Published in final edited form as:

Vitam Horm. 2009 ; 80: 541–562. doi:10.1016/S0083-6729(08)00618-3.

Matrix Metalloproteinases, T Cell Homing and β -Cell Mass in Type 1 Diabetes

Alexei Y. Savinov and Alex Y. Strongin

Burnham Institute for Medical Research, Inflammatory and Infectious Disease Center, La Jolla, CA 92037

Abstract

The pathogenesis of type 1 diabetes begins with the activation of autoimmune T killer cells and is followed by their homing into the pancreatic islets. After penetrating the pancreatic islets, T cells directly contact and destroy insulin-producing β cells. This review provides an overview of the dynamic interactions which link T cell membrane type-1 matrix metalloproteinase (MT₁-MMP) and the signaling adhesion CD₄₄ receptor with T cell transendothelial migration and the subsequent homing of the transmigrated cells to the pancreatic islets. MT₁-MMP regulates the functionality of CD₄₄ in diabetogenic T cells. By regulating the functionality of T cell CD₄₄, MT₁-MMP mediates the transition of T cell adhesion to endothelial cells to the transendothelial migration of T cells, thus, controlling the rate at which T cells home into the pancreatic islets. As a result, the T cell MT₁-MMP-CD₄₄ axis controls the severity of the disease. Inhibition of MT₁-MMP proteolysis of CD₄₄ using highly specific and potent synthetic inhibitors, which have been clinically tested in cancer patients, reduces the rate of transendothelial migration and the homing of T cells. Result is a decrease in the net diabetogenic efficiency of T cells and a restoration of β cell mass and insulin production in NOD mice. The latter is a reliable and widely used model of type I diabetes in humans. Overall, existing experimental evidence suggests that there is a sound mechanistic rationale for clinical trials of the inhibitors of T cell MT₁-MMP in human type 1 diabetes patients.

I. Matrix Metalloproteinases and Their Natural Protein Inhibitors

A. MMPs

Historically, interstitial collagenase (MMP-1) was the first identified member of the now extensive matrix metalloproteinase (MMP) family. MMP-1 was initially discovered in the course of studying collagen remodeling during the metamorphosis of a tadpole into a frog and much later the presence of this enzyme was confirmed in humans (Gross and Lapiere, 1962; Stocker and Bode, 1995). Because collagens, especially type I collagen, represent the major structural proteins of all tissues and serve as the main barrier to migrating cells, more than three decades ago an innovative hypothesis was postulated and this hypothesis has now been proven correct. According to this hypothesis, collagenolytic enzymes including MMP-1 play pivotal roles in multiple physiologic and, especially pathologic processes, which involve both extensive and aberrant collagenolysis. Recent scientific discoveries have vastly expanded our knowledge of MMPs' structures and functions. These discoveries directly implicate a number of the individual MMPs, including MMP-1, in multiple diseases of the cardiovascular, pulmonary, renal, endocrine, gastrointestinal, musculoskeletal, visual, and hematopoietic systems in humans.

MMPs belong to a zinc endopeptidase, metzincin superfamily (Gomis-Ruth, 2003). This superfamily is distinguished from other proteinases by the presence of a strictly conserved HEXHXXGXX(H/D) histidine sequence motif. This motif exhibits three histidine residues

that chelate the active site zinc and also a canonical methionine residue which is the C-terminal to the conserved histidine sequence. The canonical methionine is part of a tight 1,4-beta-turn that loops the polypeptide chain beneath the catalytic zinc ion, forming a hydrophobic floor to the zinc ion binding site. The metzincin family is normally divided into four subfamilies: seralysins, astacins, adamalysins [ADAMs (proteins with a disintegrin and a metalloproteinase domain) and ADAM-TS (ADAM with thrombospondin-like motif)] and MMPs. Although our knowledge of MMP biology is rapidly expanding, we do not as yet understand precisely how these enzymes regulate various cellular functions.

The human MMP family is comprised of 24 currently known zinc-containing enzymes which share several common functional domains. MMPs are often referred to by a descriptive name such as gelatinases (MMP-2 and MMP-9) and collagenases [MMP-1, MMP-8, MMP-13, MMP-14/membrane type-1 matrix metalloproteinase (MT1-MMP) and, somewhat conclusively, MMP-18] and this name is generally based on a preferred substrate. Collagenases are the only known mammalian enzymes capable of degrading triple-helical fibrillar collagen into distinctive 3/4 and 1/4 fragments. An additional and widely accepted MMP numbering system based on the order of discovery is also in use (Fig. 18.1) (Egeblad and Werb, 2002; Nagase and Woessner, 1999).

In general, MMPs may be described as multifunctional enzymes capable of cleaving the extracellular matrix components (collagens, laminin, fibronectin, vitronectin, aggrecan, enactin, versican, perlecan, tenascin, elastin, and many others), growth factors, cytokines and cell surface-associated adhesion and signaling receptors. Because of their high degrading activity and potentially disastrous effect on the cell microenvironment, cellular MMPs are expressed in small amounts, and their cellular localization and activity are tightly controlled, either positively or negatively, at both the transcriptional and the posttranscriptional levels by cytokines, including interleukins (IL-1, IL-4, and IL-6), growth factors (epidermal growth factor, hepatocyte growth factor and transforming growth factor- β), and tumor necrosis factor- α) (Sternlicht and Werb, 2001; Zucker *et al.*, 2003). In a feedback loop, some of these regulatory factors themselves are proteolytically activated or inactivated by the individual MMPs (McQuibban *et al.*, 2000).

MMPs are synthesized as latent zymogens. The active site zinc of the MMP catalytic domain is coordinated with the three histidines of the active site and with the cysteine of the "cysteine switch" motif of the N-terminal prodomain (Van Wart and Birkedal-Hansen, 1990). To become functionally potent proteinases, the zymogens of MMPs require proteolytic activation. In the process of this activation, the N-terminal inhibitory prodomain is removed and the catalytic site of the emerging enzyme becomes liberated and exposed. The activation of MMPs may occur both intracellularly and extracellularly (Murphy *et al.*, 1999; Pei and Weiss, 1995). MMPs including MMP-11, MMP-28, and several MT-MMPs with the furin cleavage motif RXX/RR in their propeptide sequence are normally activated in the trans-Golgi network by serine proteinases such as furin and certain additional members of the proprotein convertase family. Activation of MMPs which are secreted in the extracellular milieu is frequently mediated by serine proteases, including plasmin, by the membrane type MMPs (e.g., activation of the latent soluble MMP-2 proenzyme by MT1-MMP) or by other preexisting active MMPs (e.g., activation of the latent soluble enzymes of MMP-1 and MMP-9 by the soluble MMP-3 enzyme).

With the exception of the activated MMP-7 and MMP-26 enzymes, which are represented by the catalytic domain alone, all other MMPs have a C-terminal hemopexin-like domain. This domain regulates the activity and the specificity of the catalytic domain function. The hemopexin domain is separated from the catalytic domain by a flexible hinge region. Membrane-tethered MMPs are distinguished from soluble MMPs by an additional

transmembrane domain and a short cytoplasmic tail (MMP-14/MT1-MMP, MMP-15/MT2-MMP, MMP-16/MT3-MMP, and MMP-24/MT5-MMP). In contrast to these four MT-MMPs, MMP-17/MT4-MMP, and MMP-25/MT6-MMP are attached to the cell membrane via a glycosylphosphatidyl inositol (GPI) anchor (Fig. 18.1) Historically, MMPs were initially characterized by their extensive ability to degrade extracellular matrix proteins including collagens, laminin, fibronectin, vitronectin, aggrecan, enactin, tenascin, elastin, and proteoglycans. More recently, it has been recognized that MMPs cleave in addition to the extracellular matrix components, many other protein types including cytokines and cell adhesion signaling receptors.

