and limited patient compliance because of parenteral administration have often been cited as limitations for their development. Nevertheless, monoclonal-antibody derivatives[25] are promising drugs to be used as therapeutics, especially if a high MMP specificity is required[26]. TIMPs that have affinities for MMPs in the picomolar range seem ideal inhibitors but they lack good selectivity and possess other biological functions, which could lead to side effects[27].

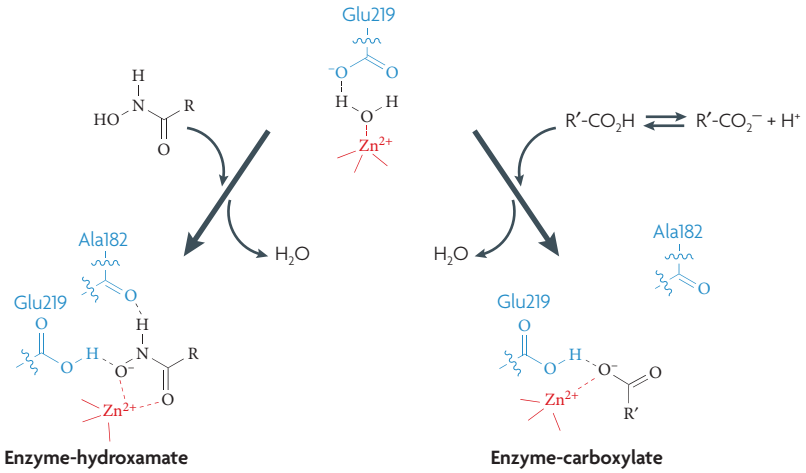


Figure 3 | **A proposed mechanistic model of the molecular interaction between a hydroxamate or carboxylate inhibitor and the active-site zinc ion of a metalloproteinase.** The numbering of enzyme residues (in blue) follows that of human matrix metalloproteinase 1 (MMP1). This model is based on the data and discussions presented in REFS 24,32,142 with modifications.

Many small-molecular-mass inhibitors have been discovered and some of these have been evaluated for clinical use, but in general these seem to be unselective and to cause side effects[4,28]. Detailed reviews on the medicinal chemistry of MMP inhibitors, including natural medicinal products, have been published recently[29–31]. Here, we summarize the characteristics of the major chemical classes of MMPIs. For details on specific molecules, the reader is referred to Supplementary information S1 (box).

The structures of MMPs, in particular the catalytic sites, have been extensively studied with high-resolution X-ray crystallography and nuclear magnetic resonance analyses[32,33]. As demonstrated in FIG. 1, the members of the family possess similar domain arrangements and all MMPs, the structures of which (catalytic domains) have been solved, possess similar core elements. A schematic representation of a substrate-based inhibitor bound to an MMP active site is given in FIG. 2. As different MMPs have similar core structures in the active sites, and as the insertion of the gelatin-binding fibronectin domain between the catalytic and zinc-binding parts in the gelatinases does not change the three-dimensional structure of the active site[34], it is reasonable to apply this information to the design of inhibitors against several MMPs.

Substrate versus structure-based drug design. The principal approach taken for developing synthetic MMPIs was the substrate-based design of peptides and analogues derived from the information of the sequence around the substrate-cleavage site. Later on, non-peptide-based inhibitors were identified by gradually increasing the affinity of the inhibitor. However, these days, most MMPIs that are discovered by the process of structure-based design are analogues of compounds obtained by the substrate-based approach. The requirements for a small molecule to be an effective MMPI are summarized in FIG. 2. If one compares the literature on inflammation and vascular disorders for each individual MMP, the number of publications on MMP9 is the largest[8]. In Supplementary information S2 (table), a summary of the biological effects of MMPIs on MMP9 is provided as an example and scaffold for the future development of MMPIs.

Structure–activity relationships of MMPI classes

Hydroxamate-based MMPIs. Most small-molecule MMPIs use hydroxamate as their zinc-binding group. The hydroxamate acts as a bidentate ligand with the active-site zinc ion to form a slightly distorted trigonal-bipyramidal coordination geometry. In MMP1 inhibition, the hydroxamate oxyanion forms a strong, short hydrogen bond to the carboxylate oxygen of the catalytical Glu219 that is orientated towards the unprimed binding regions (FIGS 2,3). Also contributing to the binding is another hydrogen bond between the hydroxamate-NH group and the carbonyl oxygen of Ala182. Thus, a set of interactions is achieved at the site without any significant unfavourable contact[32]. In particular, the hydroxamate MMPIs can be further grouped as substrate-analogue peptides (see below), succinyl, sulphonamide, phosphinamide hydroxamates and derivatives.

Succinyl hydroxamate MMPIs are among the early MMPIs, of which the three best known examples are batimastat, BB-1101 and marimastat (TABLE 1). They are all broad-spectrum inhibitors and display efficacy in animal models of human diseases. Marimastat was found to be orally available, partially owing to the increased aqueous solubility achieved by the introduction of the α-hydroxyl group. The α-substituent such as the allyl (in BB-1101) had a beneficial effect on the inhibition of tumour-necrosis factor (TNF)-converting enzyme (TACE; also known as ADAM17). This results in a benefit in diseases that involve both inflammation and ECM remodelling[35], and hence involve MMPs and TACE. Cyclization of P_1' and P_2', for example, as in SC903, resulted in a substantial increase in aqueous solubility[36] (TABLE 1).

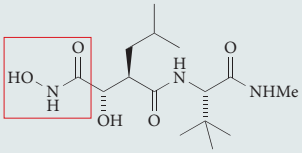
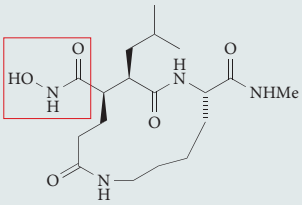
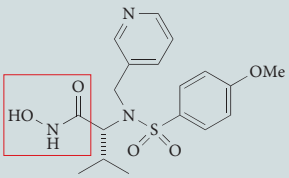
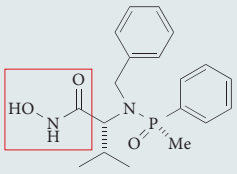
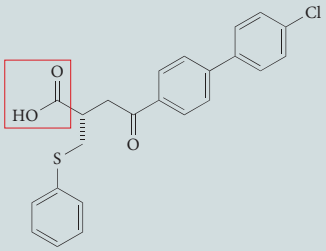
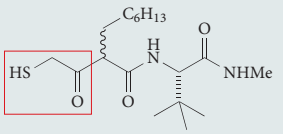
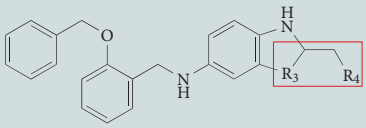
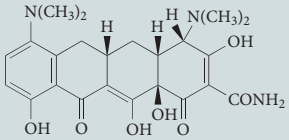
Structural data of MMPs revealed that the S_1' subsite is a deep pocket for several of the enzymes, including MMP2, MMP3, MMP8 and MMP9, but is occluded for MMP1 and MMP7. A bulky P_1' side group may therefore confer selective inhibition for MMP2, MMP8 and MMP9, a notion that has been confirmed in our own library screening work in which peptides with a biphenyl group at P_1' showed, in general, higher inhibitory activity against MMP9 (REF. 37). CGS27023A, an *N*-sulphonyl amino-acid hydroxamate, is an orally available broad-spectrum inhibitor[38] and is an example of several derivatives, which include deep-pocket MMPIs and molecules, without an α-stereocentre.

The sulphonamide moiety of CGS27023A can be replaced by a phosphinamide group[39]. This compound is a potent inhibitor of MMP3, the collagenases (MMP1, MMP8 and MMP13) and the gelatinases (MMP2 and MMP9). However, hydrolysis of the phosphinamide bond, which occurs at low pH, might limit the potential of these compounds to be developed into orally available drugs.

Chemokines
A family of structurally related, small proteins that have potent chemotactic activity and mediate migration of inflammatory cells from blood vessels to the site of inflammation and leukocyte homing.

Atherosclerosis
Atherosclerosis starts by the formation of a fatty streak, an accumulation of lipid-loaded macrophages in the tunica intima. The fatty streak evolves into an atherosclerotic plaque by the migration and proliferation of smooth muscle cells, the formation of a necrotic core and a fibrous cap with extracellular matrix components.

Table 1 | **Structural classes of matrix metalloproteinase inhibitors**

Class	Example	Structure	Benefit	Limitation	Refs
Succinyl hydroxamates	Marimastat		Orally active, nM affinity	Broad spectrum	143,144
	SC903		Aqueous solubility, nM affinity	Broad spectrum	36
Sulphonamide hydroxamates	CGS27023A		Orally active, nM affinity	Broad spectrum	38
Phosphinamide hydroxamates			nM affinity	Hydrolysis at low pH	39
Carboxylate inhibitors	BAY12-9566		Orally active	Broad spectrum, μM affinity	40
Thiol inhibitors	Compound B		Mercapto-ketone	Broad spectrum, μM to high nM affinity	145
Aminomethyl benzimidazole analogue				μM affinity	43
Peptides	Regasepin	PRCBCGE*		μM affinity	37
Tetracyclines	Minocycline		Can cross blood–brain barrier	Induces fibrosis	50,51,72, unpublished observations

*Amino-acid residues are indicated in one-letter code, B represents biphenylalanine.

Substrate-based design
Substrate-based design of hydrolase inhibitors is done on the basis of the definition of characteristics of preferred substrates. Shared properties of such substrates are used as anchor points in the design of inhibitors. Usually the scissile bond between P_1 and P_1' is chemically modified to prevent the hydrolysis of the substrate.

Structure-based design
Structure-based design of enzyme inhibitors uses the three-dimensional structural information of the catalytic site of an enzyme. With the use of molecular-modelling techniques, series of possible small-inhibitor structures are docked into the active site and selected.

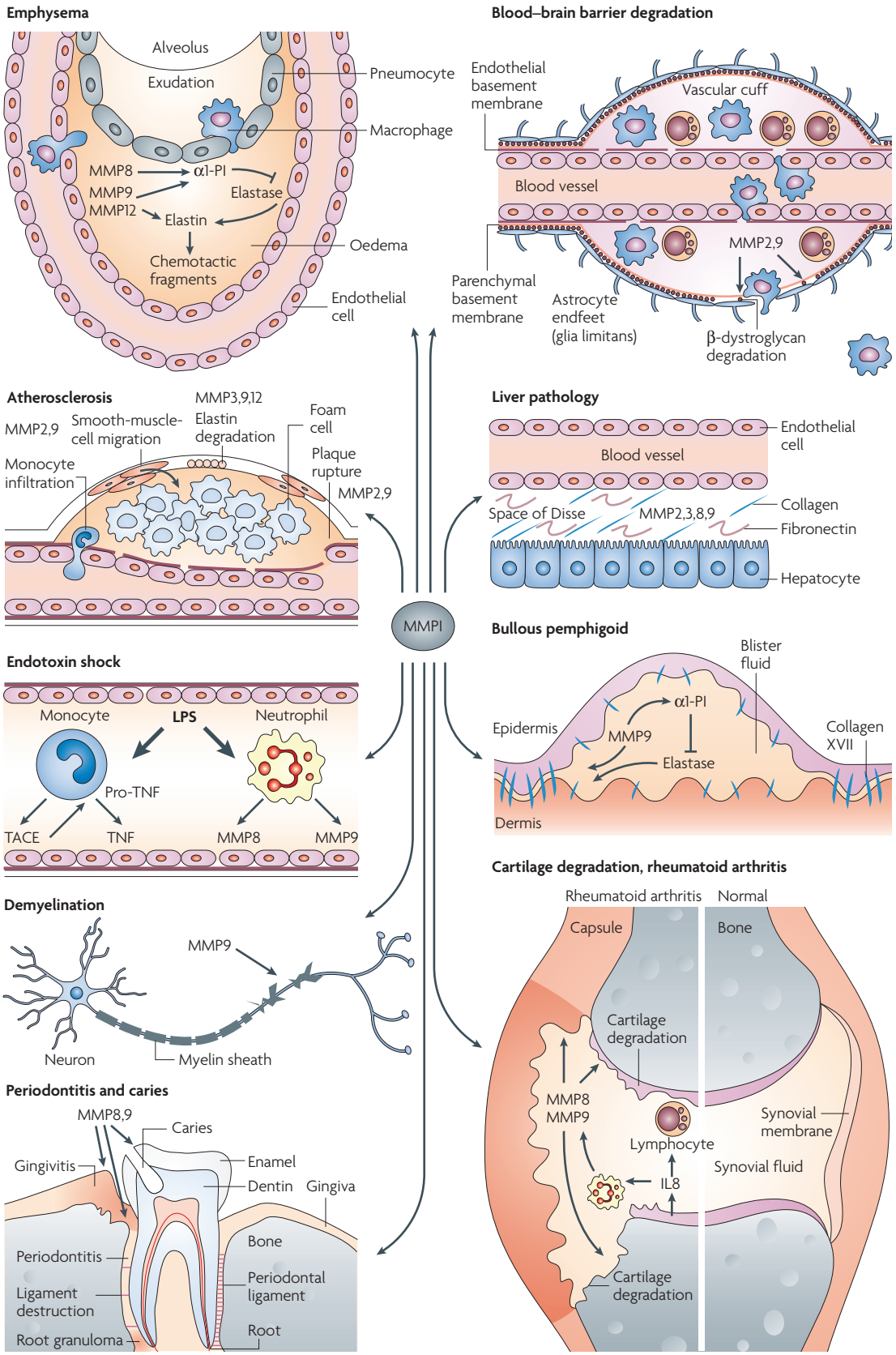


Figure 4 | **MMPs in inflammation and influence by MMPIs.** Both acute inflammation as well as chronic inflammatory processes constitute targets for matrix metalloproteinase inhibitors (MMPIs). A number of disease processes are named and pathogenetic MMPs and key substrates are indicated. α1-PI, α1 proteinase inhibitor; IL8, interleukin 8; LPS, lipopolysaccharide; TACE, TNF-converting enzyme (also known as ADAM17); TNF, tumour-necrosis factor.

Non-hydroxamate-based MMPIs. Most hydroxamate inhibitors lacked specificity and inhibited non-MMP zinc-based enzymes (BOX 1). Thus, new compounds were discovered that made use of alternative zinc-binding groups, such as carboxylic acids, thiols, phosphorous-based and other novel zinc-binding groups.

