Proteases

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Abstract The macrophage, as a gatekeeper to both the innate and acquired immune systems, has great potential as a therapeutic target for such diverse human disease states as bacterial and viral infection, autoimmunity, inflammatory diseases, and cancer. The phenotype of macrophages in different tissues varies markedly between tissues. While this characteristic creates technical challenges in terms of isolation and characterization of resident tissue macrophages, it opens the possibility of targeting individual tissue-specific macrophage populations for pharmacologic intervention. The proteases are among the most numerous and abundant of enzyme classes, representing 1%–4% of all proteins encoded by eukaryotic genomes. Proteases are particularly abundant in macrophages, where they are critical players in many key functions of the macro-

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phage, such as degradation of exogenous, potentially pathogenic proteins; digestion of both foreign and self proteins into peptides for presentation by MHC class I and II; and functional regulation of target proteins, for example by removal of a regulatory domain or a transmembrane anchor. This chapter reviews some of the proteases expressed in macrophages, and discusses what functional roles have been shown for, or postulated for, these enzymes. The enzymes discussed here are divided into two main groups: ectoproteases, which cleave amino acids from either end of a protein or peptide, and endoproteases, which cleave proteins at internal sites. Examples are given illustrating the actions of proteases within the macrophage, at the cell surface, and after secretion into the extracellular milieu.

Keywords Aminopeptidase, Angiotensin converting enzyme, Carboxypeptidase, Caspase, Cathepsin, CPVL, Matrix metalloprotease, TNF-? converting enzyme

1 Introduction

The role of macrophages lies at the interface of the innate and adaptive immune systems. Innate immune functions include phagocytosis of both unopsonized and opsonized pathogens, release of toxic free radicals, and secretion of inflammatory mediators such as cytokines, chemokines, and a large variety of other extracellular signaling molecules. Within the adaptive immune response, macrophages process and present antigen to T cells, and are capable of providing both immunogenic and tolerogenic signaling through secretion of cytokines and other soluble mediators. This unique immunomodulatory role of macrophages makes them ideal candidates for pharmacological intervention, with the potential to treat a highly diverse set of human diseases, including bacterial and viral infections, autoimmunity, inflammatory conditions, and cancer. An important hallmark of macrophages is their ability to adapt to their cellular surroundings, leading to extreme phenotypic diversity of macrophages. While this diversity presents the researcher with certain challenges, it also represents a unique opportunity for pharmacological intervention. If the nature of this diversity is understood, it may be possible to treat restricted subsets of macrophages without affecting others, thus greatly increasing drug specificity.

The proteases are among the most numerous and abundant of enzyme classes. The MEROPS database (Rawlings et al. 2002) (http://www.merops.sanger.ac.uk) lists nearly 400 different human proteases for which a chromosomal location has been mapped, representing more than 1% of the human genome. Analysis of the more completely characterized eukaryotic genomes has shown that proteases comprise between 1.7% and 3.9% of expressed genes, suggesting the presence of a large number of unknown or poorly characterized human proteases. Proteases can be subdivided functionally into those that can cleave internal polypeptide sequences (endopeptidases) and those that cleave only from

one end of the substrate molecule (ectopeptidases). The ectopeptidases are further divided into enzymes cleaving from the N and C termini, called aminopeptidases and carboxypeptidases, respectively. Proteases perform a wide variety of physiological functions throughout the body, both inside and outside the cell. From a pharmacological standpoint, a comprehensive understanding of protease function is critical, both for identification of new drug targets and to anticipate and ameliorate the side effects associated with protease inhibition. Known protease functions include: (1) intracellular destruction of proteins that are senescent, misfolded, or expressed cyclically; (2) breakdown of foreign, potentially pathogenic proteins; (3) digestion of both foreign and self proteins into peptides for presentation by MHC class I and II; (4) activation and execution of cell death cascades, acting either on itself or on an adjacent target cell; (5) functional activation and/or inactivation of enzymes, bioactive peptides, and many other types of proteins by proteolytic removal of a regulatory domain; (6) release of proteins from the plasma membrane or other membrane-bound compartment by cleavage of a transmembrane domain; and (7) digestion of proteins in the digestive tract for nutritional purposes.