Because the individual MMPs have overlapping substrate cleavage preferences, MMP knockouts and inactivating mutations in individual MMP genes in mice, with the exception of MT1-MMP, do not elicit an easily recognized phenotype and are non-lethal, at least up to the first few weeks after birth, suggesting functional redundancy among MMP family members. MT1-MMP knockout, however, has a profound effect: MT1-MMP null mice develop dwarfism, extensive bone malformations and die before adulthood, thus supporting an important role of MT1-MMP in collagen type I turnover (Holmbeck *et al.*, 1999, 2003, 2004). Mice lacking both MMP-2 and MT1-MMP die immediately after birth of respiratory failure, abnormal blood vessels, and immature muscle fibers reminiscent of central core disease (Oh *et al.*, 2004).

B. Tissue inhibitors of matrix metalloproteinases

Once activated, MMPs are normally inhibited by tissue inhibitors of metalloproteinases (TIMPs). Four individual species of TIMPs are known in humans (TIMP-1, -2, -3, and -4) (Nagase *et al.*, 2006) (Fig. 18.2). MMP/TIMP balance is believed to be a major factor in the regulation of the net proteolytic activity of the individual activated MMPs. Structurally, TIMPs contain two domains. The inhibitory N-terminal domain binds non-covalently and stoichiometrically to the active site of the active mature MMPs, blocking access of substrates to the catalytic site. The C-terminal domain of TIMP-1 and TIMP-2 binds to the hemopexin domain of the proenzymes of MMP-9 and MMP-2, respectively. The latter binding is essential for the cell surface activation of MMP-2 by MMP-14/MT1-MMP.

In this well-characterized unconventional activation mechanism, MMP-14/MT1-MMP on the cell surface acts as a receptor for TIMP-2. TIMP-2 binds via its N-terminal domain to the active site of MT1-MMP. This binary complex then acts as a receptor for the MMP-2 proenzyme, with the TIMP-2 C-terminal domain binding to the C-terminal hemopexin domain of MMP-2 and with the formation of a trimolecular MT1-MMP-TIMP-2-MMP-2 complex. A TIMP-2-free, second MT1-MMP molecule which is close to the trimolecular complex then cleaves the N-terminal propeptide of the MMP-2 proenzyme, generating an intermediate species. Further proteolysis of the MMP-2 propeptide through an autocatalytic mechanism generates the fully active enzyme of MMP-2 which is then released from the complex.

II. T Cell Membrane Type-1 Matrix Metalloproteinase

A. Structure and function of MT₁-MMP

MT1-MMP, a prototypic membrane-type MMP, is distinguished from soluble MMPs by a C-terminal transmembrane domain and a cytoplasmic tail (Egeblad and Werb, 2002). In the human genome, MT1-MMP is encoded by a single copy gene located on chromosome 14. MT1-MMP is widely expressed and its presence has been documented in multiple cell types. Because both the expression and the activity of MT1-MMP are elevated in tumor cells and because high levels of MT1-MMP directly correlate with enhanced cell migration, this proteinase is generally considered pro-invasive and pro-tumorigenic.

Because the prodomain part of MT1-MMP has the furin-cleavage motif, furin is believed to be an essential component of the activation pathway that results in the generation of the active, mature cellular MT1-MMP (Pei and Weiss, 1995; Yana and Weiss, 2000). MT1-MMP was originally thought to exhibit a single function as a membrane activator of soluble MMPs, including MMP-2 (Sato *et al.*, 1994; Strongin *et al.*, 1995) and MMP-13 (Knauper *et al.*, 2002). Recent data, however, has provided evidence that, in addition, MT1-MMP degrades multiple components of the extracellular matrix and a number of cell adhesion and signaling receptors (Strongin, 2006). MT1-MMP is regulated both as a proteinase and as a membrane protein at the transcriptional and posttranscriptional levels by multifaceted, tightly controlled and well-coordinated mechanisms. These multidimensional mechanisms regulate MT1-MMP spatially and temporally, and they are essential not only for the proper functioning of MT1-MMP alone but also for the performance of the normal multiple biological functions of the entire cell. The regulatory mechanisms which control the functional activity of MT1-MMP include control of the extent of activation of the MT1-MMP proenzyme by furin, the level of inhibition of MT1-MMP by TIMPs and self-proteolytic inactivation, a homophilic complex formation involving the hemopexin domain and the cytoplasmic tail, the efficiency of trafficking of MT1-MMP through the cell compartment to the plasma membrane, the rate of the internalization of MT1-MMP into the transient compartment inside the cells and, lastly, the extent of the recycling of MT1-MMP back to the plasma membrane (Itoh and Seiki, 2006; Seiki, 2003; Strongin, 2006).

Internalization via clathrin-coated pits and also through caveolae is also recognized as a important mechanism to regulate MT1-MMP activity (Galvez *et al.*, 2004; Jiang *et al.*, 2001; Labrecque *et al.*, 2004; Rozanov *et al.*, 2004). The “up/down” switch may have been built into the peptide sequence of the MT1-MMP’s cytoplasmic tail to regulate the recruitment to the plasma membrane and to target the protease to the invasive front in migrating cells. Transient changes in subcellular compartmentalization of MT1-MMP, which occur in its trafficking and internalization pathways, are, probably, the underlying mechanisms which specifically control the functions of MT1-MMP in malignant cells (Wang *et al.*, 2004a,b).

TIMP-2, TIMP-3, and TIMP-4 are highly potent inhibitors of MT1-MMP. TIMP-1, however, is a very poor inhibitor of this proteinase (Nagase and Woessner, 1999; Nagase *et al.*, 2006; Will *et al.*, 1996). Current evidence suggests that the activity of cellular MT1-MMP is short-lived and that the half-life of active, mature MT1-MMP attached to the plasma membrane is approximately 1 h (Deryugina *et al.*, 2004; Wang *et al.*, 2004b). During this time period, active MT1-MMP is either inactivated by TIMPs or autolytically degraded or internalized with only a subsequent partial recycling (Osenkowski *et al.*, 2004). Because MT1-MMP, in addition to its role in matrix degradation, is directly involved in the cleavage of cell surface receptors, this short-lived proteinase, exerts a long-lasting effect on cell behavior and functions in cancer cells as the main mediator of proteolytic events on the cell surface. Our data and the results of others show that the proteolysis of CD44, integrins, tissue transglutaminase, the low density lipoprotein receptor-related protein (LRP1), E-cadherin and related cell-associated adhesion signaling receptors is the important role of MT1-MMP (Strongin, 2006). By cleaving these receptors, the short-lived MT1-MMP has a long lasting effect on the cell microenvironment and cell behavior. In addition, MT1-MMP, as opposed to the soluble MMPs, is ideally positioned to regulate pericellular proteolysis and the functionality of the neighboring cell receptors, including CD44 (Seiki, 2003).

B. Adhesion and signaling CD₄₄ receptor

The transmembrane, cell adhesion signaling receptor CD44 is the principal receptor for hyaluronan (Turley *et al.*, 2002). CD44 plays a critical role in cell functions, including adhesion, migration, invasion, and survival (Cichy and Pure, 2003). The C-end cytoplasmic tail interacts with ezrin, radixin and moesin and links CD44 to the actin cytoskeleton. CD44

also interacts with the Rho-family GTPases and induces the rearrangement of the cytoskeleton (Turley *et al.*, 2002). The binding of CD44 to hyaluronan stimulates the downstream signaling pathways and leads to the activation of protein kinase Ca (Slevin *et al.*, 2002). Antigenic stimulation of T cells induces the cell surface expression of the highly active form of CD44 which binds hyaluronan with high affinity (Lesley *et al.*, 1993). The presence of high concentrations of hyaluronan in the microcapillaries is consistent with the importance of CD44 in T cell trafficking (Aruffo *et al.*, 1990; Bennett *et al.*, 1995; DeGrendele *et al.*, 1997; Estess *et al.*, 1999; Mohamadzadeh *et al.*, 1998). CD44 is a potent adhesion receptor and it facilitates T cell adhesion on the endothelium and the subsequent transmigration events (Avigdor *et al.*, 2004; Savinov and Strongin, 2007; Savinov *et al.*, 2005, 2006, 2007; Weiss *et al.*, 2000).