Under many physiological conditions, hydroxamate inhibitors are more potent than carboxylate inhibitors. One of the most important factors for this might be the difference in acidity. Carboxylate inhibitors bind more tightly to MMPs when the pH is in the acidic range, whereas hydroxamate binding is essentially pH independent over a range of pH 5–8. A proposed model of binding of MMP1 by a hydroxamate or a carboxylate inhibitor is shown in FIG. 3. At neutral pH, the carboxylate inhibitors are much weaker than hydroxamic acid inhibitors. During inflammatory and ischaemic vascular conditions when the pH is lowered to 5 the carboxylates might become more potent MMP inhibitors than when they are at pH 7.4.

Many hydroxamic acid MMPIs were prepared by conversion from a carboxylic acid precursor. Although the carboxylate group is a less effective binding group towards zinc, many carboxylates have been shown to be effective MMPIs and some appear to have promise as therapeutics.

The carboxylate BAY12-9566 (TABLE 1) was tested in trials for osteoarthritis and cancer[40,41]. The compound exhibited modest *in vitro* activity against most MMPs. It inhibited the invasion of human HT1080 fibrosarcoma cells *in vitro* and it was not toxic. Oral treatment of mice (50 mg per kg for 7 days) inhibited angiogenesis that was induced by Matrigel (a commercial solubilized basement membrane preparation) and basic fibroblast-growth factor (bFGF; also known as FGF2) in a subcutaneous-pellet assay.

From the initial observation on the amino-acid α-mercaptoamides a series of mercaptoalcohols and mercaptoketones were prepared. The mercaptoalcohols exhibited modest activity against MMP1, MMP3 and MMP9, whereas the equivalent mercaptoketones could be optimized to become active broad-spectrum inhibitors[42].

High-throughput screening efforts led to the discovery of other zinc-binding MMPIs. A collection of potential zinc binders were assayed against murine MMP9. Among the compounds tested, aminomethyl benzimidazole showed promising activity. This compound is also advantageous for further optimization because diversity can be introduced at multiple positions of its scaffold using efficient synthetic chemistry. In addition, aminomethyl benzimidazole binds to the enzyme active site rather than chelating the zinc. A completely non-peptidic gelatinase B inhibitor, an aminomethyl benzimidazole analogue (TABLE 1), was identified with modest inhibitory activity against MMP9 (IC_{50}=13 μM)[43].

A group of completely novel and promising type of MMPIs are the mechanism-based inhibitors developed by the group of Mobashery[44]. The first prototype was named SB-3CT and is highly selective for the gelatinases. Specifically, this compound contains a thiirane group that coordinates with the active-site zinc. In turn, this leads to a conformational change and a covalent attachment to the active-site Glu[45]. Therefore, this class of inhibitor is also named suicide inhibitors. New variants were recently designed, synthesized and proved to be active *in vivo*, one of which exclusively inhibits MMP2 (REF. 46).

Synthesized peptides with natural and non-natural amino acids can also inhibit MMPs. The histidine-ε-amino-caproic-acid-β-alanine-histidine (His-εAhx-βAla-His) sequence was found to inhibit both MMP2 and MMP9. The length of the spacer between the two terminal histidines was found to be crucial for the inhibitory potential[47]. The cyclic peptide (Cys-Thr-Thr-His-Trp-Gly-Phe-Thr-Leu-Cys) is a selective inhibitor of MMP2 and MMP9, which was found by phage-display technology. Although the inhibitory mechanism is not yet clear, it is speculated that the Trp residue in the His-Trp-Gly-Phe motif may bind to the hydrophobic pocket of the substrate cleft in the enzyme and that the His residue may act as a ligand for the catalytic zinc ion[48]. Serendipitously, we found that polyhistidine in the micromolar range can inhibit both MMP2 and MMP9 (REF. 25), presumably by chelating the active-site zinc and by simulating His-εAhx-βAla-His.

Tetracyclines are antibiotics that also inhibit the breakdown of connective tissue. Chemically modified tetracyclines (CMTs) without antibiotic activities have several potential advantages over conventional tetracyclines. Long-term systemic administration of CMTs does not result in gastrointestinal side effects or toxicity, and higher plasma concentrations can be reached for prolonged periods of time[49]. In addition, on the basis of their chemical properties, tetracyclines and CMTs may cross anatomical barriers such as the blood–brain barrier and blood–retina barrier. The proposed mechanisms of action of CMTs are their ability to bind metal ions such as calcium and zinc, and to affect *MMP* gene transcription. The first study on the structure–activity relationships of CMTs as MMPIs appeared a decade ago[50] and demonstrated that minocycline is one of the best CMT inhibitors of the gelatinases (TABLE 1). Minocycline is an inexpensive MMPI and is currently being evaluated as a lead compound for the treatment of multiple sclerosis and vascular neurological disorders, as it crosses the blood–brain barrier easily[51,52].

Tetracyclines and derivatives. Tetracyclines and chemically modified tetracyclines (CMTs) have been used to counteract extracellular matrix turnover and as anti-inflammatory agents. The binding of tetracycline and CMTs with pro- or active matrix metalloproteinases (MMPs) results in the disruption of the normal conformation of the protein structure and leaves the enzymes inactive and therefore vulnerable to degradation into small molecule fragments. CMTs have also been shown to downregulate gene expression of particular MMPs.

MMPIs in inflammatory and vascular diseases

Because of their potential in therapeutic approaches, MMPIs have been tested in many animal models of acute and chronic inflammation (FIG. 4), as well as in invasive cancer. The literature is extensive as exemplified in a recent overview of models used to study the pathophysiology of MMP9 (REF. 8). Many inflammatory and vascular animal models for human diseases have been developed and the effects of inhibitors have been tested, as exemplified by Supplementary information S2 (table) on MMP9 inhibitors. For this reason, we have organized this part by describing prototypic examples of animal models as a scaffold for further studies. A comprehensive overview of relevant knockout studies grouped according to organ system in TABLE 2a–d is accompanied by a critical note in BOX 2.

Table 2a | **Inflammatory phenotypes of *Mmp*-knockout mice according to organ system**

Mmp deficiency	Phenotypes	Genetic background	Refs
Respiratory system			
Mmp2$^{-/-}$	No effect on obliterative airway disease after tracheal allograft	C57BL/6 (H-2^{b})	146
Mmp2$^{-/-}$	Increased allergen-induced asphyxiation, reduced intraluminal leukocytes	C57BL/6	114
Mmp3$^{-/-}$	Decreased immune-complex-induced lung injury and neutrophil counts	129/SvEv × C57BL/6J	147
Mmp7$^{-/-}$	Decreased reorganization of cell functions and transepithelial neutrophil migration in bleomycin-induced lung injury	C57BL/6	127,148
Mmp7$^{-/-}$	Reduction of re-epithelialization in wounded trachea	> 99% C57BL/6	149
Mmp8$^{-/-}$	Reduction of inflammatory cell apoptosis, increased neutrophils in BAL in asthma	C57BL/6 × 129	113
Mmp9$^{-/-}$	Reduced respiratory failure and lung enlargement induced by IL13, increased BAL leukocyte numbers	N.D.	150
Mmp9$^{-/-}$	Reduced alveolar bronchiolization after bleomycin insult	129 SvEv	151
Mmp9$^{-/-}$	Decreased peribronchial cell infiltration and BAL lymphocytes after allergic challenge, decreased carbachol hyperresponsiveness	N.D.	116
Mmp9$^{-/-}$	Increased BAL-cell recruitment after allergic challenge	129SvEv × C57BL/6	152
Mmp9$^{-/-}$	Decreased lung injury by immune complexes	129SvEv	147
Mmp9$^{-/-}$	Decreased obliterative airway disease, increased T-cell alloreactivity	129SvEv (H-2^{b})	146
Mmp9$^{-/-}$ or *Mmp9*$^{-/-}$*Mmp2*$^{-/-}$	Reduced inflammatory cell influx to BAL fluid in asthma	C57BL/6	118
Mmp12$^{-/-}$	Reduced respiratory failure and lung enlargement induced by IL13	N.D.	150
Mmp12$^{-/-}$	No effect on bleomycin-induced BAL-cell composition	C57BL/6	126
Mmp12$^{-/-}$	Resistance against cigarette-smoke-induced emphysema	C57BL/6*	108–110
Mmp12$^{-/-}$	Reduced acute lung injury by immune complexes	129SvEv	153
Mt1–Mmp$^{-/-}$	Spontaneous retarded lung alveolar development and diminished surface area	129Rej/NIH Black Swiss	154,155
Gastrointestinal system			
Mmp2$^{-/-}$	Increased dextran-sulphate-induced colitis	C57BL/6	156
Mmp2$^{-/-}$	Protection and reduced hepatocyte apoptosis and necrosis in TNF-induced hepatitis	C57BL/6	129
Mmp3$^{-/-}$	Protection and reduced hepatocyte apoptosis and necrosis in TNF-induced hepatitis	C57BL6 × C57BL10 × RIII	129
Mmp7$^{-/-}$	Decreased innate immunity by decreased processing of defensin	C57BL/6	157
Mmp8$^{-/-}$	Protection against TNF-induced acute hepatitis, impaired hepatic leukocyte influx	C57BL6 × 129Sv	115
Mmp9$^{-/-}$	Protection and reduced hepatocyte apoptosis and necrosis in TNF-induced hepatitis	129Sv × CD1	129
Mmp9$^{-/-}$	Impaired PMN infiltration 6 hours after zymosan peritonitis induction	C57BL/6	158
Mmp9$^{-/-}$	Enhanced early vascular permeability by zymosan peritonitis	C57BL/6	159
Mmp-9$^{-/-}$	Reduced resistance against *Escherichia coli* peritonitis, reduced leukocyte recruitment	FVB/N	160
Mmp9$^{-/-}$	Decreased dextran-sulphate-induced colitis	FVB	161
Mmp12$^{-/-}$	No protection against TNF-induced hepatitis	129Sv × C57BL6	129
Mmp13$^{-/-}$	Suppressed liver fibrosis induced by cholestasis	129 × C57BL6	162
Mmp20$^{-/-}$	Postponed mineralization of mantle dentin	HM-1 × C57BL/6‡	163,164

*For references 109 and 110. ‡For reference 163. BAL, bronchoalveolar lavage; IL13, interleukin 13; Mmp, matrix metalloproteinase; Mt, membrane-type; N.D., not disclosed; PMN, polymorphonuclear; TNF, tumour-necrosis factor.

A model of acute inflammation

Endotoxin shock. Bacteraemia, septic and endotoxin shock are among the most frequent causes of mortality in modern hospitals. These clinical syndromes, which are characterized by multi-organ failure, are caused by a rapid and excessive host-inflammatory response to the invading microorganisms and their products[53,54]. Bacterial cell-wall constituents, such as endotoxins/lipopolysaccharides (LPS) and peptidoglycans, induce a systemic inflammatory response (which often leads to shock) by activation of the toll-like receptors (TLRs)[55]. In endotoxinaemia, this leads to the excessive production of inflammatory cytokines and enzymes. Genetic defects in TLR, cytokine and enzyme genes have therefore been found to result in resistance against endotoxin shock. For instance, mice deficient in MMP9 have an altered resistance to LPS-induced toxicity[56], whereas mice deficient in protease inhibitors are more susceptible to LPS shock[57]. Similarly, the complement cascade[58] and the plasminogen-activator-plasmin system[59] have been implicated in the pathogenesis of endotoxinaemia.

A peptide inhibitor, regasepin1 with the sequence Pro-Arg-Cys-Bip-Cys-Gly-Glu (Bip is biphenylalanine) against MMP9 was developed in our laboratory and shows an inhibitory effect against MMP8 and MMP9 (REF. 37). This peptide inhibitor also inhibits TACE activity. Intravenous injection with regasepin1 was shown to protect mice against lethal endotoxin shock[60]. Regasepin2 with the sequence Pro-Pyr·Ala-Cys-Bip-Arg-Gly-Glu (Pyr·Ala represents pyridylalanine) is another peptide inhibitor with a similar inhibitory potential *in vitro* and *in vivo* against lethal endotoxin shock as regasepin1 (REF. 61).

Models of chronic inflammation

Multiple sclerosis. Multiple sclerosis (MS) is a multifactorial disease that is influenced by genetic predisposition, environmental factors and important immunological effector mechanisms that damage the central nervous system

Table 2b | **Inflammatory phenotypes of *Mmp*-knockout mice according to organ system**

Mmp deficiency	Phenotypes	Genetic background	Refs
Musculoskeletal system			
Mmp2$^{-/-}$	Increased immune-complex-induced arthritis	BALB/c	165
Mmp3$^{-/-}$	No effect on collagen-induced arthritis	129SvEv × C57BL/6 × B10R111 (H-2^{r})	166
Mmp9$^{-/-}$	Decreased immune-complex-induced arthritis	BALB/c	165
Mmp9$^{-/-}$	Increased bacterial arthritis, decreased bacterial clearance	C57BL/6	167
Mmp9$^{-/-}$	Spontaneous delayed growth-plate angiogenesis and apoptosis of hypertrophic chondrocytes	129/Sv × CD1 or 129/Sv × Black Swiss	168,169
Mmp9$^{-/-}$	Reduced incidence of induced chondrodermatitis	E14-J × C57BL/6	64
Mmp12$^{-/-}$	Reduced macrophage numbers in ligament-injury repair	N.D.	170
Mmp13$^{-/-}$	Spontaneous abnormal growth plate, increased trabecular bone	FVB/N	171
Mt1–Mmp$^{-/-}$	Impaired osteocyte processes and collagen cleavage	HM-1 × Black Swiss	172
Nervous system			
Mmp2$^{-/-}$	Reduced functional recovery from spinal-cord injury	FVBn	173
Mmp9$^{-/-}$	Increased collagenase-induced brain haemorrhage and injury	N.D.	174
Mmp9$^{-/-}$	Reduced remyelination after trauma of spinal cord	129SvEv	75
Mmp9$^{-/-}$	Spontaneous deficient myelination of corpus callosum, fewer oligodendrocytes	129/SvEv	175
Mmp9$^{-/-}$	Reduced EAE in young mice	E14-J × C57BL/6	64
Mmp9$^{-/-}$*Mmp2*$^{-/-}$	Reduced EAE development	C57BL/6	65
Mmp9$^{-/-}$	Reduced ischaemic lesion volume after permanent focal ischaemia	CD1	176
Mmp9$^{-/-}$	Reduced survival of *Sod1*-knockout mice	C57 BL/6 SOD1^{G93A}	177
Mmp12$^{-/-}$	Improved functional recovery from spinal cord compression	129/SvEv (CD1 outbred mice)	178
Mmp12$^{-/-}$	Increased maximal EAE severity	129/SvEv	74
Mmp12$^{-/-}$	Spontaneous deficient myelination of corpus callosum, fewer oligodendrocytes	129/SvEv	175
Mmp12$^{-/-}$	Retarded *in vitro* differentiation of oligodendrocytes	129/SvEv	179
Mt5–Mmp$^{-/-}$	Decreased nerve-fibre sprouting and neural invasion	C57Bl/6J	180

EAE, experimental autoimmune encephalomyelitis; Mmp, matrix metalloproteinase; Mt, membrane-type; N.D., not disclosed; *Sod1*, superoxide dismutase 1.