Since the vast array of proteases in the human body display widely variable specificities and inhibitor sensitivities, the value of proteolysis as a pharmacologic target is clear. Targeting proteolytic pathways has led to such drug successes as angiotensin converting enzyme inhibitors for high blood pressure (Douglas 1985) (see below), the plasmin-targeted thrombolytic agents such as streptokinase and urokinase (Reilly 1985), and HIV protease inhibitors as part of combination therapy for HIV infection (Hammer et al. 1997; Gulick et al. 1997). Proteases are particularly abundant in macrophages, where they perform a wide variety of functions. These functions, which will be described in more detail below, include destruction of phagocytosed material, trimming of peptides for presentation by MHC class II molecules, alterations of extracellular matrix components, and a variety of regulatory roles. This chapter will provide an overview of the properties of proteases found in macrophages, and will attempt to highlight some areas of interest for further basic biochemical study and potential pharmacologic intervention.

2 Ectoproteases

Ectoproteases differ from the more abundant endoproteases in that they cleave substrates only at the carboxy or amino terminus. This functional difference has a structural basis: the substrate binding sites of ectoproteases tend to be solvent-accessible at only one end, thus allowing cleavage at the end of a protein but not in the middle of a polypeptide loop. This difference has important implications in design of synthetic inhibitors. Properties of ectoproteases found in macrophages are discussed below. Alternate names are shown in parentheses.

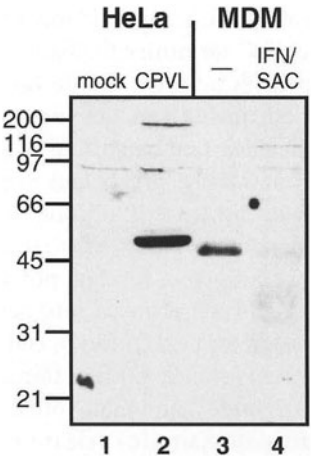

Fig. 1 Regulation of CPVL expression by macrophage activation state. Polyclonal anti-CPVL Western blot of lysates from mock- and CPVL-transfected HeLa cells (*lanes 1 and 2*) shows a major specific band of about 48 kDa. Human monocyte-derived macrophages (*MDM, lanes 3 and 4*) were cultured for approximately 10 days in RPMI 1640 plus 10% fetal bovine serum, either alone or with IFN-γ and *Staphylococcus aureus* cells (*SAC*, a source of lipopolysaccharide). Activation led to complete loss of the immunoreactive band. Equal protein loading was confirmed by Ponceau S staining (not shown). Note that recombinant CPVL migrates slightly more slowly because of an N-terminal epitope tag

2.1 Carboxypeptidase, Vitellogenic-Like (CPVL, CP-Mac)

CPVL (MEROPS ID S10.003) is a 476 amino acid serine carboxypeptidase, discovered as a result of a differential display polymerase chain reaction (PCR) screen for novel macrophage-specific genes (Mahoney et al. 2001). RT-PCR, Northern blot, and Western blot analysis confirm that, among hematopoietic cells, CPVL is indeed restricted to the monocytic lineage. CPVL mRNA was readily detected throughout the lineage, whereas protein expression was absent or low in monocytes and relatively abundant in mature monocyte-derived macrophages. Outside the immune system, however, a wider expression pattern was apparent. High levels of expression, as judged by Northern blot, were apparent in kidney and heart, two organs with few macrophages, while lung and liver, which have much larger macrophage populations, expressed little or no CPVL mRNA. We postulate that CPVL has two distinct expression profiles, one in a subset of tissue macrophages and a second one, presumably representing a separate function, in organs of the cardiovascular system. This pattern is similar to the expression pattern of angiotensin-converting enzyme (ACE, see below), which also shows significant expression in macrophages, heart, and kidney, although ACE is detectable in virtually all organs because of its presence in vascular endothelial cells (Dzau et al. 2001). Moreover, an alternate isoform of ACE is highly expressed in testis. Surveys of the human expressed sequence tags (EST) database suggest that CPVL is also expressed in testis.