CD44 is a marker of activated T cells (DeGrendele *et al.*, 1997; Estess *et al.*, 1998). CD44 is heavily glycosylated (Katoh *et al.*, 1995). Glycosylation regulates the oligomerization and the movement of CD44 across the plasma membrane. CD44 is a target of MT1-MMP proteolysis in tumor cells (Kajita *et al.*, 2001; Mori *et al.*, 2002; Murai *et al.*, 2004; Nakamura *et al.*, 2004; Suenaga *et al.*, 2005). There are three cleavage sites in the ectodomain stem region of CD44 (Mori *et al.*, 2002; Nakamura *et al.*, 2004). MT1-MMP cleaves the $\text{SGG}^{192}\downarrow\text{Y}^{193}\text{IF}$ sequence in the CD44 molecule while two additional sites ($\text{SGG}^{233}\downarrow\text{S}^{234}\text{HT}$ and $\text{HGS}^{249}\downarrow\text{Q}^{250}\text{EG}$) are cleaved either by MT1-MMP or ADAM/ADAM-TS proteases or by both. The inactivation of CD44 functionality by MT1-MMP proteolysis stimulates the migration of cancer cells. Conversely, the inactivation of MT1-MMP by TIMPs (excluding TIMP-1 which is a poor inhibitor MT1-MMP) or synthetic inhibitors protect cell surface-associated CD44 from MT1-MMP proteolysis and these events correlate with migration recess.

C. Synthetic antagonists of MT₁-MMP

In many cancer types, MMPs including MT1-MMP are up-regulated and because these proteinases are considered pro-invasive and tumorigenic, considerable effort has been devoted to the development of synthetic MMP inhibitors. This activity has led to the design of a variety of compounds including hydroxamate-based inhibitors the ligand hydroxamate group of which coordinates to the catalytic zinc ion thus rendering MMPs inactive. Hydroxamate inhibitors have showed great promise in animal models of cancer and certain inhibitors have been tested in cancer patients.

Several hydroxamate-based inhibitors, clinically tested in cancer patients, including GM6001 and AG3340, are potent against MT1-MMP. The hydroxamate AG3340 (trade name Prinomastat; 2-[[[(hydroxyamino)methyl]-5,6-dimethyl-4-(4-pyridin-4-yloxyphenyl)sulfonyl-morpholine-3-thione] (Agouron-Pfizer) inhibits MT1-MMP with a K_i of 40 pM (Shalinsky *et al.*, 1999; Zucker *et al.*, 2000). The K_i values of AG3340 against MMP-2, MMP-3, and MMP-13 are approximately 100, 300, and 200 pM, respectively. Other individual MMPs are significantly less sensitive to AG3340 inhibition (e.g., the K_i values for MMP-1 and MMP-7 are 10 and 55 nM, respectively). AG3340/Prinomastat was used as an oral angiogenic drug in phase I–III clinical trials in humans with advanced non-small cell lung cancer and prostate cancer (Hande *et al.*, 2004). The trials were halted because of the drug's lack of effectiveness in patients with late-stage disease (Cappuzzo *et al.*, 2003) but there was no question about patient safety. The hydroxamate peptidomimetic inhibitor GM6001 (Galardin; Ilomastat; *N*-[2R]-2-(hydroxamidocarbonylmethyl)-4-methyl-pentanoyl]-L-tryptophan methylamide) with a K_i value against MT1-MMP also in the sub-nanomolar range was tested on ulcer patents.

A recently designed thiirane inhibitor SB-3CT [(4-phenoxyphenylsulfonyl) butane-1,2-dithiol] exhibits a dithiolate moiety that chelates the active site zinc (Celenza *et al.*, 2008;

Lee *et al.*, 2007; Sodek *et al.*, 2007). SB-3CT was specifically designed to target MMP-2/MMP-9 gelatinases, not MT1-MMP. Whereas SB-3CT is an effective and selective gelatinase inhibitor, it either does not inhibit or it poorly inhibits other MMPs and the closely related metalloproteinase TACE (tumor necrosis factor- α converting enzyme) (Ikejiri *et al.*, 2005a,b). The efficacy of SB3-CT was demonstrated in several distinct models where the functional activity of gelatinases was relevant to the disease. Thus, intraperitoneal treatment with SB-3CT (50 mg/kg) inhibited intraosseous growth of human PC3 cells within the marrow of human fetal femur fragments previously implanted in SCID mice (Bonfil *et al.*, 2006). In a transient focal cerebral ischemia model of stroke in mice, MMP-9 contributes directly to neuron apoptosis and brain damage (Gu *et al.*, 2005) by degrading the extracellular matrix laminin. SB-3CT blocks MMP-9 activity, including MMP-9-mediated laminin cleavage, thus rescuing neurons from apoptosis.

A natural compound epigallocatechin gallate (EGCG), a major catechin of green tea, also exhibits inhibitory, albeit largely non-specific, effects on MMP (Annabi *et al.*, 2002; Chiang *et al.*, 2006; Dell'Aica *et al.*, 2002; El Bedoui *et al.*, 2005; Kim *et al.*, 2004; Lee *et al.*, 2005; Song *et al.*, 2004; Vayalil and Katiyar, 2004).

III. Rodent Model of Human Type 1 Diabetes

A. Type I diabetes

Type 1 diabetes (T1D) is a major debilitating human disease with an early childhood onset. Lymphocyte infiltration into the islets of Langerhans is a hallmark of T1D in humans and in rodent models. The pathogenesis of T1D begins with the activation of autoimmune T killer cells which then home into the pancreatic islets. After penetrating the pancreatic islets, T cells directly contact and destroy insulin-producing β cells. Autoreactive IS CD8⁺ T killer cells are specific for islet-derived insulin antigen (Savinov *et al.*, 2003a,b; Verdager *et al.*, 1997; Wong *et al.*, 1996). This T cell subpopulation is the key player in the destruction of the pancreatic β cells. The transmigration of IS-CD8⁺ T killer cells from the bloodstream through the pancreatic endothelium and into the islets of Langerhans is essential for the T cell- β cell contact and the subsequent destruction of β cells. The specific molecular mechanisms governing these processes are not, as yet, completely understood (Springer, 1994; Weber, 2003; Worthylake and Burridge, 2001) and, therefore, therapeutic interventions leading to β cell regeneration and the reversal of established T1D are exceedingly limited.

The transendothelial migration of T cells consists of the following basic steps: initial adhesion followed by tethering and rolling, arrest of rolling that translates into firm adhesion, extravasation and migration through the endothelial cell barrier (Butcher and Picker, 1996; Butcher *et al.*, 1999). CD44 and other adhesion receptors including selectins, cadherins, immunoglobulin superfamily cell adhesion molecules (CAMs, and, specifically, VCAM, ICAM-1, and ICAM-2) and integrins, which are expressed in both T cells and endothelial cells, contribute to the adhesion of T cells to the endothelium (Constantin *et al.*, 2000; Stein *et al.*, 2000; Yang *et al.*, 1996). Initial adhesion and slow rolling of T cells on endothelial cells is largely mediated by L-selectin, ligands for tissue-specific P and E selectins, and integrins. The slow rolling of T cells on the endothelial surface is followed by the activation of T cell integrins and firm adhesion. The interactions of T cell CD44 with its abundant endothelial ligand (hyaluronan) are essential for firm adhesion (Butcher *et al.*, 1999; Nandi *et al.*, 2004; Sallusto *et al.*, 2000; Weber, 2003). Thus, neutralizing antibodies to CD44 protect NOD mice from diabetes (Weiss *et al.*, 2000). Chemokines, expressed in a tissue-specific manner, mediate homing specificity by activating cognate G protein-coupled receptors on the T cells (Campbell and Butcher, 2000; Savinov *et al.*, 2003b). G protein-coupled chemokine receptors participate in integrin activation (Campbell *et al.*, 1998; Stein

et al., 2000; von Andrian and Mackay, 2000). The transition from firm adhesion to extravasation and diapedesis, which involves the cytoskeletal rearrangement of endothelial cells and the proteolytic degradation of the endothelial cell junctions and sub-endothelial basement membrane, is not yet completely understood. It is clear, however, that the enhancement of firm adhesion will decrease transmigration and diapedesis. Weak adhesion and the inability of T cells to establish firm adhesion will also result in diminished transmigration and diapedesis.