(CNS). Pathogenetic mechanisms such as chemotaxis, subsequent activation of autoreactive lymphocytes and skewing of the extracellular proteinase balance are targets for new therapies.

MMP9 is an important immune effector molecule in MS pathogenesis[62]. It functions in cell migration through connective tissues and vessel walls and damages the blood–brain barrier[65]. It also lyses protein substrates, such as myelin proteins, cell-adhesion molecules, cytokines and chemokines that are relevant in MS and other neurological diseases[62]. Additional evidence, which supports a detrimental role of MMP9 in inflammatory CNS damage, has been obtained with animal models. In murine experimental autoimmune encephalomyelitis (EAE), a model for MS, both gelatinases, MMP2 and MMP9, become upregulated during the development of the disease syndrome[63]. Young *Mmp*9-deficient mice were resistant to the development of EAE, whereas adult mice seemed

Table 2c | **Inflammatory phenotypes of *Mmp*-knockout mice according to organ system**

Mmp deficiency	Phenotypes	Genetic background	Refs
Cardiovascular system			
Mmp2$^{-/-}$	Prolonged cardiac allograft survival	C57BL/6 (H-2^{b})	181
Mmp2$^{-/-}$	Delayed inflammation-associated corneal neovascularization	97% C57BL/6J and 3% 129	182
Mmp2$^{-/-}$	Reduced extraretinal neovascularization after oxygen-induced retinopathy	C57BL/6	183
Mmp2$^{-/-}$	Reduced choroidal neovascularization after fundus photocoagulation	97% C57BL/6 and 3% 129	184
Mmp2$^{-/-}$ or *Mmp9*$^{-/-}$	Reduced abdominal aorta injury, elastin degeneration and calcification	N.D.	185
Mmp2$^{-/-}$ *Mmp9*$^{-/-}$	Reduced laser-induced choroidal neovascularization	N.D.	186
Mmp2$^{-/-}$ or *Mmp9*$^{-/-}$	Reduced induction of aortic aneurysm	129/SvEv or FVB (Mmp9); C57BL/6 (Mmp2)	187,188
Mmp2$^{-/-}$	No effect on retinal vascular development or pathological retinal vascularization	97% C57BL/6 × 3% 129	189
Mmp2$^{-/-}$*Apoe*$^{-/-}$	Reduction of atherosclerotic plaque and smooth muscle cells	C57BL/6	190
Mmp3$^{-/-}$	Larger myocardial scar volume 3 days post injury	C57BL/6	191
Mmp7$^{-/-}$	Increased corneal neovascularization after injury and during wound healing	> 96% C57BL/6	192
Mmp9$^{-/-}$	Suppressed elastase-induced aneurysmal degeneration	129/SvEv	193
Mmp9$^{-/-}$	Delayed vessel formation in ischaemic limb model	CD1	194
Mmp9$^{-/-}$	Reduced endocardial endothelial cell apoptosis and blood-pressure levels after induction by arteriovenous fistula	FVB.Cg	195
Mmp9$^{-/-}$	Reduced left-ventricle dilatation and cardiac fibrosis after pressure overload	50% CD1 × 50% 129Sv	196
Mmp9$^{-/-}$	Reduced cardiac rupture post myocardial infarction	N.D.	100
Mmp9$^{-/-}$	Improved left-ventricular function after myocardial infarction, increased neovascularization in remodelling myocardium	FVB	197
Mmp9$^{-/-}$	Decreased capillary branching after ischaemic insult	129 SvEv or C57BL/6J	198
Mmp9$^{-/-}$	Decreased cardiac allograft survival	129SvEv (H-2^{b})	181
Mmp19$^{-/-}$	Increased early angiogenetic response and tumour-cell invasion	C57Bl6 × 129Ola	199
Mmp9$^{-/-}$	Decreased capillary formation	129/SvEv	200
Mmp9$^{-/-}$ or *Apoe*$^{-/-}$*Mmp9*$^{-/-}$	Decreased atherosclerotic burden, impaired macrophage infiltration	68.75% C57Bl/6, 12.5% 129SvJ, 12.5% CDI and 6.25% 129SJ*; C57BL6 × 129 SvEv‡	99,201
Mmp11$^{-/-}$	Enhanced neointima formation after vascular injury	129SV or 129SV/Bl6	97
Mmp12$^{-/-}$	Reduced $CaCl_2$-induced aortic aneurym and less elastin breakdown	129SvJ	202
Mmp12$^{-/-}$ *Apoe*$^{-/-}$	Decreased transmedial elastin degradation in atherosclerosis	84.37% C57Bl/6, 12.5% 129 SvJ and 3.13% 129SvEvTac	99

*For reference 99. ‡For reference 201. Apoe, apolipoprotein E; Mmp, matrix metalloproteinase; N.D., not disclosed.

to lose this effect; both young and adult mice controls developed severe EAE[64] (TABLE 2b). Double *Mmp2*/*Mmp9*-knockout mice were completely resistant against the development of EAE[65]. As in human MS, MMP2 is a constitutive enzyme in the cerebrospinal fluid[66], and so selective MMP9 inhibition therefore might be appropriate, especially in view of the fact that MMP9 kills the activity of interferon-β, one of the drugs used to treat MS[67].

Pharmacological inhibition of MMP activity improved the course of EAE in several studies that used MMPIs with different degrees of selectivity. Specifically, the hydroxamate-type inhibitors have been found to be protective in animal models of EAE[68,69]. The sulphydryl-containing

Table 2d | **Inflammatory phenotypes of *Mmp*-knockout mice according to organ system**

Mmp deficiency	Phenotypes	Genetic background	Refs
Defence system (skin and leukocytes)			
Mmp2$^{-/-}$	Prolonged cardiac allograft survival	C57BL/6 (H-2^{b})	85,181
Mmp3$^{-/-}$	Impaired skin-contact hypersensitivity	B10.R111	203
Mmp3$^{-/-}$	Delayed clearance of bacteria, delayed appearance of T cells	B10.R111	204
Mmp7$^{-/-}$	Lower epithelial cell apoptosis, reduced Fasl processing	Sv129 × C57BL/6	205
Mmp7$^{-/-}$	Impaired transepithelial migration of neutrophils	C57BL/6	127
Mmp7$^{-/-}$	Lower TNF processing and MMP3 production by macrophages *in vitro*	129SvEv	206,207
Mmp8$^{-/-}$	Increased incidence of skin cancers in male and ovariectomized mice	129/SvJ × C57BL/6	208
Mmp9$^{-/-}$ *SP/D*$^{-/-}$	Reduced shedding of endotoxin receptor CD14	N.D.	209
Mmp9$^{-/-}$	Reduced corneal epithelial permeability and desquamation	129SvEv × CD1	210
Mmp9$^{-/-}$	Dispensable for G-CSF and EPO-induced leukocyte precursor mobilization	–	211
Mmp9$^{-/-}$	Decreased inflammatory cell invasion	129/SvEv	200
Mmp9$^{-/-}$	Decreased bacterial clearance	C57BL/6	167
Mmp9$^{-/-}$	Reduced resistance against *Escherichia coli* peritonitis	FVB/N	160
Mmp9$^{-/-}$	Resistance against experimental bullous pemphigoid	129/Sv × CD1 or 129/Sv × Black Swiss	212
Mmp9$^{-/-}$	Resistance against endotoxin shock	E14-J × C57BL/6 (75% C57BL/6)	56
Mmp9$^{-/-}$	Reduced migration of Langerhans cells from skin explants *in vitro*	129/SvEv	213
Mmp9$^{-/-}$	Prolonged skin-contact hypersensitivity	129/Sv	203
Mmp9$^{-/-}$	Decreased cardiac and tracheal allograft survival	129SvEv (H-2^{b})	146,181
Mmp12$^{-/-}$	Reduced matrix degradation by macrophages after subcutaneous implantation	N.D.	214
Mmp12$^{-/-}$ *SP/D*$^{-/-}$	Reduced shedding of endotoxin receptor CD14	N.D.	209
Miscellaneous			
Mmp3$^{-/-}$	Deficient production of macrophage chemoattractant in disc hernia *in vitro*	129SvEv	206
Mmp7$^{-/-}$	Deficient release of TNF from peritoneal macrophages *in vitro*	129SvEv	207
Mmp9$^{-/-}$	Tetracycline inhibits ischaemia-induced cerebral damage but not in *Mmp9*$^{-/-}$ mice	C57Bl/6 or E14-J × C57BL/6 (75% C57Bl/6)	215
Mmp9$^{-/-}$ *Rag1*$^{-/-}$	Impaired recruitment of bone-marrow-derived leukocytes into neuroblastoma	FVB/N	216
Mmp9$^{-/-}$	Reduced platelet adhesion to cold injured liver sinusoidal epithelial cells *in vitro*	129/SvEv	217
Mmp11$^{-/-}$	Increased fat excess and adipogenesis	BALB/c	218
Mmp19$^{-/-}$	Spontaneous adipocyte hypertrophy and decreased clinical carcinogenesis	C57BL/6 × 129Ola	219

EPO, erythropoietin; Fasl, Fas ligand (Tnf superfamily, member 6); G-CSF, granulocyte colony-stimulating factor; Mmp, matrix metalloproteinase; N.D., not disclosed; *Rag1*, recombination activating gene 1; TNF, tumour-necrosis factor.

Box 2 | **Knockout mouse studies knockout valuable information**

Knockout mouse models are often used to predict the phenotypes of enzyme inhibition for the development of enzyme inhibitors (TABLE 2a–d). However, as recently outlined, these types of studies do not necessarily or always have predictive value[28]. One aspect that has been constantly overlooked is the fact that knockout mouse lines can differ and thus, depending on the recombination events, the phenotypes can be divergent. These differences are not only based on variation of genetic backgrounds, which is often cited as an explanation, but also on the generated transgenes. For instance, we developed serendipitously two matrix metalloproteinase 9 (*Mmp9*)-deficient mouse lines: a leaky one and a non-leaky knockout one. The former was tolerant to immunization with recombinant MMP9, whereas from the latter knockout mice monoclonal antibodies were developed[139]. The fact that neutralizing antibodies against MMPs could be formed in non-leaky-knockout mice, which results in paradoxical effects, has been overlooked so far when extrapolating the biological effects observed in knockout mice. This has important implications for the development of MMP inhibitors. In addition, compensatory mechanisms may fade or brighten phenotypes in knockout mice. One logical conclusion is that inhibitors that possess multiple MMP targets will generate complex outcomes, particularly with prolonged use. As long-term inhibitor treatment will also touch the activity of constitutive homeostatic MMPs, it is predicted that side effects of inhibitor use will be observed that might not be detected in the knockout mouse equivalent(s).

D-penicillamine as an unselective MMP9 inhibitor protected mice against the development of acute EAE[70], but caused side effects in patients with MS during a combination trial with metacycline[71]. Minocycline is one of the most potent tetracycline derivatives with MMP9-inhibitory activity and is effective against EAE[50,72,73]. A first step in the direction of clinical application is a Phase I trial with an MMPI in MS (TABLE 3).

Most EAE experiments have been done on a short-term basis. We caution, therefore, that before studies with MMPIs are initiated in human MS, the long-term effects of MMPIs need to be established in animals. Besides MMP9, several other MMPs are increased in EAE, including MMP3, MMP8, MMP10 and MMP12. It has become clear that different MMPs can have different roles in the pathogenesis of EAE. In particular, it was found that MMP12 plays a protective role by favouring a T-helper 2 (T_H2) bias, thereby reducing the T_H1 autoimmune response[74]. This is another example of how specific MMPIs might be more useful than their broad-spectrum counterparts. In addition, selective MMP inhibition might be restricted to specific disease phases; for example, although MMP9 is detrimental in the onset of MS, it plays a beneficial role in remyelination[75].

Rheumatoid arthritis. Rheumatoid arthritis is characterized by the autoimmune inflammation of the joints and degradation of the joint cartilage (FIG. 4). In severe cases, it is also accompanied by irreversible bone erosion. Collagenases (MMP1, MMP8 and MMP13) are responsible for the first cleavage of collagen II, an important structural component of cartilage and a major autoantigen. Subsequently, the resulting quarter and three-quarter fragments are degraded by MMP9, which leads to the exposure and release of immunodominant epitopes[76,77]. In addition, MMPs are important for the migration of inflammatory leukocytes. This suggests that MMPIs might be useful in the therapy of rheumatoid arthritis, a notion that was confirmed in different animal models. Arthritis that was induced in rats with complete Freund's adjuvant was inhibited by the hydroxamate GI168, which resulted in a reduction of paw swelling and protection against degradation of bone and cartilage, pannus formation, and abnormal bone deposition[78]. GI168 does not inhibit TACE, although TNF inhibitors are currently being used in the clinic against rheumatoid arthritis. Therefore, use of the hydroxamates against both MMPs and TACE, such as BB-94, BB-1101, TMI-1 and the retrohydroxamate GW3333, led to better outcomes in different animal models[79–82]. Besides hydroxamates, carboxylates and CMTs were found to be effective in adjuvant arthritis. BAY12-9566, a carboxylate specific for MMP2, MMP3 and MMP9, inhibits neutrophil infiltration and paw swelling[40]. CMTs were found to diminish bone loss and stiffness but not inflammation and paw swelling. This could be overcome by combining CMTs with non-steroidal anti-inflammatory drugs[83].