The physiological function of CPVL is not currently known. Both primary sequence analysis and pulse-chase experiments (unpublished studies) suggest a lumenal and/or secreted distribution. In preliminary immunocytochemical studies using confocal microscopy, CPVL showed cytoplasmic expression in a vesicular pattern that did not coincide with endosomes, lysosomes, or MHC class II peptide-loading compartments (B. Ntolosi, R. DaSilva, and S. Gordon,

personal communication). Protein levels of CPVL are strikingly modulated by inflammatory stimuli: Culture of developing macrophages in the presence of interferon (IFN)-γ and lipopolysaccharide causes a dramatic downregulation of cellular CPVL expression (Fig. 1). While a great deal more work is required to ascertain the importance of this macrophage-restricted protease, we speculate that CPVL may play a role in the macrophage inflammatory response.

2.2 Carboxypeptidase M (CPM)

CPM (MEROPS ID M14.006) is a 439-amino acid glycosylphosphatidyl inositol-linked, membrane-bound metallo-carboxypeptidase (Tan et al. 1989) with preference for removal of lysine or arginine. While it is found in several different cell types, it is strongly expressed in monocytic lineage cells. Just as is the case with CPVL, CPM expression is upregulated during maturation of monocytes to macrophages (Rehli et al. 1995). CPM cleaves the C-terminal basic amino acid from a variety of biologically active peptide substrates, including bradykinin, dynorphin A(1–13), and enkephalins (Skidgel et al. 1989). It has been suggested, though not yet explicitly shown, that CPM would cleave and thus inactivate the anaphylatoxins C3a/C4a/C5a, in the same way as the related liver enzyme carboxypeptidase N (Rehli et al. 2000).

The level of CPM expression in vivo is highly dependent on the activation and/or differentiation state of the cells. Expression in macrophages of secondary lymph organs is low, but macrophage CPM expression during rejection of kidney transplants is much higher, and this elevated expression is inhibited by cyclosporin treatment (Andreesen et al. 1988). Moreover, monocytes from patients with aplastic anemia (Andreesen et al. 1989) or HIV (Andreesen et al. 1990) do not display maturation-induced CPM upregulation, even when cultured with healthy serum. These data suggest that CPM activation may be part of a macrophage inflammatory process. While these types of data show a compelling correlation between CPM levels and macrophage activation, proof of the importance of CPM in macrophage activation will require more understanding of its physiologically relevant substrates.

2.3 CD13 (Aminopeptidase N, Alanine aminopeptidase)

CD13 (MEROPS ID M01.001) is, like CPM, a plasma membrane-bound ectoprotease, consisting of 967 amino acids and one N-terminal transmembrane domain (Olsen et al. 1988). CD13 is ubiquitously expressed, and highly expressed in monocytic and granulocytic cells. As an aminopeptidase, CD13 removes single amino acids from the N termini of proteins and peptides. Like most ectopeptidases, CD13 is capable of removing amino acids from small bioactive peptides. Strikingly, removal of a single amino terminal residue from the chemokine monocyte chemotactic protein (MCP)-1 converts this basophil-activating che-

mokine into an eosinophil-activating one (Weber et al. 1996), taking advantage of the alternate expression of chemokine receptors with overlapping specificity in these cell types. However, its primary role appears to be the trimming of antigenic peptides bound to MHC molecules (Larsen et al. 1996). Therefore, the relative efficiency of CD13 to perform this function on antigen-presenting cells may significantly affect the balance of epitopes presented to T cells, with important ramifications in autoimmunity.

In addition to these physiologic roles, CD13 is also the receptor for coronaviruses to attach to endothelial cells of the upper respiratory tract (Yeager et al. 1992). Cytomegalovirus also uses CD13 as its receptor, as evidenced by the inhibition of both viral binding and infection with anti-CD13 antibodies in vitro (Soderberg et al. 1993). Cytomegalovirus binding to targets can lead to production of chronic graft-versus-host disease and pathogenic anti-CD13 autoantibodies (Soderberg et al. 1996).