B. NOD mice and T1D

Mice of the NOD inbred strain develop a spontaneous disease closely resembling human T1D, and are widely and successfully used as a model of T1D (Gallegos and Bevan, 2004). CD8⁺ T lymphocytes are involved in diabetogenesis in NOD mice. NOD mice lacking CD8⁺ T cells do not develop diabetes (Serreze *et al.*, 1994). Prior work has demonstrated that the IS-CD8⁺ T cell clone itself is highly diabetogenic, and, after injection, reliably causes the onset of diabetes in NOD mice in a matter of 5–7 days (Savinov *et al.*, 2003b; Wong *et al.*, 1999). After injection into animals, IS-CD8⁺ T cells recapitulate the natural development of T1D in humans. These cells efficiently adhere to the endothelium and then transmigrate through the pancreatic endothelium. Transmigrated IS-CD8⁺ T cells destroy β cells and cause diabetes in NOD mice. IS-CD8⁺ T cells are a convenient, reliable and cost-effective tool to study the regulation of tissue-specific homing of diabetogenic T cells. The IS-CD8⁺ T cells/NOD mice adoptive transfer model mimics the effector stage of T1D in humans. We believe that inhibitory approaches that use the IS-CD8⁺ T cells/NOD mice model will greatly increase our understanding of T1D in humans.

IV. T Cell MT1-MMP and CD44 in T1D

A. The MT₁-MMP/CD₄₄ axis

Recently, we demonstrated that MT1-MMP dynamically regulates the functionality of the cell surface-associated signaling and adhesion receptor CD₄₄ in diabetogenic IS-CD8⁺ T cells (Savinov and Strongin, 2007; Savinov *et al.*, 2005, 2006, 2007). The importance of the MT1-MMP-CD₄₄ axis in T1D has been identified both in diabetes transfer model NOD mice and in freshly diabetic NOD mice. By regulating the functionality of CD₄₄, MT1-MMP mediates the transition of T cell adhesion to endothelial cells to the transmigration of T cells which controls the rate of homing of T cells into the pancreatic islets and thus controls the severity of the disease.

Shedding of cellular CD44 by external recombinant MT1-MMP reduces adhesion to the pancreatic endothelium and, as a result, reduces the level of transendothelial migration and the homing of IS-CD8⁺ T cells into the islets. To establish the role of endogenous T cell MT1-MMP in the proteolysis of CD44 and in the adhesion, the transmigration and the homing of T cells into the pancreatic islets, we determined that endogenous MT1-MMP was latent in non-adherent IS-CD8⁺ cells, while adhesion of IS-CD8⁺ cells induced the activation of MT1-MMP, the cleavage of CD44 and the stimulation of T cell transmigration. According to our RT-PCR analysis of the gene expression in the adherent and non-adherent cells, three proprotein convertase family proteinases (furin, PC7 and PACE4) are up-regulated after the adhesion of IS-CD8⁺ T cells to gelatin-coated plastic. PC7 and, especially, furin and PACE4, are potent in the proteolytic processing and the activation of the proenzyme of MT1-MMP in *in vitro* tests (Remacle *et al.*, 2006). Accordingly, we believe that these three proprotein convertases are largely responsible for the activation of MT1-MMP in the adherent T cells, thus leading to the MT1-MMP-dependent proteolysis of T cell CD44. We have also established that CD44 is the primary receptor involved in the adhesion of IS-CD8⁺ T cells to the pancreatic endothelium. Thus, inhibition of the CD44

function by the anti-CD44 function-blocking monoclonal antibody led to a 75–80% decrease of IS-CD8⁺ T cell adhesion leading, in turn, to a decrease of transmigration and homing. We have also demonstrated that MT1-MMP plays the primary role in shedding of T cell CD44. Thus, we have established that MT1-MMP is responsible for 90% of the shedding of CD44 observed in the adherent IS-CD8⁺ T cells while the combined action of proteinases distinct from MT1-MMP is responsible for only 10% of the shedding.

In agreement with the primary role of MT1-MMP in the shedding of CD44, both AG3340 and GM6001 blocked the functional activity of MT1-MMP and the proteolysis of CD44 in the adherent IS-CD8⁺ T cells. These events led to the aberrantly extended rather than the temporal adhesion of IS-CD8⁺ T cells on the pancreatic endothelium. The continuous immobilization on the pancreatic endothelium impeded the transmigration efficiency of the diabetogenic T cells into the pancreas. Overall, the inhibition of MT1-MMP proteolysis of CD44 by either GM6001 or AG3340 reduced the diabetogenic efficiency of T cells by immobilizing the adherent diabetogenic, cytotoxic, T cells on the vascular endothelium, thus preventing T cell homing into the islets. The diminished transmigration led to decreased levels of the homing of IS-CD8⁺ T cells and this event delayed the transferred diabetes onset in NOD mice which received AG3340 (Savinov and Strongin, 2007; Savinov *et al.*, 2005, 2006, 2007).

B. The specific role of T cell MT1-MMP in T1D

It was shown previously in the model of the onset of type 2 diabetes that MMP-2, MMP-12 and MT1-MMP were up-regulated in diabetic male and high-fat-fed female Zucker diabetic fatty rats as compared to their non-diabetic lean counterparts (Zhou *et al.*, 2005). PD166793 [(S)-2-(4'-bromobiphenyl-4-sulfonylamino)-3-methyl-butyric acid] (a broad-range inhibitor with EC₅₀ values of 6100, 47, 12, 7200, 7900, 8, and 240 nM against MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-13, and MT1-MMP, respectively) (O'Brien *et al.*, 2000; Peterson *et al.*, 2001) preserved β cell mass, presumably, by decreasing the turnover of islet extracellular matrix molecules. The study suggested that pancreatic MMPs play a role in the maintenance of β cell mass. We, in turn, chose to focus our studies on the T cell MT1-MMP instead of on MMPs from the pancreata.

To validate the specific role of T cell MT1-MMP as well as to elucidate the potential significance of other MMPs in the NOD model of T1D, we used AG3340 and two additional inhibitors, EGCG and SB-3CT. While both EGCG and SB-3CT are poor inhibitors of MT1-MMP, they are capable of targeting MMPs distinct from MT1-MMP. As a result of our cell-based assays and *in vivo* tests employing both a diabetes transfer model in NOD mice and *in vivo* visualization of IS-CD8⁺ T cells pre-labeled with a fluorescence dye, didodecyl-tetramethylindocarbocyanine perchlorate, and then injected in NOD mice only AG3340, the antagonist of MT1-MMP, delivered clinically-relevant effects. In contrast, EGCG and SB-3CT, were without effect in our studies. Because of the wide-range specificity of the MMP inhibitors, a simultaneous assessment of AG3340, SB-3CT, and EGCG permitted us to conclude that only T cell MT1-MMP plays a significant role in T1D. The combined effect of all other MMPs, including MMP-2 and MMP-9, both of which are efficiently inhibited by SB-3CT, is far less important. We conclude that only MT1-MMP antagonists such as AG3340, as opposed to other broad-range inhibitors of MMPs, are efficient in delaying T1D transfer to NOD mice. We believe that these results confirm the functional importance of the MT1-MMP-CD44 axis in mediating the efficiency of transendothelial migration and the homing of diabetogenic T cells to the pancreatic islets.