As in chronic neuroinflammation, MMPs also possess beneficial effects in joint physiology. As for the maintenance of the functional integrity, the action of MMPs is essential. This finding was borne out of clinical studies of patients with cancer who developed tendinitis with long-term use of MMPIs. This indicates that selective MMPIs without causing these side effects are needed, in particular for long-term treatment. Several companies have followed these ideas and Phase II clinical trials with MMPIs for arthritic diseases are underway (TABLE 3).

Vascular diseases

Atherosclerosis and related diseases, including myocardial infarction and stroke, have often been compared with chronic inflammatory diseases[84]. This is based on histopathological findings such as the activation of foamy macrophages, the local production of cytokines and chemokines, and the involvement of MMPs (FIG. 4). The use of animal models with genetically altered mice (both transgenic and knockout mice) (TABLE 2c) has only strengthened the view that MMPs are key players in vascular pathologies[85,86]. On the basis of the available data, inhibition of MMPs with selective inhibitors opens new perspectives for treatment. However, as these are chronic processes, long-term treatment will be necessary and side effects need to be minimized and treatment compliance maximized. In other words, in these situations the need for oral drug treatment is mandatory. As outlined above, the combination of an orally available selective MMPI for each situation is at present difficult to achieve, and expensive long-term studies in animal models might not reflect the situation in humans.

Almost the opposite may be true for life-threatening acute ischaemic diseases. The roles played by MMPs in these diseases can presently be assessed in mouse, rat and other mammalian species and so one could discover which MMPs are detrimental and which ones are beneficial in which phases of the disease process (TABLE 2c). For instance, it has recently been found that serine proteases and MMPs, involved in the same proteinase cascade, play differential roles in the evolution of cerebral infarction in stroke. This research paves the way to define therapeutic windows to improve the net result by diminishing neuronal loss[87–90]. If one takes into account that saving small

Neutrophils
Neutrophils are the most abundant cell type in the human circulation. Lipopolysaccharide acts directly on these cells in endotoxinaemia. This results in the release of pro-matrix metalloproteinase 8 (MMP8) and pro-MMP9, which are activated by, for example, reactive oxygen intermediates, and of lysozyme, which generates peptidoglycan fragments. An important aspect of endotoxinaemia and septic shock is its acuteness, based on fast degranulation of the neutrophils.

Foamy macrophages
Macrophages in the arterial wall that ingest oxidized low-density lipoprotein and assume a foamy appearance. These cells secrete various substances involved in further plaque growth.

Table 3 | **Matrix metalloproteinase (MMP) inhibitors in clinical trials of inflammation and malignancies***

Compound	Company	Indication and clinical phase	MMP classes known to be inhibited
Doxycycline hyclate (Dermostat, Periostat)	CollaGenex Pharmaceuticals	Launched: Periodontal disorders Phase III: Rosacea Phase III: Controlled-release formulation for periodontal disorders Phase II: Acne	Collagenase
AZD 8955	AstraZeneca	Phase II: Osteoarthritis	Collagenase
PCK 3145	Ambrilia Biopharma	Phase II: Prostate cancer	MMP9
Apratastat	Amgen/ Wyeth	Phase II: Rheumatoid arthritis	MMP1, MMP9, MMP13, TACE
Incyclinide	CollaGenex Pharmaceuticals	Phase II: Acne Phase II: Brain cancer Phase II: Kaposi's sarcoma Phase I: Cancer metastases Phase I: Solid tumours	MMP2
ABT 518	Abbott Laboratories	Phase I: Solid tumours	Unknown
MPC 2130	Myriad Pharmaceuticals	Phase I: Cancer Phase I: Haematological malignancies	Unknown
MMP12 inhibitor	Merck Serono	Phase I: Multiple sclerosis	MMP12

*Data was obtained from the Adis R&D Insight Database. TACE, tumor-necrosis factor, converting enzyme (also known as ADAM17).

percentages of neurons can in the end make the difference between, for instance, paralysis, aphasia or rescue of these functions, patients and medical practitioners will have no objections against the use of for example, recombinant serine-proteinase inhibitors or synthetic MMPIs, even if these have to be infused parenterally for days or a week. However, as MMPs are also required for neurovascular remodelling, prolonged MMP inhibition is detrimental by impairing functional recovery and therefore has to be avoided[91].

Vascular remodelling also has a central role in (re)stenosis, plaque rupture and aneurysm formation, and is dependent on the action of several MMPs. Restenosis can occur after interventions for treatment of atherothrombosis, such as stenting and balloon angioplasty, and is caused by the proliferation and migration of vascular smooth muscle cells from the tunica media into the tunica intima, which leads to neointima formation and constriction of the arterial lumen. Plaque rupture can cause obstruction of the blood vessel, whereas loss of elasticity in the blood-vessel wall can lead to aneurysm formation (FIG. 4).

The *in vivo* migration of smooth muscle cells was shown to be dependent on MMP2 and MMP9 (REFS 92,93). In addition, neointima formation was increased in TIMP1-deficient mice[94]. Broad-spectrum inhibitors such as GM6001 and marimastat, but not batimastat, were able to inhibit in-stent intimal hyperplasia, and batimastat and marimastat could inhibit constrictive arterial remodelling after balloon dilation (reviewed by Sluijter *et al.*[95]). Unfortunately, a clinical study with batimastat-coated stents yielded negative results[96], which demonstrates that the role of the different MMPs in vascular remodelling is more complex than originally thought. A further indication for the need of more specific MMPIs is the fact that in MMP11-deficient mice, neointima formation is enhanced instead of decreased[97].

Selective disruption of MMP3 also results in larger atherosclerotic lesions. MMP3 also displays detrimental roles by enhancing the infiltration of macrophages in the lesions, possibly through enhancing plaque instability[98]. Macrophage infiltration and collagen deposition are similarly promoted by MMP9 (REF. 99). MMP3, MMP9 and MMP12 have important roles in the degradation of elastic lamina and aneurysm formation. All together, these studies indicate paradoxical roles of different MMPs in different pathological processes in vascular diseases. Nevertheless, a number of broad-spectrum MMPIs showed some positive effects in animal models, providing the proof-of-concept that MMP inhibition might be therapeutically useful. It can therefore be foreseen that the development and use of more specific inhibitors might lead to effective therapies in these vascular pathologies. However, it seems mandatory to perform more and better basic research studies before embarking on expensive clinical trials. For example, through the careful study of animal models it has recently been found that MMPs have pivotal roles in the processes following arterial occlusion and that the damage (to the brain, heart and so on) can be reduced with MMPIs such as tetracycline derivatives[51].

Sudden death after myocardial infarction can occur by cardiac rupture, a process in which MMPs are involved. In mouse model studies, the critical role of gelatinases in balance with TIMPs was demonstrated[100]. This pathology could be reversed by treating mice with an oral inhibitor of MMP2 (REF. 101).

Examples of organ-specific inflammations

The use of tetracyclines as inhibitors of MMPs was pioneered by Golub and colleagues, who studied collagenases in periodontitis[102]. ECM and dentin degradation are important processes in the pathogenesis of periodontitis, peri-implantitis and in the formation of caries lesions

(FIG. 4). Originally, microbial proteases were held responsible, but increasing evidence indicates that host MMPs, mainly MMP8 and MMP9, are essential in these processes (reviewed by Sorsa *et al.*[103,104]). In a rat model of LPS-induced periodontal-tissue destruction[102] and in human pathology[105], CMTs proved to limit the disease. The mechanisms of action involved are decreases in MMP activity and in pro-inflammatory cytokines (TNF, interleukin-1β (IL1β) and IL6) and an increase in IL10. Combination of a CMT with the bisphosphonate clodronate resulted in a synergizing potency that inhibited tissue and bone loss[106]. Also a mercaptoacyl-containing MMPI and a sulphonamide-containing hydroxamate-based MMPI were effective in reducing LPS-induced periodontal bone loss[107]. MMPIs are already being tested in Phase III studies or are launched for the treatment of periodontal disorders (TABLE 3).

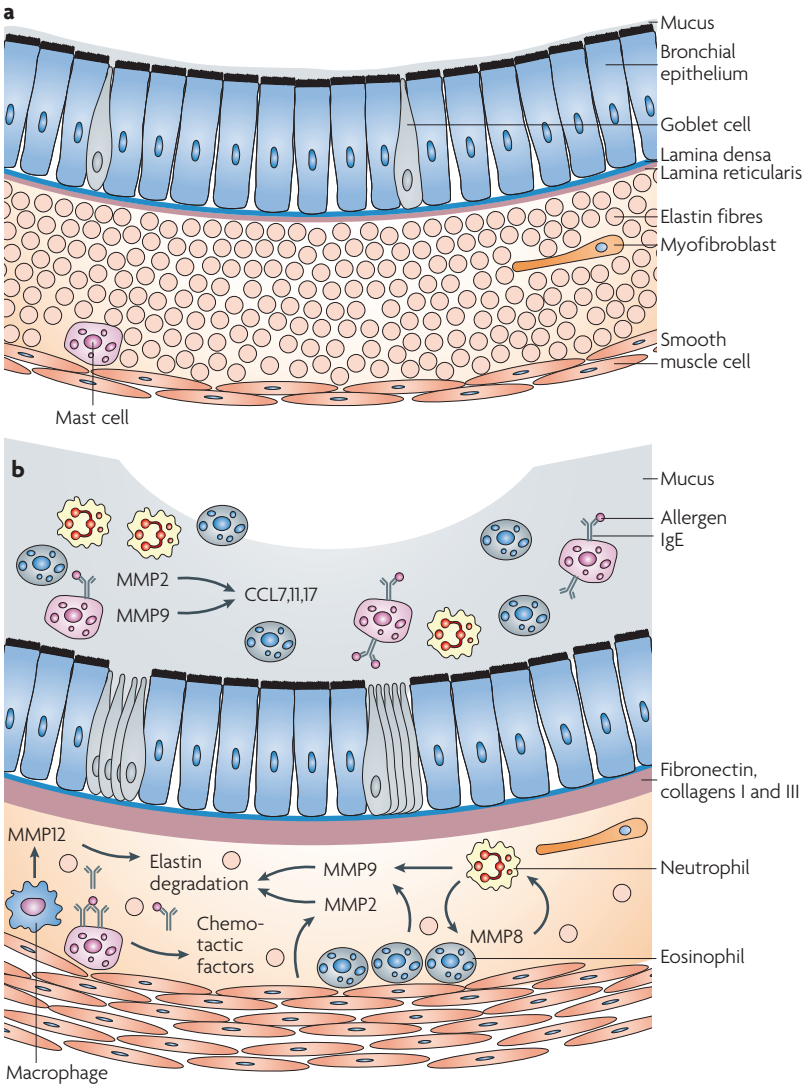


Figure 5 | **Roles of MMPs in asthma. a** | At the luminal side of normal bronchi and bronchioli, epithelial cells are covered by a thin mucus layer, which is produced by goblet cells. At the basal side, they are supported by the extracellular matrix, that is, a basement membrane and elastin fibres. Within this connective tissue, blood vessels can supply all types of inflammatory leukocytes, including mast cells. Physiological bronchoconstriction is controlled by the action of smooth muscle cells. **b** | In asthma, allergens bind to specific cytophilic immunoglobulin E (IgE) molecules. The immune complexes stimulate mast cells to degranulate chemotactic factors for eosinophils, macrophages and neutrophils. These leukocytes, together with resident cells (for example, smooth muscle cells), produce matrix metalloproteinases (MMPs), which cleave various substrates, including elastin (leading to structural destruction and loss of elasticity) and chemokines (resulting in enhanced chemotactic activity to the airway lumen). Smooth-muscle-cell proliferation, fibrosis of the lamina reticularis and mucus hypersecretion by proliferating goblet cells lead to narrowing of the airway lumen and inhibited air flow. CCL7,11,17, chemokine (C-C motif) ligand 7,11,17.

Chronic obstructive pulmonary disease. Chronic obstructive pulmonary disease (COPD) is the result of chronic inflammation of the lungs, and can result in emphysema. An essential aspect of COPD and emphysema is the degradation of elastin by proteases. In particular, intrapulmonary instillation of elastases leads to emphysema, and *Mmp12*-knockout mice are resistant to cigarette-smoke-induced emphysema[108]. The resulting elastin peptides are chemotactic for monocytes, which contribute to the perpetuation of the inflammation[109]. Also, TNF release and degradation of the α1-proteinase inhibitor by several MMPs contribute to the disease, by activation of neutrophils and increased activity of serine proteases such as neutrophil elastase[110,111]. In accordance to these results, treatment of guinea pigs with a broad-spectrum MMPI resulted in a delay of tobacco-smoke-induced emphysema[112].

Asthma. MMPs have important, mainly protective, roles in asthma (FIG. 5). In particular, MMP8 deficiency leads to enhanced granulocytic inflammation after allergen exposure, presumably because MMP8 has a role in the apoptosis of granulocytes[113]. Both MMP2 and MMP9 are essential for the movement of inflammatory cells into the airway lumen, which prevents lethal asphyxiation[114,118]. One explanation for this finding is that MMP2 and MMP9 cleave chemotactic factors in the luminal fluid, which results in enhanced chemotactic activities[128]. In addition, MMP9 is also important for the movement of dendritic cells into the lumen, which can also contribute to the production of chemotactic factors[117]. Whether MMP9 deficiency affects airway hyperreactivity in a positive or negative sense is not clear, as different groups report conflicting results[116–118]. Such differences might be due to different protocols for allergy induction, and also to the use of different mice strains and different levels of backcrossing (TABLE 2a, BOX 2). Nevertheless, it remains important to pinpoint the role of each involved MMP to define optimal pharmacological targets. One of the currently used treatments are corticosteroids, which decrease the inflammation and therefore also MMP production. Inhibition of MMPs with doxycycline, with a hydroxamate MMPI or with TIMP2 has been reported to decrease airway damage, hyperresponsiveness and inflammation[119–122]. However, treatment with GM6001 results in decreased leukocyte migration, which indicates that MMP inhibition in asthma can also lead to asphyxiation[114]. More animal studies are therefore needed to evaluate whether specific inhibition of MMPs can be used for therapy.