2.4 Lysosomal Protective Protein (LPP, Cathepsin A, PPCA, Lysosomal Carboxypeptidase A)

LPP (MEROPS ID S10.002) is a lysosomally localized serine carboxypeptidase that, along with CPVL and a smooth muscle cell protein called RISC (Chen et al. 2001), make up the only three serine carboxypeptidases known in mammals. LPP was first isolated as the gene mutated in the human lysosomal storage disease galactosialidosis (Galjart et al. 1988), a syndrome caused by instability of lysosomal beta galactosidase and neuraminidase in the absence of a 54-kDa "protective protein," which normally protects these enzymes from degradation in the harsh lysosomal environment. Phenotypes vary by exact mutation, but generally include dwarfism, mental retardation, and a macular cherry-red spot. Sequence analysis showed similarity to serine proteases, and serine carboxypeptidase activity was soon confirmed but, importantly, shown not to be required for the protective function (Galjart et al. 1991).

LPP has three distinct enzymatic activities: an esterase activity, a deamidase activity, and a carboxypeptidase activity (Jackman et al. 1990). The protein is made as a 452 amino acid, 54-kDa precursor, which is then cleaved into 32-kDa and 20-kDa subunits (Pshezhetsky 1998). Mature LPP cleaves a variety of bioactive peptides in vitro, including bradykinin, endothelin I, substance P, and oxytocin (Jackman et al. 1990; Pshezhetsky 1998). However, the physiologically relevant substrates are not known.

Significant progress has recently been made in the treatment of a galactosialidosis model disease in mice (LPP knockout mice), which raises the possibility that this disease may be treatable in humans. D'Azzo and colleagues transplanted LPP knockout mice with bone marrow cells transduced to overexpress LPP under the influence of the colony stimulating factor-1 promoter, which directs expression to monocytes and macrophages. Since bone marrow-derived monocytic lineage cells traffic to essentially all tissues and secrete LPP, resident cells

may take up the secreted LPP, thus curing the defect in their own lysosomes. The treated mice showed marked reduction in symptoms and histopathology, with virtually all but the loss of cerebellar Purkinje cells corrected (Hahn et al. 1998). Their most recent model used murine stem cell virus to stably infect bone marrow cells with LPP and green fluorescent protein from a bicistronic vector (Leimig et al. 2002). The treated mice showed marked improvement for many months, including sparing of Purkinje cells. As this impressive work continues on the protective role LPP and its link to galactosialidosis, little information has emerged so far on the physiological role of the enzyme activities of this protein. Lysosomal storage disorders are discussed in detail in Chap. 11.

2.5
Angiotensin Converting Enzyme (ACE, Peptidyl Dipeptidase, Kininase II)

ACE (MEROPS ID X06.001) is a 1,306 amino acid cell surface bound protein containing two independent metalloprotease domains (Soubrier et al. 1988). It is widely expressed in somatic cells, and an alternate form with only the C-terminal protease domain is expressed only in male germ cells. ACE is a dicarboxypeptidase, cleaving two amino acids from the C terminus of the inactive angiotensin I, thus creating the powerful vasoconstrictor angiotensin II. Inhibitors of ACE such as captopril, enalapril, and numerous others have been extremely valuable agents for controlling hypertension (Douglas 1985).

Macrophages express high levels of ACE, and several reports within the past few years have emphasized the pathophysiological importance of macrophage ACE. It has long been known that ACE inhibition is beneficial in the treatment of atherosclerosis. Diet et al. showed that ACE accumulates in atherosclerotic plaques, and that the main source of ACE is foam cells, the characteristic lipid-laden macrophages of atherosclerosis (Diet et al. 1996). Moreover, they showed that differentiation of the monocytic cell line THP-1 into a macrophage phenotype led to an increase in ACE activity, and that increase was potentiated by addition of acetylated LDL. The mechanism of this effect is unknown, but probably involves inhibition of inflammatory mediators such as MCP-1 and interleukin (IL)-12 in the macrophages (Hernandez-Presa et al. 1997; Constantinescu et al. 1998). Finally, treatment of human mononuclear cells with ACE inhibitors in vitro decreased the synthesis of tissue factor, the clotting cascade initiator implicated in arterial thrombosis (Napoleone et al. 2000).