C. Potential clinical relevance of targeting T cell MT1-MMP in T1D

Consistent with our biochemical model of the MT1-MMP-CD44 interactions, low dosages of AG3340 (1–5 mg/kg) injected jointly with insulin specifically inhibit T cell intra-islet transmigration, restore β cell functionality, increase insulin-producing β cell mass and alleviate the severity of T1D in acutely diabetic NOD mice. As a result, acutely diabetic NOD mice do not require insulin injections for survival for a significant 30-day time period thus providing a promising clue to finding the means to reverse IDDM in humans. The extensive morphometric analyses and the measurements of both the C peptide blood levels and the proinsulin mRNA levels in the islets confirmed the highly beneficial effects of the inhibitor. Diabetes transfer experiments suggest that the inhibitor specifically represses the T cell transmigration and homing processes as opposed to causing general immunosuppression. According to relevant recent publications (Hao *et al.*, 2006) and in agreement with our observations, endothelial precursor stem cells instead of the β -cells themselves are the source of the regenerated, functional, β -cells.

To prove that insulin-producing β -cells were regenerated, NOD mice were allowed to develop T1D. Diseased mice then received insulin alone or insulin jointly with AG3340 for 40 days. Insulin injections were then suspended. Mice which received insulin after the onset of the disease became hyperglycemic in a matter of 2–3 days and were then sacrificed. In contrast, mice which received insulin jointly with the inhibitor restored the pool of insulin-producing β -cells. When insulin injections were cancelled, this β -cell pool was sufficient for the survival of these mice which continued to be normoglycemic/mildly hyperglycemic for several weeks without the use of external insulin.

In contrast to the exocrine pancreatic cells, β cells do not deposit a conventional basement membrane. Instead, they rely on the endothelial cells, which form capillaries with a vascular basement membrane next to the β cells (Nikolova *et al.*, 2006; Yoshitomi and Zaret, 2004). Endothelial basement membranes are rich in laminins, collagen type IV and fibronectin. It is noteworthy that the islet capsule represents an alternative, nonvascular source of laminin and collagen. Because of the spheroid shape of the islets, a large number of β cells is not in contact with the islet capsule, and, therefore, β cells are normally in contact with the vascular basement membrane (Olsson and Carlsson, 2006). Signals from the vascular basement membrane regulate the expression of insulin and control the proliferation of β cells and their progenitors (Duvillie *et al.*, 2002; Lammert *et al.*, 2001, 2003). Specifically, laminin-411 ($\alpha 4\beta 1\gamma 1$) and laminin-511 ($\alpha 5\beta 1\gamma 1$) are the major laminin species which are present in the vascular basement membrane (Hallmann *et al.*, 2005; Sixt *et al.*, 2001). Plating on laminin-111, -411, and -511 but not on collagen type I, collagen type IV or fibronectin, significantly increases the expression of both the *Ins1* and *Ins2* genes and stimulates the proliferation of murine β cells. Soluble laminins exhibit only a fraction of this effect, suggesting that the interaction of β cells with the laminin matrix is required for the stimulation of these processes (Nikolova *et al.*, 2006). The effects of laminins require the presence of $\beta 1$ integrin in β cells. This integrin is predominantly represented in β cells by the $\alpha 6\beta 1$ integrin, which is a laminin receptor, and by the $\alpha 1\beta 1$ integrin, which is a collagen receptor (Kaido *et al.*, 2004). The function-blocking $\beta 1$ antibody reduces the proliferative cell response and the expression of insulin in β cells plated on laminin-111. Furthermore, collagen type IV, which is normally secreted by the islet endothelial cells, interacts with β cell integrin $\alpha 1\beta 1$ and this interaction also stimulates insulin secretion by β cells (Kaido *et al.*, 2004). We suggest that the inhibitors of MT1-MMP rescue the laminin-stimulated $\beta 1$ integrin signaling in β cells and that this event additionally contributes to survival, rejuvenation and insulin production by β cells (Sixt *et al.*, 2001; Jin *et al.*, 2007).

In summary, we are now confident that AG3340 provides diabetes protection by effectively controlling islet-destructive autoimmunity and stimulating the functional recovery of

insulin-producing β -cells and the regeneration of the pancreatic islets, thus providing a sound mechanistic rationale for clinical trials of the inhibitors of MT1-MMP, including AG3340, in T1D in humans. Overall, our current and prior findings (Savinov and Strongin, 2007; Savinov *et al.*, 2005, 2006, 2007) provide a working hypothesis for the novel, antidiabetic, application of the existing inhibitors of MT1-MMP (Fig. 18.2). Our data suggest that inhibition of T cell MT1-MMP is a key to the design of novel and effective therapies of T1D. Because the inhibitor excess to MMPs localized in the poorly angiogenic tumors is limited relative to the cell-surface protease T cell protease, it is highly unlikely that the bioavailability of the T cell MT1-MMP targeting drugs will pose a problem in T1D treatment. Based on the results we obtained with NOD mice, we hypothesize that the pharmacological inhibition of MT1-MMP by specific antagonists, including AG3340, will diminish the homing of T killer cells into the islets and that this event will stimulate the regeneration of insulin-producing β cells leading to a favorable outcome for T1D patients (Chong *et al.*, 2006).

Acknowledgments

This work was supported by National Institutes of Health Grants CA83017, CA77470 and DK071956 (to Strongin).

REFERENCES

- Annabi B, Lachambre MP, Bousquet-Gagnon N, Page M, Gingras D, Beliveau R. Green tea polyphenol (–)-epigallocatechin 3-gallate inhibits MMP-2 secretion and MT1-MMP-driven migration in glioblastoma cells. *Biochim. Biophys. Acta* 2002;1542:209–220. [PubMed: 11853893]
- Aruffo A, Stamenkovic I, Melnick M, Underhill CB, Seed B. CD44 is the principal cell surface receptor for hyaluronate. *Cell* 1990;61:1303–1313. [PubMed: 1694723]
- Avigdor A, Goichberg P, Shvitiel S, Dar A, Peled A, Samira S, Kollet O, Hershkovich R, Alon R, Hardan I, Ben-Hur H, Naor D, et al. CD44 and hyaluronic acid cooperate with SDF-1 in the trafficking of human CD34 stem/progenitor cells to bone marrow. *Blood* 2004;103:2981–2989. [PubMed: 15070674]
- Bennett KL, Modrell B, Greenfield B, Bartolazzi A, Stamenkovic I, Peach R, Jackson DG, Spring F, Aruffo A. Regulation of CD44 binding to hyaluronan by glycosylation of variably spliced exons. *J. Cell Biol* 1995;131:1623–1633. [PubMed: 8522617]
- Bonfil RD, Sabbota A, Nabha S, Bernardo MM, Dong Z, Meng H, Yamamoto H, Chinni SR, Lim IT, Chang M, Filetti LC, Mobashery S, et al. Inhibition of human prostate cancer growth, osteolysis and angiogenesis in a bone metastasis model by a novel mechanism-based selective gelatinase inhibitor. *Int. J. Cancer* 2006;118:2721–2726. [PubMed: 16381009]
- Butcher EC, Picker LJ. Lymphocyte homing and homeostasis. *Science* 1996;272:60–66. [PubMed: 8600538]
- Butcher EC, Williams M, Youngman K, Rott L, Briskin M. Lymphocyte trafficking and regional immunity. *Adv. Immunol* 1999;72:209–253. [PubMed: 10361577]
- Campbell JJ, Butcher EC. Chemokines in tissue-specific and microenvironment-specific lymphocyte homing. *Curr. Opin. Immunol* 2000;12:336–341. [PubMed: 10781407]
- Campbell JJ, Hedrick J, Zlotnik A, Siani MA, Thompson DA, Butcher EC. Chemokines and the arrest of lymphocytes rolling under flow conditions. *Science* 1998;279:381–384. [PubMed: 9430588]
- Cappuzzo F, Bartolini S, Crino L. Emerging drugs for non-small cell lung cancer. *Expert Opin. Emerg. Drugs* 2003;8:179–192. [PubMed: 14610920]
- Celenza G, Villegas-Estrada A, Lee M, Boggess B, Forbes C, Wolter WR, Suckow MA, Mobashery S, Chang M. Metabolism of (4-phenoxyphenylsulfonyl) methylthiirane, a selective gelatinase inhibitor. *Chem. Biol. Drug. Des* 2008;71:187–196. [PubMed: 18221479]
- Chiang WC, Wong YK, Lin SC, Chang KW, Liu CJ. Increase of MMP-13 expression in multi-stage oral carcinogenesis and epigallocatechin-3-gallate suppress MMP-13 expression. *Oral. Dis* 2006;12:27–33. [PubMed: 16390465]