Box 3 | **Treatment with matrix metalloproteinase inhibitors at a glance**

- In contrast to microbiological targets, matrix metalloproteinases (MMPs) are host enzymes. This implies that normal physiological functions will be intrinsically blocked by MMP inhibitors (MMPIs) with subsequent side effects.
- The therapeutic benefits of MMPIs should be tested in animal studies of inflammation rather than cancer because cancer is genetically unstable and less predictable.
- The risk of side effects caused by short-term inhibition of inducible and homeostatic enzymes (by MMPIs with low selectivity) is lower than in cases of prolonged use of MMPIs in chronic inflammation, whereas long-term blockage of homeostatic enzymes will enhance fibrotic processes.
- For life-threatening acute inflammations, oral use and high selectivity of MMPIs are not necessary. As a consequence, recombinant protein and peptide inhibitors are worthwhile to develop.
- For chronic inflammatory diseases, high selectivity and preferentially oral availability are in demand, as these diseases constitute major targets for the pharmaceutical industry. Therefore, major basic and animal research efforts need to be continued.

Lung fibrosis. Lung fibrosis is characterized by an increase in the number of fibroblasts in the interalveolar septa together with an increase in collagen and elastin deposition. This thickening of the septa results in the loss of elasticity and decreased gas exchange. The most severe forms of lung fibrosis (for example, idiopathic pulmonary fibrosis) are fatal. Corbel *et al.* found that BB-94 ameliorates the outcome of bleomycin-induced fibrosis in mice[123]. Similarly, GM6001 decreased lung fibrosis in a model of asbestos-induced injury[124]. *Mmp7* was found to be one of the most upregulated genes in the lungs of bleomycin-treated mice, and *Mmp7*-knockout mice were less sensitive to the induced fibrosis[125]. MMP7 was found to be essential to generate a chemotactic gradient for neutrophils from the perivascular space to the alveolar lumen, by cleaving the proteoglycan syndecan-1, which associates with the chemokine KC[127]. Recently, *Mmp12*-knockout mice were reported to be protected against bleomycin- or transforming growth factor-β (TGFβ)-induced fibrosis[220], but another study failed to detect any difference between wild-type and *Mmp12*-knockout mice[126].

Hepatitis. Hepatitis is also associated with the expression of MMPs. In one study of acute hepatitis, TNF-induced lethality and influx of leukocytes into the liver were prevented by the hydroxamate MMPI BB-94. In addition, mice deficient in *Mmp2, Mmp3 or Mmp9* had lower levels of apoptosis and necrosis of hepatocytes and showed better survival[129]. In hepatitis B virus (HBV)-transgenic mice which were injected with HBV-specific cytotoxic T lymphocytes, liver disease was ameliorated by the reduction of myeloid cells and also by the use of the TIMP1 as an MMPI[130]. Similarly, it was recently shown that MMP8 plays an important role in acute lethal hepatitis induced by TNF[115]. These examples are reminiscent of the picture of endotoxin shock, which also has a major implication of tissue damage by neutrophils, which mainly produce MMP9 and MMP8 (REFS 56,60,61).

Pancreatitis and meningitis. Other organ-specific clinical entities of inflammation with a pathogenetic involvement of MMPs are pancreatitis and bacterial meningitis. The expression levels of MMPs and TIMPs were evaluated in an animal model of acute cerulein-induced pancreatitis. Mainly *Mmp3* was induced in the regenerative phase, although *Mmp2*, *Mmp9* and *Timp2* were also induced during the disease[131]. Local trypsin was shown to activate pro-MMP9 *in vivo* in cerulein-induced pancreatitis[132], adding another physiological link to the MMP-activation cascade[8]. The production of active MMP9 in pancreatitis could also have effects on the endocrine functions of the pancreas in acute and chronic inflammation, as MMP9 destroys insulin[133].

The involvement of MMP9 in bacterial or viral meningitis was described a decade ago[66]. These observations were followed by the finding of blood–brain barrier damage by MMP8 and MMP9 in bacterial meningitis[134]. Furthermore, in a rat model of bacterial meningitis it was demonstrated that inhibition of MMPs with the hydroxamate BB-1101 showed beneficial effects if proper antibiotic therapy was instated[135]. Reciprocally, it needs to be noted that in *Mmp9*-knockout mice the host defence against bacteria is impaired if antibiotic treatment is omitted[136]. As a result, it can be deduced that adjuvant therapy with MMPIs in severe cases of meningitis might reduce the disease symptoms and sequelae by stabilization of the blood–brain barrier, much like in the case of acute forms of EAE.

Conclusions and future perspectives

Irrespective of the considerable efforts carried out by both academic institutions and industrial laboratories to develop MMPIs, little has been achieved for the clinical practitioner. Is it necessary to introduce first orally available drugs to the market? Or is it a worthwhile exercise to show proof-of-principle with highly selective biotechnological drugs? We contemplate that in the cancer studies the objectives were set too high by combining oral availability and high selectivity. In addition, the choice of invasive or metastatic cancer as the clinical readout was not a good one. Inflammatory and vascular diseases, because of their genetic stability, constitute better candidate diseases for treatment with MMPIs. Through recent data it became evident that in some acute pathological situations, strict selectivity is not necessary (BOX 3). Furthermore, it is justified to test well-chosen available MMPIs in animal studies of inflammation, and, if therapeutically active, to start clinical trials. Along this line, CMTs are the most developed for the treatment of vascular and inflammatory diseases. The short-term use of parenteral MMPIs in severe illnesses with high mortality, such as lethal shock syndromes, or with high morbidity, such as sequelae of stroke, seems to have a bright future. Is it possible to generate MMPIs for both acute and chronic inflammatory or vascular diseases? We suggest that in the setting of an acute inflammation, blocking both constitutive and inducible MMPs for a limited time interval might help to rescue critical tissues, whereas by long-term inhibition in chronic diseases, the inhibition of constitutive enzymes might cause damage and side effects. Hence, for prolonged clinical use, the development of highly selective and preferentially orally active MMPIs will be

crucial. The necessary tools of recombinant enzymes, high-throughput screening methods, combinatorial synthetic chemistry, structure-based design and others are in place, and recently Phase I, II and III clinical trials have been started with MMPIs. However, further developments will be needed to achieve the combined goals of high selectivity, ideal oral uptake and acceptable pharmacokinetics.

1. Barrett, A. J., Rawlings, N. D. & Woessner J. F. *Handbook of Proteolytic Enzymes* (Academic Press, London, 2004).
2. Egeblad, M. & Werb, Z. New functions for the matrix metalloproteinases in cancer progression. *Nature Rev. Cancer* **2**, 161–174 (2002).
3. Kobayashi, Y., Matsumoto, M., Kotani, M. & Makino, T. Possible involvement of matrix metalloproteinase-9 in Langerhans cell migration and maturation. *J. Immunol.* **163**, 5989–5993 (1999).
4. Coussens, L. M., Fingleton, B. & Matrisian, L. M. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. *Science* **295**, 2387–2392 (2002).
5. Overall, C. M. & Lopez-Otin, C. Strategies for MMP inhibition in cancer: innovations for the post-trial era. *Nature Rev. Cancer* **2**, 657–672 (2002).
6. Van Wart, H. E. & Birkedal-Hansen, H. The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. *Proc. Natl Acad. Sci. USA* **87**, 5578–5582 (1990).
7. Kleifeld, O. *et al.* Structural characterization of the catalytic active site in the latent and active natural gelatinase B from human neutrophils. *J. Biol. Chem.* **275**, 34335–34343 (2000).
8. Van den Steen, P. E. *et al.* Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9). *Crit. Rev. Biochem. Mol. Biol.* **37**, 375–536 (2002).
9. Coussens, L. M. & Werb, Z. Inflammation and cancer. *Nature* **420**, 860–867 (2002).
An excellent review on the similarities of the molecular processes between inflammation and cancer.
10. Creighton, C. & Hanash, S. Expression of matrix metalloproteinase 9 (MMP-9/gelatinase B) in adenocarcinomas strongly correlated with expression of immune response genes. *In Silico Biol.* **3**, 301–311 (2003).
11. Opdenakker, G. & Van Damme, J. Cytokines and proteases in invasive processes: molecular similarities between inflammation and cancer. *Cytokine* **4**, 251–258 (1992).
12. Opdenakker, G. & Van Damme, J. The countercurrent principle in invasion and metastasis of cancer cells. Recent insights on the roles of chemokines. *Int. J. Dev. Biol.* **48**, 519–527 (2004).
13. Heissig, B. *et al.* Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. *Cell* **109**, 625–637 (2002).
14. Chantrain, C. F. *et al.* Stromal matrix metalloproteinase-9 regulates the vascular architecture in neuroblastoma by promoting pericyte recruitment. *Cancer Res.* **64**, 1675–1686 (2004).
15. Cuzner, M. L. and Opdenakker, G. Plasminogen activators and matrix metalloproteases, mediators of extracellular proteolysis in inflammatory demyelination of the central nervous system. *J. Neuroimmunol.* **94**, 1–14 (1999).
16. Pruijt, J. F. M. *et al.* Prevention of interleukin-8-induced mobilization of hematopoietic progenitor cells in rhesus monkeys by inhibitory antibodies against the metalloproteinase gelatinase B (MMP-9). *Proc. Natl Acad. Sci. USA* **96**, 10863–10868 (1999).
17. Opdenakker, G., Fibbe, W. E. & Van Damme, J. The molecular basis of leukocytosis. *Immunol. Today* **19**, 182–189 (1998).
18. Kollet, O. *et al.* Osteoclasts degrade endosteal components and promote mobilization of hematopoietic progenitor cells. *Nature Med.* **12**, 657–664 (2006).
19. Parks, W. C., Wilson, C. L. & Lopez-Boado, Y. S. Matrix metalloproteinases as modulators of inflammation and innate immunity. *Nature Rev. Immunol.* **4**, 617–629 (2004).
20. Van den Steen, P. E., Proost, P., Wuyts, A., Van Damme, J. & Opdenakker, G. Neutrophil gelatinase B potentiates interleukin-8 tenfold by aminoterminal processing, whereas it degrades CTAP-III, PF-4, and GRO-α and leaves RANTES and MCP-2 intact. *Blood* **96**, 2673–2681 (2000).
Together with references 21–23, 67 and 157, describes important regulatory effects of MMPs on chemokine and cytokine functions.
21. Van den Steen, P. E. *et al.* Gelatinase B/MMP-9 and neutrophil collagenase/MMP-8 process the chemokines human GCP-2/CXCL6, ENA-78/CXCL5 and mouse GCP-2/LIX and modulate their physiological activities. *Eur. J. Biochem.* **270**, 3739–3749 (2003).
22. McQuibban, G. A. *et al.* Inflammation dampened by gelatinase A cleavage of monocyte chemoattractant protein-3. *Science* **289**, 1202–1206 (2000).
23. McQuibban, G. A. *et al.* Matrix metalloproteinase processing of monocyte chemoattractant proteins generates CC chemokine receptor antagonists with anti-inflammatory properties *in vivo*. *Blood* **100**, 1160–1167 (2002).
24. Whittaker, M., Floyd, C. D., Brown, P. & Gearing, A. J. Design and therapeutic application of matrix metalloproteinase inhibitors. *Chem. Rev.* **99**, 2735–2776 (1999).
25. Hu, J., Van den Steen, P. E., Houde, M., Ilenchuk, T. T. & Opdenakker, G. Inhibitors of gelatinase B/matrix metalloproteinase-9 activity comparison of a peptidomimetic and polyhistidine with single-chain derivatives of a neutralizing monoclonal antibody. *Biochem. Pharmacol.* **67**, 1001–1009 (2004).
26. Martens, E. *et al.* A monoclonal antibody inhibits gelatinase B/MMP-9 by selective binding to part of the catalytic domain and not to the fibronectin or zinc binding domains. *Biochim. Biophys. Acta* **1770**, 178–186 (2007).
27. Chirco, R., Liu, X. W., Jung, K. K. & Kim, H. R. Novel functions of TIMPs in cell signaling. *Cancer Metastasis Rev.* **25**, 99–113 (2006).
28. Overall, C. M. & Kleifeld, O. Tumour microenvironment — opinion: validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy. *Nature Rev. Cancer* **6**, 227–239 (2006).
29. Kontogiorgis, C. A., Papaioannou, P. & Hadjipavlou-Litina, D. J. Matrix metalloproteinase inhibitors: a review on pharmacophore mapping and (Q)SARs results. *Curr. Med. Chem.* **12**, 339–355 (2005).
30. Sang, Q. X. *et al.* Matrix metalloproteinase inhibitors as prospective agents for the prevention and treatment of cardiovascular and neoplastic diseases. *Curr. Top. Med. Chem.* **6**, 289–316 (2006).
Together with references 24 and 29, constitutes an excellent update on the medicinal chemistry of MMPIs.
31. Breuer, E., Frant, J. & Reich, R. Recent non-hydroxamate matrix metalloproteinase inhibitors. *Expert Opin. Ther. Patents* **15**, 253–269 (2005).
32. Babine, R. E. & Bender, S. L. Molecular recognition of protein–ligand complexes: applications to drug design. *Chem. Rev.* **97**, 1359–1472 (1997).
33. Maskos, K. Crystal structures of MMPs in complex with physiological and pharmacological inhibitors. *Biochimie* **87**, 249–263 (2005).
34. Morgunova, E. *et al.* Structure of human pro-matrix metalloproteinase-2: activation mechanism revealed. *Science* **284**, 1667–1670 (1999).
35. Clements, J. M. *et al.* Matrix metalloproteinase expression during experimental autoimmune encephalomyelitis and effects of a combined matrix metalloproteinase and tumour necrosis factor-α inhibitor. *J. Neuroimmunol.* **74**, 85–94 (1997).
36. Xue, C. B. *et al.* Design and synthesis of cyclic inhibitors of matrix metalloproteinases and TNF-α production. *J. Med. Chem.* **41**, 1745–1748 (1998).
37. Hu, J., Fiten, P., Van den Steen, P. E., Chaltin, P. & Opdenakker, G. Simulation of evolution-selected propeptide by high-throughput selection of a peptidomimetic inhibitor on a capillary DNA sequencer platform. *Anal. Chem.* **77**, 2116–2124 (2005).
38. MacPherson, L. J. *et al.* Discovery of CGS 27023A, a non-peptidic, potent, and orally active stromelysin inhibitor that blocks cartilage degradation in rabbits. *J. Med. Chem.* **40**, 2525–2532 (1997).
39. Pikul, S. *et al.* Design and synthesis of phosphinamide-based hydroxamic acids as inhibitors of matrix metalloproteinases. *J. Med. Chem.* **42**, 87–94 (1999).
40. Hamada, T., Arima, N., Shindo, M., Sugama, K. & Sasaguri, Y. Suppression of adjuvant arthritis of rats by a novel matrix metalloproteinase-inhibitor. *Br. J. Pharmacol.* **131**, 1513–1520 (2000).
41. Gatto, C. *et al.* BAY 12–9566, a novel inhibitor of matrix metalloproteinases with antiangiogenic activity. *Clin. Cancer Res.* **5**, 3603–3607 (1999).
42. Levin, J. I. *et al.* The asymmetric synthesis and *in vitro* characterization of succinyl mercaptoalcohol and mercaptoketone inhibitors of matrix metalloproteinases. *Bioorg. Med. Chem. Lett.* **8**, 1163–1168 (1998).
43. Wang, X., Choe, Y., Craik, C. S. & Ellman, J. A. Design and synthesis of novel inhibitors of gelatinase B. *Bioorg. Med. Chem. Lett.* **12**, 2201–2204 (2002).
44. Brown, S. *et al.* Potent and selective mechanism-based inhibition of gelatinases. *J. Am. Chem. Soc.* **122**, 6799–6800 (2000).
45. Kleifeld, O. *et al.* X-ray absorption studies of human matrix metalloproteinase-2 (MMP-2) bound to a highly selective mechanism-based inhibitor. Comparison with the latent and active forms of the enzyme. *J. Biol. Chem.* **276**, 17125–17131 (2001).
46. Ikejiri, M. *et al.* Design, synthesis, and evaluation of a mechanism-based inhibitor for gelatinase A. *J. Org. Chem.* **70**, 5709–5712 (2005).
47. Ferry, G., Boutin, J. A., Atassi, G., Fauchere, J. L. & Tucker, G. C. Selection of a histidine-containing inhibitor of gelatinases through deconvolution of combinatorial tetrapeptide libraries. *Mol. Divers.* **2**, 135–146 (1997).
48. Koivunen, E. *et al.* Tumor targeting with a selective gelatinase inhibitor. *Nature Biotech.* **17**, 768–774 (1999).
49. Acharya, M. R., Venitz, J., Figg, W. D. & Sparreboom, A. Chemically modified tetracyclines as inhibitors of matrix metalloproteinases. *Drug Resist. Updat.* **7**, 195–208 (2004).
50. Paemen, L. *et al.* The gelatinase inhibitory activity of tetracyclines and chemically modified tetracycline analogues as measured by a novel microtiter assay for inhibitors. *Biochem. Pharmacol.* **52**, 105–111 (1996).
The first study on the relation of structure and function of tetracyclines and CMTs as MMPIs.
51. Yrjanheikki, J. *et al.* A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. *Proc. Natl Acad. Sci. USA* **96**, 13496–13500 (1999).
52. Yong, V. W., Power, C., Forsyth, P. & Edwards, D. R. Metalloproteinases in biology and pathology of the nervous system. *Nature Rev. Neurosci.* **2**, 502–511 (2001).
53. Cohen, J. The immunopathogenesis of sepsis. *Nature* **420**, 885–891 (2002).
54. Hotchkiss, R. S. & Karl, I. E. The pathophysiology and treatment of sepsis. *N. Engl. J. Med.* **348**, 138–150 (2003).
55. Beutler, B. Toll-like receptors: how they work and what they do. *Curr. Opin. Hematol.* **9**, 2–10 (2002).
56. Dubois, B. *et al.* Gelatinase B deficiency protects against endotoxin shock. *Eur. J. Immunol.* **32**, 2163–2171 (2002).
57. Nakamura, A. *et al.* Increased susceptibility to LPS-induced endotoxin shock in secretory leukoprotease inhibitor (SLPI)-deficient mice. *J. Exp. Med.* **197**, 669–674 (2003).
58. Riedemann, N. C. *et al.* Regulation by C5a of neutrophil activation during sepsis. *Immunity* **19**, 193–202 (2003).
59. Abraham, E. *et al.* Urokinase-type plasminogen activator potentiates lipopolysaccharide-induced neutrophil activation. *J. Immunol.* **170**, 5644–5651 (2003).
60. Hu, J., Van den Steen, P. E., Dillen, C. & Opdenakker, G. Targeting neutrophil collagenase/matrix metalloproteinase-8 and gelatinase B/matrix metalloproteinase-9 with a peptidomimetic inhibitor protects against endotoxin shock. *Biochem. Pharmacol.* **70**, 535–544 (2005).