3
Endoproteases

3.1
Cathepsins

The term cathepsins does not refer to a group of proteins related by evolution, but rather by location and function. Cathepsins are a group of lysosomal pro-

teases, most of which are involved in the degradation of phagocytosed or endocytosed products. They can be of any enzyme class, although most are cysteine proteases. Many of the cathepsins are ubiquitously expressed, but as the numbers of known cathepsins increases, some cell type-specific examples are emerging.

Cathepsins B and D (MEROPS IDs C01.060, A01.009) have been implicated in the degradation of apolipoproteins in macrophages (Kuroda et al. 1994). Apolipoproteins from oxidized LDL particles are not digested well by macrophages, potentially leading to accumulation of foam cell macrophages and atherosclerosis. This inhibition of apolipoprotein digestion appears to be mediated by oxidized LDL inhibition of cathepsin B, via an unknown mechanism (Hoppe et al. 1994).

Cathepsin K (MEROPS ID C01.036) is one of the most cell type-specific of this group. While cathepsin K was originally thought to be expressed only on osteoclasts, the macrophage lineage cells responsible for bone resorption (see Chap. 19, this volume), recent evidence suggests that macrophages involved in foreign body responses, such as multinucleated giant cells or epithelioid cells in granulomas, also express it (Buhling et al. 2001). Resident tissue macrophages did not express cathepsin K, whereas cathepsins B and L were expressed on both resident and foreign body-elicited macrophages.

A final example of macrophage-specific cathepsin function comes from the study of processing of the invariant chain, Ii, by antigen-presenting cells. The invariant chain associates with nascent MHC class II molecules, to prevent binding of endogenous antigens. When the class II molecule enters the endosomal compartment, Ii is cleaved by a cathepsin, leaving only the small class II-associated invariant-chain peptide (CLIP) in the MHC groove, to be exchanged for a newly processed antigenic peptide. Chapman and colleagues showed that cathepsin S (MEROPS ID C01.034) is required for Ii cleavage in B cells and dendritic cells (Shi et al. 1999). They went on to show that MHC class II presentation in macrophages of cathepsin S knockout mice was normal, and identified a novel protease, cathepsin F (MEROPS ID C01.018), responsible for this activity in macrophages (Shi et al. 2000).

3.2
Caspase-1 (Interleukin 1β Converting Enzyme, ICE)

Caspases are cysteine endoproteases that cleave after Asp residues, in the context of a four amino acid recognition motif, in a wide variety of protein substrates. Caspases are best known for their involvement in the apoptosis cascade (Earnshaw et al. 1999). However, a subset of caspases are primarily involved in proteolytic release of cytokine precursors from the membrane, for action at a distant site. The best-known example is caspase-1 (MEROPS ID C14.001), which was identified by its ability to release IL-1β from monocytes and macrophages (Thornberry et al. 1992). Mice deficient in caspase-1 cannot release IL-1β, and thus are resistant to septic shock, an IL-1β-dependent process (Li et al. 1995). It was subsequently shown that caspase-1 also catalyzes the release of IL-18, or in-

terferon γ inducing factor (Ghayur et al. 1997; Gu et al. 1997). These data raise the possibility that a specific inhibitor of caspase-1 may be a useful treatment for sepsis. In the meantime, this area has provided an explanation for the regulatory effect of nitric oxide on inflammatory cytokine release. Nitric oxide potently inhibits cysteine proteases by S-nitrosylation of the active site cysteine. Kim et al. showed that activated macrophages treated in vitro with a nitric oxide synthase inhibitor released fourfold more IL-1β than those untreated (Kim et al. 1998). Furthermore, mice deficient in inducible nitric oxide synthase produced more IL-1β and more interferon γ in response to challenge with endotoxin.