- Chong AS, Shen J, Tao J, Yin D, Kuznetsov A, Hara M, Philipson LH. Reversal of diabetes in non-obese diabetic mice without spleen cell-derived beta cell regeneration. *Science* 2006;311:1774–1775. [PubMed: 16556844]
- Cichy J, Pure E. The liberation of CD44. *J. Cell Biol* 2003;161:839–843. [PubMed: 12796473]
- Constantin G, Majeed M, Giagulli C, Piccio L, Kim JY, Butcher EC, Laudanna C. Chemokines trigger immediate beta2 integrin affinity and mobility changes: Differential regulation and roles in lymphocyte arrest under flow. *Immunity* 2000;13:759–769. [PubMed: 11163192]
- DeGrendele HC, Estess P, Siegelman MH. Requirement for CD44 in activated T cell extravasation into an inflammatory site. *Science* 1997;278:672–675. [PubMed: 9381175]
- Dell'Aica I, Dona M, Sartor L, Pezzato E, Garbisa S. (–)Epigallocatechin-3-gallate directly inhibits MT1-MMP activity, leading to accumulation of nonactivated MMP-2 at the cell surface. *Lab. Invest* 2002;82:1685–1693. [PubMed: 12480918]
- Deryugina EI, Ratnikov BI, Yu Q, Baciu PC, Rozanov DV, Strongin AY. Prointegrin maturation follows rapid trafficking and processing of MT1-MMP in Furin-Negative Colon Carcinoma LoVo Cells. *Traffic* 2004;5:627–641. [PubMed: 15260832]
- Duvillie B, Currie C, Chrones T, Bucchini D, Jami J, Joshi RL, Hill DJ. Increased islet cell proliferation, decreased apoptosis, and greater vascularization leading to beta-cell hyperplasia in mutant mice lacking insulin. *Endocrinology* 2002;143:1530–1537. [PubMed: 11897712]
- Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. *Nat. Rev. Cancer* 2002;2:161–174. [PubMed: 11990853]
- El Bedoui J, Oak MH, Anglard P, Schini-Kerth VB. Catechins prevent vascular smooth muscle cell invasion by inhibiting MT1-MMP activity and MMP-2 expression. *Cardiovasc. Res* 2005;67:317–325. [PubMed: 15885676]
- Estess P, DeGrendele HC, Pascual V, Siegelman MH. Functional activation of lymphocyte CD44 in peripheral blood is a marker of autoimmune disease activity. *J. Clin. Invest* 1998;102:1173–1182. [PubMed: 9739051]
- Estess P, Nandi A, Mohamadzadeh M, Siegelman MH. Interleukin 15 induces endothelial hyaluronan expression *in vitro* and promotes activated T cell extravasation through a CD44-dependent pathway *in vivo*. *J. Exp. Med* 1999;190:9–19. [PubMed: 10429666]
- Gallegos AM, Bevan MJ. Driven to autoimmunity: The nod mouse. *Cell* 2004;117:149–151. [PubMed: 15084253]
- Galvez BG, Matias-Roman S, Yanez-Mo M, Vicente-Manzanares M, Sanchez-Madrid F, Arroyo AG. Caveolae are a novel pathway for membrane-type 1 matrix metalloproteinase traffic in human endothelial cells. *Mol. Biol. Cell* 2004;15:678–687. [PubMed: 14657245]
- Gomis-Ruth FX. Structural aspects of the metzincin clan of metalloendopeptidases. *Mol. Biotechnol* 2003;24:157–202. [PubMed: 12746556]
- Gross J, Lapiere CM. Collagenolytic activity in amphibian tissues: A tissue culture assay. *Proc. Natl. Acad. Sci. USA* 1962;48:1014–1022. [PubMed: 13902219]
- Gu Z, Cui J, Brown S, Fridman R, Mobashery S, Strongin AY, Lipton SA. A highly specific inhibitor of matrix metalloproteinase-9 rescues laminin from proteolysis and neurons from apoptosis in transient focal cerebral ischemia. *J. Neurosci* 2005;25:6401–6408. [PubMed: 16000631]
- Hallmann R, Horn N, Selg M, Wendler O, Pausch F, Sorokin LM. Expression and function of laminins in the embryonic and mature vasculature. *Physiol. Rev* 2005;85:979–1000. [PubMed: 15987800]
- Hande KR, Collier M, Paradiso L, Stuart-Smith J, Dixon M, Clendeninn N, Yeun G, Alberti D, Binger K, Wilding G. Phase I and pharmacokinetic study of prinomastat, a matrix metalloprotease inhibitor. *Clin. Cancer Res* 2004;10:909–915. [PubMed: 14871966]
- Hao E, Tyrberg B, Itkin-Ansari P, Lakey JR, Geron I, Monosov EZ, Barcova M, Mercola M, Levine F. Beta-cell differentiation from nonendocrine epithelial cells of the adult human pancreas. *Nat. Med* 2006;12:310–316. [PubMed: 16491084]
- Holmbeck K, Bianco P, Caterina J, Yamada S, Kromer M, Kuznetsov SA, Mankani M, Robey PG, Poole AR, Pidoux I, Ward JM, Birkedal-Hansen H. MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. *Cell* 1999;99:81–92. [PubMed: 10520996]