61. Hu, J. *et al.* Inhibition of lethal endotoxin shock with an L-pyridylalanine containing metalloproteinase inhibitor selected by high-throughput screening of a new peptide library. *Comb. Chem. High Throughput Screen.* **9**, 599–611 (2006).
62. Opdenakker, G., Nelissen, I. & Van Damme, J. Functional roles and therapeutic targeting of gelatinase B and chemokines in multiple sclerosis. *Lancet Neurol.* **2**, 747–756 (2003).
63. Gijbels, K. *et al.* Gelatinase B is present in the cerebrospinal fluid during experimental autoimmune encephalomyelitis and cleaves myelin basic protein. *J. Neurosci. Res.* **36**, 432–440 (1993).
64. Dubois, B. *et al.* Resistance of young gelatinase B-deficient mice to experimental autoimmune encephalomyelitis and necrotizing tail lesions. *J. Clin. Invest.* **104**, 1507–1515 (1999).
65. Agrawal, S. *et al.* Dystroglycan is selectively cleaved at the parenchymal basement membrane at sites of leukocyte extravasation in experimental autoimmune encephalomyelitis. *J. Exp. Med.* **203**, 1007–1019 (2006).
Together with references 64, 165 and 212, gives prototypic examples of knockout studies that demonstrate the role of MMPs in autoimmune diseases.
66. Paemen, L., Olsson, T., Söderström, M., Van Damme, J. & Opdenakker, G. Evaluation of gelatinases and IL-6 in the cerebrospinal fluid of patients with optic neuritis, multiple sclerosis and other inflammatory neurological diseases. *Eur. J. Neurol.* **1**, 55–63 (1994).
67. Nelissen, I. *et al.* Gelatinase B/matrix metalloproteinase-9 cleaves interferon-β and is a target for immunotherapy. *Brain* **126**, 1371–1381 (2003).
68. Gijbels, K., Galardy, R. E., Steinman, L. Reversal of experimental autoimmune encephalomyelitis with a hydroxamate inhibitor of matrix metalloproteases. *J. Clin. Invest.* **94**, 2177–2182 (1994).
Together with reference 101, illustrates the potential of MMPIs in autoimmune and cardiovascular diseases, respectively.
69. Hewson, A. K., Smith, T., Leonard, J. P. & Cuzner, M. L. Suppression of experimental allergic encephalomyelitis in the Lewis rat by the matrix metalloproteinase inhibitor Ro31-9790. *Inflamm. Res.* **44**, 345–349 (1995).
70. Norga, K. *et al.* Prevention of acute autoimmune encephalomyelitis and abrogation of relapses in murine models of multiple sclerosis by the protease inhibitor D-penicillamine. *Inflamm. Res.* **44**, 529–534 (1995).
71. Dubois, B. *et al.* Toxicity in a double-blind, placebo-controlled pilot trial with D-penicillamine and metacycline in secondary progressive multiple sclerosis. *Mult. Scler.* **4**, 74–78 (1998).
72. Brundula, V., Rewcastle, N. B., Metz, L. M., Bernard, C. C. & Yong, V. W. Targeting leukocyte MMPs and transmigration: minocycline as a potential therapy for multiple sclerosis. *Brain* **125**, 1297–1308 (2002).
73. Popovic, N. *et al.* Inhibition of autoimmune encephalomyelitis by a tetracycline. *Ann. Neurol.* **51**, 215–223 (2002).
74. Weaver, A. *et al.* An elevated matrix metalloproteinase (MMP) in an animal model of multiple sclerosis is protective by affecting Th1/Th2 polarization. *FASEB J.* **19**, 1668–1670 (2005).
75. Larsen, P. H., Wells, J. E., Stallcup, W. B., Opdenakker, G. & Yong, V. W. Matrix metalloproteinase-9 facilitates remyelination in part by processing the inhibitory NG2 proteoglycan. *J. Neurosci.* **23**, 11127–11135 (2003).
76. Van den Steen, P. E. *et al.* Cleavage of denatured natural collagen type II by neutrophil gelatinase B reveals enzyme specificity, post-translational modifications in the substrate, and the formation of remnant epitopes in rheumatoid arthritis. *FASEB J.* **16**, 379–389 (2002).
77. Van den Steen, P. E. *et al.* Generation of glycosylated remnant epitopes from human collagen type II by gelatinase B. *Biochemistry* **43**, 10809–10816 (2004).
78. Conway, J. G. *et al.* Inhibition of cartilage and bone destruction in adjuvant arthritis in the rat by a matrix metalloproteinase inhibitor. *J. Exp. Med.* **182**, 449–457 (1995).
79. DiMartino, M. *et al.* Anti-arthritic activity of hydroxamic acid-based pseudopeptide inhibitors of matrix metalloproteinases and TNF α processing. *Inflamm. Res.* **46**, 211–215 (1997).
80. DiMartino, M. J., High, W., Galloway, W. A. & Crimmin, M. J. Preclinical antiarthritic activity of matrix metalloproteinase inhibitors. *Ann. N. Y. Acad. Sci.* **732**, 411–413 (1994).
81. Zhang, Y. *et al.* Identification and characterization of 4-[[4-(2-butynyloxy)phenyl]sulfonyl]-*N*-hydroxy-2,2-dimethyl-(3S)thiomorpho linecarboxamide (TMI-1), a novel dual tumor necrosis factor-α-converting enzyme/matrix metalloprotease inhibitor for the treatment of rheumatoid arthritis. *J. Pharmacol. Exp. Ther.* **309**, 348–355 (2004).
82. Conway, J. G. *et al.* Inhibition of tumor necrosis factor-α (TNF-α) production and arthritis in the rat by GW3333, a dual inhibitor of TNF-α-converting enzyme and matrix metalloproteinases. *J. Pharmacol. Exp. Ther.* **298**, 900–908 (2001).
83. Zernicke, R. F. *et al.* Administration of systemic matrix metalloproteinase inhibitors maintains bone mechanical integrity in adjuvant arthritis. *J. Rheumatol.* **24**, 1324–1331 (1997).
84. Hansson, G. K. & Libby, P. The immune response in atherosclerosis: a double-edged sword. *Nature Rev. Immunol.* **6**, 508–519 (2006).
A comprehensive review on atherosclerosis as an inflammatory disease.
85. Janssens, S. & Lijnen, H. R. What has been learned about the cardiovascular effects of matrix metalloproteinases from mouse models? *Cardiovasc. Res.* **69**, 585–594 (2006).
86. Tayebjee, M. H., Lip, G. Y. & MacFadyen, R. J. Matrix metalloproteinases in coronary artery disease: clinical and therapeutic implications and pathological significance. *Curr. Med. Chem.* **12**, 917–925 (2005).
87. Pawlak, R. *et al.* Tissue plasminogen activator and plasminogen mediate stress-induced decline of neuronal and cognitive functions in the mouse hippocampus. *Proc. Natl Acad. Sci. USA* **102**, 18201–18206 (2005).
88. Lebeurrier, N. *et al.* The brain-specific tissue-type plasminogen activator inhibitor, neuroserpin, protects neurons against excitotoxicity both *in vitro* and *in vivo*. *Mol. Cell. Neurosci.* **30**, 552–558 (2005).
89. Yang, Y., Estrada, E. Y., Thompson, J. F., Liu, W. & Rosenberg, G. A. Matrix metalloproteinase-mediated disruption of tight junction proteins in cerebral vessels is reversed by synthetic matrix metalloproteinase inhibitor in focal ischemia in rat. *J. Cereb. Blood Flow Metab.* **27**, 697–709 (2007).
90. Jian, L. K. & Rosenberg, G. A. Matrix metalloproteinases and free radicals in cerebral ischemia. *Free Radic. Biol. Med.* **39**, 71–80 (2005).
91. Zhao, B. Q. *et al.* Role of matrix metalloproteinases in delayed cortical responses after stroke. *Nature Med.* **12**, 441–445 (2006).
92. Galis, Z. S. *et al.* Targeted disruption of the matrix metalloproteinase-9 gene impairs smooth muscle cell migration and geometrical arterial remodeling. *Circ. Res.* **91**, 852–859 (2002).
93. Cho, A. and Reidy, M. A. Matrix metalloproteinase-9 is necessary for the regulation of smooth muscle cell replication and migration after arterial injury. *Circ. Res.* **91**, 845–851 (2002).
94. Lijnen, H. R., Soloway, P. & Collen, D. Tissue inhibitor of matrix metalloproteinases-1 impairs arterial neointima formation after vascular injury in mice. *Circ. Res.* **85**, 1186–1191 (1999).
95. Sluijter, J. P., de Kleijn, D. P. & Pasterkamp, G. Vascular remodeling and protease inhibition — bench to bedside. *Cardiovasc. Res.* **69**, 595–603 (2006).
96. Sousa, J. E., Serruys, P. W. & Costa, M. A. New frontiers in cardiology: drug-eluting stents: Part II. *Circulation* **107**, 2383–2389 (2003).
97. Lijnen, H. R. *et al.* Accelerated neointima formation after vascular injury in mice with stromelysin-3 (*MMP-11*) gene inactivation. *Arterioscler. Thromb. Vasc. Biol.* **19**, 2863–2870 (1999).
98. Silence, J., Lupu, F., Collen, D. & Lijnen, H. R. Persistence of atherosclerotic plaque but reduced aneurysm formation in mice with stromelysin-1 (*MMP-3*) gene inactivation. *Arterioscler. Thromb. Vasc. Biol.* **21**, 1440–1445 (2001).
99. Luttun, A. *et al.* Loss of matrix metalloproteinase-9 or matrix metalloproteinase-12 protects apolipoprotein E-deficient mice against atherosclerotic media destruction but differentially affects plaque growth. *Circulation* **109**, 1408–1414 (2004).
100. Heymans, S. *et al.* Inhibition of plasminogen activators or matrix metalloproteinases prevents cardiac rupture but impairs therapeutic angiogenesis and causes cardiac failure. *Nature Med.* **5**, 1135–1142 (1999).
101. Matsumura, S. *et al.* Targeted deletion or pharmacological inhibition of MMP-2 prevents cardiac rupture after myocardial infarction in mice. *J. Clin. Invest.* **115**, 599–609 (2005).
102. Golub, L. M. *et al.* Minocycline reduces gingival collagenolytic activity during diabetes. Preliminary observations and a proposed new mechanism of action. *J. Periodontal Res.* **18**, 516–526 (1983).
A pioneering study on the anti-inflammatory effects of tetracyclines, further illustrated in references 103–107.
103. Sorsa, T., Tjaderhane, L. & Salo, T. Matrix metalloproteinases (MMPs) in oral diseases. *Oral Dis.* **10**, 311–318 (2004).
104. Sorsa, T. *et al.* Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. *Ann. Med.* **38**, 306–321 (2006).
105. Emingil, G. *et al.* The effect of adjunctive low-dose doxycycline therapy on clinical parameters and gingival crevicular fluid matrix metalloproteinase-8 levels in chronic periodontitis. *J. Periodontol.* **75**, 106–115 (2004).
106. Llavaneras, A. *et al.* A combination of a chemically modified doxycycline and a bisphosphonate synergistically inhibits endotoxin-induced periodontal breakdown in rats. *J. Periodontol.* **72**, 1069–1077 (2001).
107. Ramamurthy, N. S. *et al.* Inhibition of alveolar bone loss by matrix metalloproteinase inhibitors in experimental periodontal disease. *J. Periodontal. Res.* **37**, 1–7 (2002).
108. Hautamaki, R. D., Kobayashi, D. K., Senior, R. M. & Shapiro, S. D. Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice. *Science* **277**, 2002–2004 (1997).
109. Houghton, A. M. *et al.* Elastin fragments drive disease progression in a murine model of emphysema. *J. Clin. Invest.* **116**, 753–759 (2006).
110. Churg, A. *et al.* Macrophage metalloelastase mediates acute cigarette smoke-induced inflammation via tumor necrosis factor-α release. *Am. J. Respir. Crit. Care Med.* **167**, 1083–1089 (2003).
111. Liu, Z. *et al.* The serpin α1-proteinase inhibitor is a critical substrate for gelatinase B/MMP-9 *in vivo*. *Cell* **102**, 647–655 (2000).
112. Selman, M. *et al.* Matrix metalloproteinases inhibition attenuates tobacco smoke-induced emphysema in guinea pigs. *Chest* **123**, 1633–1641 (2003).
113. Gueders, M. M. *et al.* Matrix metalloproteinase-8 deficiency promotes granulocytic allergen-induced airway inflammation. *J. Immunol.* **175**, 2589–2597 (2005).
114. Corry, D. B. *et al.* Decreased allergic lung inflammatory cell egression and increased susceptibility to asphyxiation in MMP2-deficiency. *Nature Immunol.* **3**, 347–353 (2002).
115. Van Lint, P. *et al.* Resistance of collagenase-2 (matrix metalloproteinase-8)-deficient mice to TNF-induced lethal hepatitis. *J. Immunol.* **175**, 7642–7649 (2005).
116. Cataldo, D. D. *et al.* Matrix metalloproteinase-9 deficiency impairs cellular infiltration and bronchial hyperresponsiveness during allergen-induced airway inflammation. *Am. J. Pathol.* **161**, 491–498 (2002).
117. Vermaelen, K. Y. *et al.* Matrix metalloproteinase-9-mediated dendritic cell recruitment into the airways is a critical step in a mouse model of asthma. *J. Immunol.* **171**, 1016–1022 (2003).
118. Corry, D. B. *et al.* Overlapping and independent contributions of MMP2 and MMP9 to lung allergic inflammatory cell egression through decreased CC chemokines. *FASEB J.* **18**, 995–997 (2004).
119. Kumagai, K. *et al.* The involvement of matrix metalloproteinases in basement membrane injury in a murine model of acute allergic airway inflammation. *Clin. Exp. Allergy.* **32**, 1527–1534 (2002).
120. Kumagai, K. *et al.* Inhibition of matrix metalloproteinases prevents allergen-induced airway inflammation in a murine model of asthma. *J. Immunol.* **162**, 4212–4219 (1999).
121. Lee, K. S., Jin, S. M., Kim, S. S. & Lee, Y. C. Doxycycline reduces airway inflammation and hyperresponsiveness in a murine model of toluene diisocyanate-induced asthma. *J. Allergy Clin. Immunol.* **113**, 902–909 (2004).
122. Lee, Y. C. *et al.* A murine model of toluene diisocyanate-induced asthma can be treated with matrix metalloproteinase inhibitor. *J. Allergy Clin. Immunol.* **108**, 1021–1026 (2001).