3.3 Proteases Secreted to Act on Their Environment

3.3.1 Macrophage Gelatinase (Matrix Metalloprotease 9, MMP9, Gelatinase B)

MMP9 (MEROPS ID M10.009) is a 707 amino acid zinc metalloprotease (Wilhelm et al. 1989) and member of the matrix metalloprotease (MMP) family, a large (>20 different genes discovered) family of zinc metalloproteases responsible for the clearance and remodeling of the extracellular matrix, with downstream effects in areas such as development and wound healing (Nagase and Woessner 1999). The MMPs are highly regulated by gene expression, by synthesis as inactive preproenzymes, and by the presence of inhibitors such as the tissue inhibitors of metalloproteases (TIMPs). Unlike some other groups of proteases, such as the cathepsins, MMPs are generally not expressed in normal tissue, but expression is induced by a variety of stimuli, such as cytokines, growth factors, and others.

MMP9 is expressed on macrophages and neutrophils, and like all gelatinases, degrades a variety of extracellular components such as collagens, elastin, and fibronectin. However, the physiological roles played by this enzyme (and the others in this section; see below) are surprisingly broad, as demonstrated by the results of knockout studies. MMP9 knockout mice showed defects in bone formation and vascularization, caused by a lack of MMP9 in chondroclasts, the multinucleated, bone marrow-derived cells that resorb cartilage (Vu et al. 1998). Other studies showed that MMP9 knockout mice had reduced capacity for outgrowth of oligodendrocyte processes in the developing brain (Oh et al. 1999), and diminished ability for exogenously implanted tumors to metastasize (Itoh et al. 1999). Intriguingly, the tumors did not express MMP9, but rather required MMP9 secreted from host cells for successful metastasis. Finally, MMP9 has been shown to cleave a short amino terminal peptide from the neutrophil chemokine IL-8, and this modified IL-8 was more than tenfold more potent in neutrophil activation and chemotaxis assays (Van den Steen et al. 2000). Therefore, MMP9 has pleiotropic effects because of its matrix proteolytic functions, and moreover, exerts immunostimulatory effects by modification of a chemokine.

3.3.2
Macrophage Metalloelastase (MME, MMP12)

MMP12 (MEROPS ID M10.009) is a 470 amino zinc metalloprotease (Shapiro et al. 1993) in the MMP family. MMP12 degrades elastin and other extracellular matrix components. Consistent with the notion that many MMPs are inducible proteins, MMP12 signal is only detected in placenta (a highly macrophage-enriched tissue) by Northern blotting (Belaaouaj et al. 1995). Experiments with MMP12 promoter constructs indicated that MMP gene expression was induced by LPS in a mouse macrophage cell line, but not in human umbilical vein endothelial cells. Knockout studies showed that MMP12 was necessary for matrix degradation by macrophages in vitro and in vivo. $MMP12^{-/-}$ macrophages had reduced proteolytic activity against insoluble elastin in vitro, and the ability to penetrate Matrigel artificial basement membranes in vitro and in vivo was abolished (Shipley et al. 1996). Strikingly, the knockout conferred complete protection in an experimental model of cigarette smoke-induced emphysema (Hautamaki et al. 1997). Control mice exposed to cigarette smoke for 3 months showed immunohistochemical evidence of MMP12-positive macrophage recruitment to the lungs, and increased mean alveolar air space. Neither effect was detectable with $MMP12^{-/-}$ mice. If macrophage recruitment of $MMP12^{-/-}$ macrophages was artificially induced by adding the chemokine MCP-1, the (MMP12-negative) macrophages were detected in the lung, but there was still no change in mean alveolar air space. These experiments suggest that an MMP12-specific inhibitor has potential therapeutic value in the setting of pathological macrophage recruitment.

3.3.3
Leukocyte Elastase (LE, Neutrophil Elastase)

LE (MEROPS ID S01.131), despite sharing many properties with MMPs 9 and 12, is not a member of the MMP family, but rather is a 218 amino acid serine endoprotease (Sinha et al. 1987). Although LE is expressed in macrophages, it is most highly expressed in neutrophils, where it can be the cause of destructive lung disease (Mitsuhashi et al. 1999). It was recently reported that cystic fibrosis patients have impaired removal of apoptotic inflammatory cells by macrophages. This defect is caused by LE-mediated cleavage of the phosphatidylserine receptor that recognizes apoptotic cells for uptake (Vandivier et al. 2002). The lost capacity to dispose of toxic mediators from apoptotic inflammatory cells may exacerbate the deleterious effects of these cells.