- Holmbeck K, Bianco P, Chrysovergis K, Yamada S, Birkedal-Hansen H. MT1-MMP-dependent, apoptotic remodeling of unmineralized cartilage: A critical process in skeletal growth. *J. Cell Biol* 2003;163:661–671. [PubMed: 14610065]
- Holmbeck K, Bianco P, Yamada S, Birkedal-Hansen H. MT1-MMP: A tethered collagenase. *J. Cell. Physiol* 2004;200:11–19. [PubMed: 15137053]
- Ikejiri M, Bernardo MM, Bonfil RD, Toth M, Chang M, Fridman R, Mobashery S. Potent mechanism-based inhibitors for matrix metalloproteinases. *J. Biol. Chem* 2005a;280:33992–34002. [PubMed: 16046398]
- Ikejiri M, Bernardo MM, Meroueh SO, Brown S, Chang M, Fridman R, Mobashery S. Design, synthesis, and evaluation of a mechanism-based inhibitor for gelatinase A. *J. Org. Chem* 2005b;70:5709–5712. [PubMed: 15989356]
- Itoh Y, Seiki M. MT1-MMP: A potent modifier of pericellular microenvironment. *J. Cell. Physiol* 2006;206:1–8. [PubMed: 15920734]
- Jiang A, Lehti K, Wang X, Weiss SJ, Keski-Oja J, Pei D. Regulation of membrane-type matrix metalloproteinase 1 activity by dynamin-mediated endocytosis. *Proc. Natl. Acad. Sci. USA* 2001;98:13693–13698. [PubMed: 11698655]
- Jin EJ, Choi YA, Kyun Park E, Bang OS, Kang SS. MMP-2 functions as a negative regulator of chondrogenic cell condensation via down-regulation of the FAK-integrin beta1 interaction. *Dev. Biol* 2007;308:474–484. [PubMed: 17604018]
- Kaido T, Yebra M, Cirulli V, Montgomery AM. Regulation of human beta-cell adhesion, motility, and insulin secretion by collagen IV and its receptor alpha1-beta1. *J. Biol. Chem* 2004;279:53762–53769. [PubMed: 15485856]
- Kajita M, Itoh Y, Chiba T, Mori H, Okada A, Kinoh H, Seiki M. Membrane-type 1 matrix metalloproteinase cleaves CD44 and promotes cell migration. *J. Cell Biol* 2001;153:893–904. [PubMed: 11381077]
- Katoh S, Zheng Z, Oritani K, Shimoizato T, Kincade PW. Glycosylation of CD44 negatively regulates its recognition of hyaluronan. *J. Exp. Med* 1995;182:419–429. [PubMed: 7543137]
- Kim HS, Kim MH, Jeong M, Hwang YS, Lim SH, Shin BA, Ahn BW, Jung YD. EGCG blocks tumor promoter-induced MMP-9 expression via suppression of MAPK and AP-1 activation in human gastric AGS cells. *Anticancer Res* 2004;24:747–753. [PubMed: 15161022]
- Knauper V, Bailey L, Worley JR, Soloway P, Patterson ML, Murphy G. Cellular activation of proMMP-13 by MT1-MMP depends on the C-terminal domain of MMP-13. *FEBS Lett* 2002;532:127–130. [PubMed: 12459476]
- Labrecque L, Nyalendo C, Langlois S, Durocher Y, Roghi C, Murphy G, Gingras D, Beliveau R. Src-mediated tyrosine phosphorylation of caveolin-1 induces its association with membrane type 1 matrix metalloproteinase. *J. Biol. Chem* 2004;279:52132–52140. [PubMed: 15466865]
- Lammert E, Cleaver O, Melton D. Induction of pancreatic differentiation by signals from blood vessels. *Science* 2001;294:564–567. [PubMed: 11577200]
- Lammert E, Gu G, McLaughlin M, Brown D, Brekken R, Murtaugh LC, Gerber HP, Ferrara N, Melton DA. Role of VEGF-A in vascularization of pancreatic islets. *Curr. Biol* 2003;13:1070–1074. [PubMed: 12814555]
- Lee JH, Chung JH, Cho KH. The effects of epigallocatechin-3-gallate on extracellular matrix metabolism. *J. Dermatol. Sci* 2005;40:195–204. [PubMed: 16102944]
- Lee M, Hesek D, Shi Q, Noll BC, Fisher JF, Chang M, Mobashery S. Conformational analyses of thiirane-based gelatinase inhibitors. *Bioorg. Med. Chem. Lett* 2007;18:3064–3067. [PubMed: 18083555]
- Lesley J, Hyman R, Kincade PW. CD44 and its interaction with extracellular matrix. *Adv. Immunol* 1993;54:271–335. [PubMed: 8379464]
- McQuibban GA, Gong JH, Tam EM, McCulloch CA, Clark-Lewis I, Overall CM. Inflammation dampened by gelatinase A cleavage of monocyte chemoattractant protein-3. *Science* 2000;289:1202–1206. [PubMed: 10947989]
- Mohamadzadeh M, DeGrendele H, Arizpe H, Estess P, Siegelman M. Proinflammatory stimuli regulate endothelial hyaluronan expression and CD44/HA-dependent primary adhesion. *J. Clin. Invest* 1998;101:97–108. [PubMed: 9421471]

- Mori H, Tomari T, Koshikawa N, Kajita M, Itoh Y, Sato H, Tojo H, Yana I, Seiki M. CD44 directs membrane-type 1 matrix metalloproteinase to lamelli-podia by associating with its hemopexin-like domain. *EMBO J* 2002;21:3949–3959. [PubMed: 12145196]
- Murai S, Umemiya T, Seiki M, Harigaya K. Expression and localization of membrane-type-1 matrix metalloproteinase, CD 44, and laminin-5gamma2 chain during colorectal carcinoma tumor progression. *Virchows Arch* 2004;445:271–278. [PubMed: 15517370]
- Murphy G, Stanton H, Cowell S, Butler G, Knauper V, Atkinson S, Gavrilovic J. Mechanisms for pro matrix metalloproteinase activation. *APMIS* 1999;107:38–44. [PubMed: 10190278]
- Nagase H, Woessner JF Jr. Matrix metalloproteinases. *J. Biol. Chem* 1999;274:21491–21494. [PubMed: 10419448]
- Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc. Res* 2006;69:562–573. [PubMed: 16405877]
- Nakamura H, Suenaga N, Taniwaki K, Matsuki H, Yonezawa K, Fujii M, Okada Y, Seiki M. Constitutive and induced CD44 shedding by ADAM-like proteases and membrane-type 1 matrix metalloproteinase. *Cancer Res* 2004;64:876–882. [PubMed: 14871815]
- Nandi A, Estess P, Siegelman M. Bimolecular complex between rolling and firm adhesion receptors required for cell arrest; CD44 association with VLA-4 in T cell extravasation. *Immunity* 2004;20:455–465. [PubMed: 15084274]
- Nikolova G, Jabs N, Konstantinova I, Domogatskaya A, Tryggvason K, Sorokin L, Fassler R, Gu G, Gerber HP, Ferrara N, Melton DA, Lammert E. The vascular basement membrane: A niche for insulin gene expression and Beta cell proliferation. *Dev. Cell* 2006;10:397–405. [PubMed: 16516842]
- O'Brien PM, Ortwine DF, Pavlovsky AG, Picard JA, Sliskovic DR, Roth BD, Dyer RD, Johnson LL, Man CF, Hallak H. Structure-activity relationships and pharmacokinetic analysis for a series of potent, systemically available biphenylsulfonamide matrix metalloproteinase inhibitors. *J. Med. Chem* 2000;43:156–166. [PubMed: 10649971]
- Oh J, Takahashi R, Adachi E, Kondo S, Kuratomi S, Noma A, Alexander DB, Motoda H, Okada A, Seiki M, Itoh T, Itohara S, et al. Mutations in two matrix metalloproteinase genes, MMP-2 and MT1-MMP, are synthetic lethal in mice. *Oncogene* 2004;23:5041–5048. [PubMed: 15064723]
- Olsson R, Carlsson PO. The pancreatic islet endothelial cell: Emerging roles in islet function and disease. *Int. J. Biochem. Cell Biol* 2006;38:492–497. [PubMed: 16162421]
- Osenkowski P, Toth M, Fridman R. Processing, shedding, and endocytosis of membrane type 1-matrix metalloproteinase (MT1-MMP). *J. Cell. Physiol* 2004;200:2–10. [PubMed: 15137052]
- Pei D, Weiss SJ. Furin-dependent intracellular activation of the human stromelysin-3 zymogen. *Nature* 1995;375:244–247. [PubMed: 7746327]
- Peterson JT, Hallak H, Johnson L, Li H, O'Brien PM, Sliskovic DR, Bocan TM, Coker ML, Etoh T, Spinale FG. Matrix metalloproteinase inhibition attenuates left ventricular remodeling and dysfunction in a rat model of progressive heart failure. *Circulation* 2001;103:2303–2309. [PubMed: 11342481]
- Remacle AG, Rozanov DV, Fugere M, Day R, Strongin AY. Furin regulates the intracellular activation and the uptake rate of cell surface-associated MT1-MMP. *Oncogene* 2006;25:5648–5655. [PubMed: 16636666]
- Rozanov DV, Deryugina EI, Monosov EZ, Marchenko ND, Strongin AY. Aberrant, persistent inclusion into lipid rafts limits the tumorigenic function of membrane type-1 matrix metalloproteinase in malignant cells. *Exp. Cell Res* 2004;293:81–95. [PubMed: 14729059]
- Sallusto F, Mackay CR, Lanzavecchia A. The role of chemokine receptors in primary, effector, and memory immune responses. *Annu. Rev. Immunol* 2000;18:593–620. [PubMed: 10837070]
- Sato H, Takino T, Okada Y, Cao J, Shinagawa A, Yamamoto E, Seiki M. A matrix metalloproteinase expressed on the surface of invasive tumour cells. *Nature* 1994;370:61–65. [PubMed: 8015608]
- Savinov AY, Strongin AY. Defining the roles of T cell membrane proteinase and CD44 in type 1 diabetes. *IUBMB Life* 2007;59:6–13. [PubMed: 17365174]
- Savinov AY, Tcherepanov A, Green EA, Flavell RA, Chervonsky AV. Contribution of Fas to diabetes development. *Proc. Natl. Acad. Sci. USA* 2003a;100:628–632. [PubMed: 12525697]