123. Corbel, M. *et al.* Inhibition of bleomycin-induced pulmonary fibrosis in mice by the matrix metalloproteinase inhibitor batimastat. *J. Pathol.* **193**, 538–545 (2001).
124. Tan, R. J. *et al.* Matrix metalloproteinases promote inflammation and fibrosis in asbestos-induced lung injury in mice. *Am. J. Respir. Cell Mol. Biol.* **35**, 289–297 (2006).
125. Zuo, F. *et al.* Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. *Proc. Natl Acad. Sci. USA* **99**, 6292–6297 (2002).
126. Manoury, B. *et al.* Macrophage metalloelastase (MMP-12) deficiency does not alter bleomycin-induced pulmonary fibrosis in mice. *J. Inflamm. (Lond)* **3**, 2 (2006).
127. Li, Q., Park, P. W., Wilson, C. L. & Parks, W. C. Matrilysin shedding of syndecan-1 regulates chemokine mobilization and transepithelial efflux of neutrophils in acute lung injury. *Cell.* **111**, 635–646 (2002).
128. Greenlee, K. J. *et al.* Proteomic identification of *in vivo* substrates for matrix metalloproteinases 2 and 9 reveals a mechanism for resolution of inflammation. *J. Immunol.* **177**, 7312–7321 (2006).
129. Wielockx, B. *et al.* Inhibition of matrix metalloproteinases blocks lethal hepatitis and apoptosis induced by tumor necrosis factor and allows safe antitumor therapy. *Nature Med.* **7**, 1202–1208 (2001).
130. Sitia, G. *et al.* MMPs are required for recruitment of antigen-nonspecific mononuclear cells into the liver by CTLs. *J. Clin. Invest.* **113**, 1158–1167 (2004).
131. Muller-Pillasch, F. *et al.* The influence of transforming growth factor β1 on the expression of genes coding for matrix metalloproteinases and tissue inhibitors of metalloproteinases during regeneration from cerulein-induced pancreatitis. *Pancreas* **15**, 168–175 (1997).
132. Descamps, F. J., Martens, E., Ballaux, F., Geboes, K. & Opdenakker, G. *In vivo* activation of gelatinase B/MMP-9 by trypsin in acute pancreatitis is a permissive factor in streptozotocin-induced diabetes. *J. Pathol.* **204**, 555–561 (2004).
133. Descamps, F. J. *et al.* Gelatinase B is diabetogenic in acute and chronic pancreatitis by cleaving insulin. *FASEB J.* **17**, 887–889 (2003).
134. Leppert, D. *et al.* Matrix metalloproteinase (MMP)-8 and MMP-9 in cerebrospinal fluid during bacterial meningitis: association with blood–brain barrier damage and neurological sequelae. *Clin. Infect. Dis.* **31**, 80–84 (2000).
135. Leib, S. L. *et al.* Inhibition of matrix metalloproteinases and tumour necrosis factor α converting enzyme as adjuvant therapy in pneumococcal meningitis. *Brain* **124**, 1734–1742 (2001).
136. Bottcher, T., Spreer, A., Azeh, I., Nau, R. & Gerber, J. Matrix metalloproteinase-9 deficiency impairs host defense mechanisms against *Streptococcus pneumoniae* in a mouse model of bacterial meningitis. *Neurosci. Lett.* **338**, 201–204 (2003).
137. Yong, V. W. Metalloproteinases: mediators of pathology and regeneration in the CNS. *Nature Rev. Neurosci.* **6**, 931–944 (2005).
Together with reference 19, gives a critical review of the protective and detrimental roles played by metalloproteinases, as deduced from knockout studies.
138. Porter, S., Clark, I. M., Kevorkian, L. & Edwards, D. R. The ADAMTS metalloproteinases. *Biochem. J.* **386**, 15–27 (2005).
139. Opdenakker, G., Van den Steen, P. E., Laureys, G., Hunninck, K. & Arnold, B. Neutralizing antibodies in gene-defective hosts. *Trends Immunol.* **24**, 94–100 (2003).
140. Grams, F. *et al.* X-ray structures of human neutrophil collagenase complexed with peptide hydroxamate and peptide thiol inhibitors. Implications for substrate binding and rational drug design. *Eur. J. Biochem.* **228**, 830–841 (1995).
Together with references 32–34 and 142, gives exemplary primary and review literature on the crystallography of MMPs and MMPIs.
141. Castelhano, A. L. *et al.* Novel indolactam-based inhibitors of matrix metalloproteinases. *Bioorg. Med. Chem. Lett.* **5**, 1415–1420 (2006).
142. Lovejoy, B. *et al.* Structure of the catalytic domain of fibroblast collagenase complexed with an inhibitor. *Science* **263**, 375–377 (1994).
143. Brown, P. D. Matrix metalloproteinase inhibitors in the treatment of cancer. *Med. Oncol.* **14**, 1–10 (1997).
144. Wojtowicz-Praga, S. *et al.* Phase I trial of Marimastat, a novel matrix metalloproteinase inhibitor, administered orally to patients with advanced lung cancer. *J. Clin. Oncol.* **16**, 2150–2156 (1998).
145. Campbell, D. A. *et al.* Malonyl α-mercaptoketones and α-mercaptoalcohols, a new class of matrix metalloproteinase inhibitors. *Bioorg. Med. Chem. Lett.* **8**, 1157–1162 (1998).
146. Fernandez, F. G. *et al.* Inhibition of obliterative airway disease development in murine tracheal allografts by matrix metalloproteinase-9 deficiency. *Am. J. Transplant.* **5**, 671–683 (2005).
147. Warner, R. L. *et al.* Role of stromelysin 1 and gelatinase B in experimental acute lung injury. *Am. J. Respir. Cell Mol. Biol.* **24**, 537–544 (2001).
148. McGuire, J. K., Li, Q., Parks, W. C. Matrilysin (matrix metalloproteinase-7) mediates E-cadherin ectodomain shedding in injured lung epithelium. *Am. J. Pathol.* **162**, 1831–1843 (2003).
149. Dunsmore, S. E. *et al.* Matrilysin expression and function in airway epithelium. *J. Clin. Invest.* **102**, 1321–1331 (1998).
150. Lanone, S. *et al.* Overlapping and enzyme-specific contributions of matrix metalloproteinases-9 and-12 in IL-13-induced inflammation and remodeling. *J. Clin. Invest.* **110**, 463–474 (2002).
151. Betsuyaku, T., Fukuda, Y., Parks, W. C., Shipley, J. M. & Senior, R. M. Gelatinase B is required for alveolar bronchiolization after intratracheal bleomycin. *Am. J. Pathol.* **157**, 525–535 (2000).
152. McMillan, S. J. *et al.* Matrix metalloproteinase-9 deficiency results in enhanced allergen-induced airway inflammation. *J. Immunol.* **172**, 2586–2594 (2004).
153. Warner, R. L. *et al.* The role of metalloelastase in immune complex-induced acute lung injury. *Am. J. Pathol.* **158**, 2139–2144 (2001).
154. Irie, K. *et al.* Impaired alveolization in mice deficient in membrane-type matrix metalloproteinase 1 (MT1-MMP). *Med. Mol. Morphol.* **38**, 43–46 (2005).
155. Atkinson, J. J. *et al.* Membrane-type 1 matrix metalloproteinase is required for normal alveolar development. *Dev. Dyn.* **232**, 1079–1090 (2005).
156. Garg, P. *et al.* Selective ablation of matrix metalloproteinase-2 exacerbates experimental colitis: contrasting role of gelatinases in the pathogenesis of colitis. *J. Immunol.* **177**, 4103–4112 (2006).
157. Wilson, C. L. *et al.* Regulation of intestinal α-defensin activation by the metalloproteinase matrilysin in innate host defense. *Science* **286**, 113–117 (1999).
Together with references 13, 127, 148, 172, 209 and 211, gives an excellent illustration of how *Mmp*-knockout mice can be used to define novel substrates. Such substrate conversion might be used in the future as a readout for testing the specificity of MMPIs *in vivo*.
158. Kolaczkowska, E. *et al.* Enhanced early vascular permeability in gelatinase B (MMP-9)-deficient mice: putative contribution of COX-1-derived PGE2 of macrophage origin. *J. Leukoc. Biol.* **80**, 125–132 (2006).
159. Kolaczkowska, E. *et al.* Gelatinase B/matrix metalloproteinase-9 contributes to cellular infiltration in a murine model of zymosan peritonitis. *Immunobiology* **211**, 137–148 (2006).
160. Renckens, R. *et al.* Matrix metalloproteinase-9 deficiency impairs host defense against abdominal sepsis. *J. Immunol.* **176**, 3735–3741 (2006).
161. Castaneda, F. E. *et al.* Targeted deletion of metalloproteinase 9 attenuates experimental colitis in mice: central role of epithelial-derived MMP. *Gastroenterology* **129**, 1991–2008 (2005).
162. Uchinami, H., Seki, E., Brenner, D. A. & D'Armiento, J. Loss of MMP 13 attenuates murine hepatic injury and fibrosis during cholestasis. *Hepatology* **44**, 420–429 (2006).
163. Caterina, J. J. *et al.* Enamelysin (matrix metalloproteinase 20)-deficient mice display an amelogenesis imperfecta phenotype. *J. Biol. Chem.* **277**, 49598–49604 (2002).
164. Beniash, E., Skobe, Z. & Bartlett, J. D. Formation of the dentino-enamel interface in enamelysin (MMP-20)-deficient mouse incisors. *Eur. J. Oral Sci.* **114** (Suppl 1), 24–29 (2006).
165. Itoh, T. *et al.* The role of matrix metalloproteinase-2 and matrix metalloproteinase-9 in antibody-induced arthritis. *J. Immunol.* **169**, 2643–2647 (2002).
166. Mudgett, J. S. *et al.* Susceptibility of stromelysin1-deficient mice to collagen-induced arthritis and cartilage destruction. *Arthritis Rheum.* **41**, 110–121 (1998).
167. Calander, A. M. *et al.* Matrix metalloproteinase-9 (gelatinase B) deficiency leads to increased severity of *Staphylococcus aureus*-triggered septic arthritis. *Microbes Infect.* **8**, 1434–1439 (2006).
168. Vu, T. H. *et al.* MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. *Cell* **93**, 411–422 (1998).
Together with the reports on *Mmp3* and *Mmp7* knockouts, a pioneering study showing the spontaneous phenotypic characteristics of *Mmp* knockouts.
169. Ortega, N., Behonick, D. J., Colnot, C., Cooper, D. N. & Werb, Z. Galectin-3 is a downstream regulator of matrix metalloproteinase-9 function during endochondral bone formation. *Mol. Biol. Cell* **16**, 3028–3039 (2005).
170. Wright, R. W. *et al.* Medial collateral ligament healing in macrophage metalloelastase (MMP-12)-deficient mice. *J. Orthop. Res.* **24**, 2106–2113 (2006).
171. Stickens, D. *et al.* Altered endochondral bone development in matrix metalloproteinase 13-deficient mice. *Development* **131**, 5883–5895 (2004).
172. Holmbeck, K. *et al.* The metalloproteinase MT1-MMP is required for normal development and maintenance of osteocyte processes in bone. *J. Cell Sci.* **118**, 147–156 (2005).
173. Hsu, J. Y. *et al.* Matrix metalloproteinase-2 facilitates wound healing events that promote functional recovery after spinal cord injury. *J. Neurosci.* **26**, 9841–9850 (2006).
174. Tang, J. *et al.* MMP-9 deficiency enhances collagenase-induced intracerebral hemorrhage and brain injury in mutant mice. *J. Cereb. Blood Flow Metab.* **24**, 1133–1145 (2004).
175. Larsen, P. H., DaSilva, A. G., Conant, K. & Yong, V. W. Myelin formation during development of the CNS is delayed in matrix metalloproteinase-9 and-12 null mice. *J. Neurosci.* **26**, 2207–2214 (2006).
176. Asahi, M. *et al.* Role for matrix metalloproteinase 9 after focal cerebral ischemia: effects of gene knockout and enzyme inhibition with BB-94. *J. Cereb. Blood Flow Metab.* **20**, 1681–1689 (2000).
177. Dewil, M. *et al.* Role of matrix metalloproteinase-9 in a mouse model for amyotrophic lateral sclerosis. *Neuroreport* **16**, 321–324 (2005).
178. Wells, J. E. *et al.* An adverse role for matrix metalloproteinase 12 after spinal cord injury in mice. *J. Neurosci.* **23**, 10107–10115 (2003).
179. Larsen, P. H. & Yong, V. W. The expression of matrix metalloproteinase-12 by oligodendrocytes regulates their maturation and morphological differentiation. *J. Neurosci.* **24**, 7597–7603 (2004).
180. Komori, K. *et al.* Absence of mechanical allodynia and Aβ-fiber sprouting after sciatic nerve injury in mice lacking membrane-type 5 matrix metalloproteinase. *FEBS Lett.* **557**, 125–128 (2004).
181. Campbell, L. G. *et al.* Different roles for matrix metalloproteinase-2 and matrix metalloproteinase-9 in the pathogenesis of cardiac allograft rejection. *Am. J. Transplant.* **5**, 517–528 (2005).
182. Samolov, B. *et al.* Delayed inflammation-associated corneal neovascularization in MMP-2-deficient mice. *Exp. Eye Res.* **80**, 159–166 (2005).
183. Ohno-Matsui, K. *et al.* Reduced retinal angiogenesis in MMP-2-deficient mice. *Invest. Ophthalmol. Vis. Sci.* **44**, 5370–5375 (2003).
184. Berglin, L. *et al.* Reduced choroidal neovascular membrane formation in matrix metalloproteinase-2-deficient mice. *Invest. Ophthalmol. Vis. Sci.* **44**, 403–408 (2003).
185. Basalyga, D. M. *et al.* Elastin degradation and calcification in an abdominal aorta injury model: role of matrix metalloproteinases. *Circulation* **110**, 3480–3487 (2004).
186. Lambert, V. *et al.* MMP-2 and MMP-9 synergize in promoting choroidal neovascularization. *FASEB J.* **17**, 2290–2292 (2003).
187. Longo, G. M. *et al.* Matrix metalloproteinases 2 and 9 work in concert to produce aortic aneurysms. *J. Clin. Invest.* **110**, 625–632 (2002).
188. Ikonomidis, J. S. *et al.* Effects of deletion of the matrix metalloproteinase 9 gene on development of murine thoracic aortic aneurysms. *Circulation* **112**, I242–I248 (2005).
189. Sarman, S., van der Ploeg, I., Seregard, S. & Kvanta, A. Retinal vascular development and pathologic retinal angiogenesis are not impaired in matrix metalloproteinase-2 deficient mice. *Curr. Eye Res.* **30**, 259–267 (2005).