3.4
Proteases Acting at the Cell Surface

3.4.1
TNF-α Converting Enzyme (TACE, ADAM17, CD156b)

Tumor necrosis factor (TNF)-α is a powerful pro-inflammatory cytokine that, like IL-1β, is synthesized in a plasma membrane-bound form, and is then released into the extracellular space by TACE (MEROPS ID M12.217). TACE is an 824 amino acid member of the ADAM (the name is derived from a disintegrin and metalloprotease) family, a group of over 40 proteins containing a disintegrin domain that binds integrins and a metalloprotease domain similar to those in the MMP family (Black et al. 1997; Moss et al. 1997). While TACE is ubiquitously expressed, it is highly expressed in some macrophage populations, where TNF-α is made and secreted. Because TNF-α is often implicated in harmful inflammatory pathways, there has been great interest in devising methods to inactivate TACE. Methods for inhibiting TACE include use of the natural inhibitor TIMP-3 (Amour et al. 1998), creation of a recombinant dominant negative form of the enzyme (Solomon et al. 1999), and development of small molecule inhibitors (Barlaam et al. 1999).

Surprisingly, attempts to create a TACE knockout mouse led to the finding that TACE has a much broader substrate specificity. Mice carrying a mutation in the Zn binding site of TACE had a large number of developmental abnormalities, and most died between embryonic day 17.5 and 1 day after birth (Peschon et al. 1998). Their mutations were reminiscent of those in mice deficient in transforming growth factor (TGF)-α, which is also released from the plasma membrane by a cleavage event. The authors went on to show that TACE is responsible for cleavage of the ectodomains of TGF-α, L-selectin, and TNF receptor p75. Subsequent work by other groups has shown that other proteins of potential therapeutic significance are also cleaved by TACE, including the amyloid protein precursor associated with Alzheimer's disease (Buxbaum et al. 1998), and the receptor for colony stimulating factor-1, the factor critical for commitment of precursor cells to the monocytic lineage (Rovida et al. 2001).

3.4.2
Macrophage Mannose Receptor Secretase

Macrophage mannose receptor (MMR) is a 180-kDa glycoprotein expressed on the plasma membrane of macrophages, some dendritic cells, and a few isolated endothelial cell types (Linehan et al. 2000). Eight C-type lectin domains mediate its functions as a phagocytic and endocytic receptor, recognizing mannose- and fucose-containing structures. A second carbohydrate recognition domain, the N-terminal cysteine-rich (CR) domain, mediates binding to sulfated sugars on ligands such as sialoadhesin and CD45 expressed on marginal zone macrophages and in germinal centers (Martinez-Pomares et al. 1996; Martinez-

Pomares et al. 1999). MMR is released from macrophages in vitro and in vivo by a metalloprotease-type secretase (Martinez-Pomares et al. 1998). Martinez-Pomares and Gordon have proposed that MMR may transport polysaccharide antigens to secondary lymphoid organs for generation of immune responses (Martinez-Pomares and Gordon 1999). To date little is known about the nature of the MMR secretase, except that it is present on macrophages, and is susceptible to hydroxamate-based inhibitors. Given these facts, one may speculate that MMR secretase is, in fact, TACE. However, since most of the over 40 members of the ADAM family share the same basic domain structure as TACE, and as yet have no described function, there is no shortage of good candidates.

4 Summary

Proteases are critical players in many of the central functions of macrophages, including digestion of phagocytosed material, processing of foreign antigens for presentation on MHC class II molecules, tissue remodeling, and regulation of immune responses by activating, inactivating, or releasing from membranes a host of immune-active proteins and peptides. The task of understanding the roles of these enzymes is complicated by the fact that many proteases have multiple and overlapping functions. However, with the appropriate tools and insight, assisted by the arrival of whole genome data sets, the prospects for major advances in understanding the pathophysiology of this system are bright, leading to significant advancements in the treatment of human disease.

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