190. Kuzuya, M. *et al*. Effect of MMP-2 deficiency on atherosclerotic lesion formation in Apoe-deficient mice. *Arterioscler. Thromb. Vasc. Biol*. **26**, 1120–1125 (2006).
191. Mukherjee, R. *et al*. Time-dependent changes in myocardial structure following discrete injury in mice deficient of matrix metalloproteinase-3. *J. Mol. Cell. Cardiol*. **39**, 259–268 (2005).
192. Kure, T. *et al*. Corneal neovascularization after excimer keratectomy wounds in matrilysin-deficient mice. *Invest. Ophthalmol. Vis. Sci*. **44**, 137–144 (2003).
193. Pyo, R. *et al*. Targeted gene disruption of matrix metalloproteinase-9 (gelatinase B) suppresses development of experimental abdominal aortic aneurysms. *J. Clin. Invest*. **105**, 1641–1649 (2000).
194. Heissig, B. *et al*. Low-dose irradiation promotes tissue revascularization through VEGF release from mast cells and MMP-9-mediated progenitor cell mobilization. *J. Exp. Med*. **202**, 739–750 (2005).
195. Ovechkin, A. V. *et al*. Role of matrix metalloproteinase-9 in endothelial apoptosis in chronic heart failure in mice. *J. Appl. Physiol*. **99**, 2398–2405 (2005).
196. Heymans, S. *et al*. Loss or inhibition of uPA or MMP-9 attenuates LV remodeling and dysfunction after acute pressure overload in mice. *Am. J. Pathol*. **166**, 15–25 (2005).
197. Lindsey, M. L. *et al*. Matrix metalloproteinase-9 gene deletion facilitates angiogenesis after myocardial infarction. *Am. J. Physiol. Heart Circ. Physiol*. **290**, H232–H239 (2006).
198. Johnson, C., Sung, H. J., Lessner, S. M., Fini, M. E. & Galis, Z. S. Matrix metalloproteinase-9 is required for adequate angiogenic revascularization of ischemic tissues: potential role in capillary branching. *Circ. Res*. **94**, 262–268 (2004).
199. Jost, M. *et al*. Earlier onset of tumoral angiogenesis in matrix metalloproteinase-19-deficient mice. *Cancer Res*. **66**, 5234–5241 (2006).
200. Sung, H. J. *et al*. Matrix metalloproteinase 9 facilitates collagen remodeling and angiogenesis for vascular constructs. *Tissue Eng*. **11**, 267–276 (2005).
201. Choi, E. T. *et al*. Matrix metalloproteinase-9 modulation by resident arterial cells is responsible for injury-induced accelerated atherosclerotic plaque development in apolipoprotein E-deficient mice. *Arterioscler. Thromb. Vasc. Biol*. **25**, 1020–1025 (2005).
202. Longo, G. M. *et al*. MMP-12 has a role in abdominal aortic aneurysms in mice. *Surgery* **137**, 457–462 (2005).
203. Wang, M. *et al*. Matrix metalloproteinase deficiencies affect contact hypersensitivity: stromelysin-1 deficiency prevents the response and gelatinase B deficiency prolongs the response. *Proc. Natl Acad. Sci. USA* **96**, 6885–6889 (1999).
204. Li, C. K. *et al*. Impaired immunity to intestinal bacterial infection in stromelysin-1 (matrix metalloproteinase-3)-deficient mice. *J. Immunol*. **173**, 5171–5179 (2004).
205. Powell, W. C., Fingleton, B., Wilson, C. L., Boothby, M. & Matrisian, L. M. The metalloproteinase matrilysin proteolytically generates active soluble Fas ligand and potentiates epithelial cell apoptosis. *Curr. Biol*. **9**, 1441–1447 (1999).
206. Haro, H. *et al*. Matrix metalloproteinase-3-dependent generation of a macrophage chemoattractant in a model of herniated disc resorption. *J. Clin. Invest*. **105**, 133–141 (2000).
207. Haro, H. *et al*. Matrix metalloproteinase-7-dependent release of tumor necrosis factor-α in a model. *J. Clin. Invest*. **150**, 143–150 (2000).
208. Balbin, M. *et al*. Loss of collagenase-2 confers increased skin tumor susceptibility to male mice. *Nature Genet*. **35**, 252–257 (2003).
209. Senft, A. P., Korfhagen, T. R., Whitsett, J. A., Shapiro, S. D. & LeVine, A. M. Surfactant protein-D regulates soluble CD14 through matrix metalloproteinase-12. *J. Immunol*. **174**, 4953–4959 (2005).
210. Pflugfelder, S. C. *et al*. Matrix metalloproteinase-9 knockout confers resistance to corneal epithelial barrier disruption in experimental dry eye. *Am. J. Pathol*. **166**, 61–71 (2005).
211. Robinson, S. N., Seina, S. M., Gohr, J. C. & Sharp, J. G. Hematopoietic progenitor cell mobilization by granulocyte colony-stimulating factor and erythropoietin in the absence of matrix metalloproteinase-9. *Stem Cells Dev*. **14**, 317–328 (2005).
212. Liu, Z. *et al*. Gelatinase B-deficient mice are resistant to experimental bullous pemphigoid. *J. Exp. Med*. **188**, 475–482 (1998).
213. Ratzinger, G. *et al*. Matrix metalloproteinases 9 and 2 are necessary for the migration of Langerhans cells and dermal dendritic cells from human and murine skin. *J. Immunol*. **168**, 4361–4371 (2002).
214. Shipley, J. M., Wesselschmidt, R. L., Kobayashi, D. K., Ley, T. J. & Shapiro, S. D. Metalloelastase is required for macrophage-mediated proteolysis and matrix invasion in mice. *Proc. Natl Acad. Sci. USA* **93**, 3942–3946 (1996).
215. Koistinaho, M. *et al*. Minocycline protects against permanent cerebral ischemia in wild type but not in matrix metalloprotease-9-deficient mice. *J. Cereb. Blood Flow Metab*. **25**, 460–467 (2005).
216. Jodele, S. *et al*. The contribution of bone marrow-derived cells to the tumor vasculature in neuroblastoma is matrix metalloproteinase-9 dependent. *Cancer Res*. **65**, 3200–3208 (2005).
217. Topp, S. A., Upadhya, G. A. & Strasberg, S. M. Cold preservation of isolated sinusoidal endothelial cells in *MMP 9* knockout mice: effect on morphology and platelet adhesion. *Liver Transpl*. **10**, 1041–1048 (2004).
218. Andarawewa, K. L. *et al*. Stromelysin-3 is a potent negative regulator of adipogenesis participating to cancer cell-adipocyte interaction/crosstalk at the tumor invasive front. *Cancer Res*. **65**, 10862–10871 (2005).
219. Pendas, A. M. *et al*. Diet-induced obesity and reduced skin cancer susceptibility in matrix metalloproteinase 19-deficient mice. *Mol. Cell. Biol*. **24**, 5304–5313 (2004).
220. Kang, H. R., Cho, S. J., Lee, C. G., Homer, R. J. & Elias, J. A. Transforming growth factor (TGF)-β stimulates pulmonary fibrosis and inflammation via a Bax-dependent, bid-activated pathway that involves matrix metalloproteinase-12. *J. Biol. Chem*. **282**, 7723–7732 (2007).

Acknowledgements
This Review focuses on inflammatory and vascular diseases rather than on cancer. This implies that major cancer biology literature is not covered, for which we apologize. The present study was supported by the Fund for Scientific Research–Flanders (FWO–Vlaanderen), the Geconcerteerde OnderzoeksActies (GOA 2007-2011), the Rega Centre of Excellence (COE 05/015), the Charcot Foundation and the Belgian Foundation against Cancer. P.V.D.S. is a postdoctoral fellow of the FWO–Vlaanderen. All authors appreciate the excellent scientific help and editorial assistance of M. D. Roycik and S. Lee of Q.-X. Sang's laboratory, and V. Dubois of G.O.'s laboratory. This article is dedicated to the late Ofer Lider of the Weizmann Institute of Science, in memory of his friendship and his insights that the environmental contexts need to be better investigated and understood before any intervention whatsoever.

Competing interests statement
The authors declare no competing financial interests.

DATABASES
The following terms in this article are linked online to:
Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
MMP1 | MMP2 | MMP3 | MMP7 | MMP8 | MMP9 | MMP10 | MMP11 | MMP12 | MMP13
OMIM:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Asthma | chronic obstructive pulmonary disease | multiple sclerosis | rheumatoid arthritis | stroke

FURTHER INFORMATION
Department of Chemistry and Biochemistry, Florida State University: http://www.chem.fsu.edu
Rega Institute for Medical Research:
http://www.kuleuven.ac.be/rega

SUPPLEMENTARY INFORMATION
See online article: S1 (box) | S2 (table)
Access to this links box is available online.