- Savinov AY, Wong FS, Stonebraker AC, Chervonsky AV. Presentation of antigen by endothelial cells and chemoattraction are required for homing of insulin-specific CD8⁺ T cells. *J. Exp. Med* 2003b; 197:643–656. [PubMed: 12615905]
- Savinov AY, Rozanov DV, Golubkov VS, Wong FS, Strongin AY. Inhibition of membrane type-1 matrix metalloproteinase by cancer drugs interferes with the homing of diabetogenic T cells into the pancreas. *J. Biol. Chem* 2005;280:27755–27758. [PubMed: 15944163]
- Savinov AY, Rozanov DV, Strongin AY. Mechanistic insights into targeting T cell membrane proteinase to promote islet beta-cell rejuvenation in type 1 diabetes. *FASEB J* 2006;20:1793–1801. [PubMed: 16940151]
- Savinov AY, Rozanov DV, Strongin AY. Specific inhibition of autoimmune T cell transmigration contributes to beta cell functionality and insulin synthesis in non-obese diabetic (NOD) mice. *J. Biol. Chem* 2007;282:32106–32111. [PubMed: 17761671]
- Seiki M. Membrane-type 1 matrix metalloproteinase: A key enzyme for tumor invasion. *Cancer Lett* 2003;194:1–11. [PubMed: 12706853]
- Serreze DV, Leiter EH, Christianson GJ, Greiner D, Roopenian DC. Major histocompatibility complex class I-deficient NOD-B2mnull mice are diabetes and insulinitis resistant. *Diabetes* 1994;43:505–509. [PubMed: 8314025]
- Shalinsky DR, Brekken J, Zou H, McDermott CD, Forsyth P, Edwards D, Margosiak S, Bender S, Truitt G, Wood A, Varki NM, Appelt K. Broad antitumor and antiangiogenic activities of AG3340, a potent and selective MMP inhibitor undergoing advanced oncology clinical trials. *Ann. NY Acad. Sci* 1999;878:236–270. [PubMed: 10415735]
- Sixt M, Engelhardt B, Pausch F, Hallmann R, Wendler O, Sorokin LM. Endothelial cell laminin isoforms, laminins 8 and 10, play decisive roles in T cell recruitment across the blood-brain barrier in experimental autoimmune encephalomyelitis. *J. Cell Biol* 2001;153:933–946. [PubMed: 11381080]
- Slevin M, Kumar S, Gaffney J. Angiogenic oligosaccharides of hyaluronan induce multiple signaling pathways affecting vascular endothelial cell mitogenic and wound healing responses. *J. Biol. Chem* 2002;277:41046–41059. [PubMed: 12194965]
- Sodek KL, Ringuette MJ, Brown TJ. MT1-MMP is the critical determinant of matrix degradation and invasion by ovarian cancer cells. *Br. J. Cancer* 2007;97:358–367. [PubMed: 17609667]
- Song XZ, Xia JP, Bi ZG. Effects of (–)-epigallocatechin-3-gallate on expression of matrix metalloproteinase-1 and tissue inhibitor of metalloproteinase-1 in fibroblasts irradiated with ultraviolet A. *Chin. Med. J* 2004;117:1838–1841. [PubMed: 15603715]
- Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: The multistep paradigm. *Cell* 1994;76:301–314. [PubMed: 7507411]
- Stein JV, Rot A, Luo Y, Narasimhaswamy M, Nakano H, Gunn MD, Matsuzawa A, Quackenbush EJ, Dorf ME, von Andrian UH. The CC chemokine thymus-derived chemotactic agent 4 (TCA-4, secondary lymphoid tissue chemokine, 6CKine, exodus-2) triggers lymphocyte function-associated antigen 1-mediated arrest of rolling T lymphocytes in peripheral lymph node high endothelial venules. *J. Exp. Med* 2000;191:61–76. [PubMed: 10620605]
- Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. *Annu. Rev. Cell Dev. Biol* 2001;17:463–516. [PubMed: 11687497]
- Stocker W, Bode W. Structural features of a superfamily of zinc-endopeptidases: The metzincins. *Curr. Opin. Struct. Biol* 1995;5:383–390. [PubMed: 7583637]
- Strongin AY. Mislocalization and unconventional functions of cellular MMPs in cancer. *Cancer Metastasis Rev* 2006;25:87–98.
- Strongin AY, Collier I, Bannikov G, Marmer BL, Grant GA, Goldberg GI. Mechanism of cell surface activation of 72-kDa type IV collagenase. Isolation of the activated form of the membrane metalloprotease. *J. Biol. Chem* 1995;270:5331–5338. [PubMed: 7890645]
- Suenaga N, Mori H, Itoh Y, Seiki M. CD44 binding through the hemopexin-like domain is critical for its shedding by membrane-type 1 matrix metalloproteinase. *Oncogene* 2005;24:859–868. [PubMed: 15558018]
- Turley EA, Noble PW, Bourguignon LY. Signaling properties of hyaluronan receptors. *J. Biol. Chem* 2002;277:4589–4592. [PubMed: 11717317]

- Van Wart HE, 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* 1990;87:5578–5582. [PubMed: 2164689]
- Vayalil PK, Katiyar SK. Treatment of epigallocatechin-3-gallate inhibits matrix metalloproteinases-2 and -9 via inhibition of activation of mitogen-activated protein kinases, c-jun and NF-kappaB in human prostate carcinoma DU-145 cells. *Prostate* 2004;59:33–42. [PubMed: 14991864]
- Verdaguer J, Schmidt D, Amrani A, Anderson B, Averill N, Santamaria P. Spontaneous autoimmune diabetes in monoclonal T cell nonobese diabetic mice. *J. Exp. Med* 1997;186:1663–1676. [PubMed: 9362527]
- von Andrian UH, Mackay CR. T-cell function and migration. Two sides of the same coin. *N. Engl. J. Med* 2000;343:1020–1034. [PubMed: 11018170]
- Wang P, Wang X, Pei D. Mint-3 regulates the retrieval of the internalized membrane-type matrix metalloproteinase, MT5-MMP, to the plasma membrane by binding to its carboxyl end motif EWV. *J. Biol. Chem* 2004a;279:20461–20470. [PubMed: 14990567]
- Wang X, Ma D, Keski-Oja J, Pei D. Co-recycling of MT1-MMP and MT3-MMP Through the trans-Golgi Network: Identification of DKV582 as a Recycling Signal. *J. Biol. Chem* 2004b;279:9331–9336. [PubMed: 14665622]
- Weber C. Novel mechanistic concepts for the control of leukocyte transmigration: Specialization of integrins, chemokines, and junctional molecules. *J. Mol. Med* 2003;81:4–19. [PubMed: 12545245]
- Weiss L, Slavin S, Reich S, Cohen P, Shuster S, Stern R, Kaganovsky E, Okon E, Rubinstein AM, Naor D. Induction of resistance to diabetes in non-obese diabetic mice by targeting CD44 with a specific monoclonal antibody. *Proc. Natl. Acad. Sci. USA* 2000;97:285–290. [PubMed: 10618410]
- Will H, Atkinson SJ, Butler GS, Smith B, Murphy G. The soluble catalytic domain of membrane type 1 matrix metalloproteinase cleaves the propeptide of procollagenase A and initiates autolytic activation. Regulation by TIMP-2 and TIMP-3. *J. Biol. Chem* 1996;271:17119–17123. [PubMed: 8663332]
- Wong FS, Visintin I, Wen L, Flavell RA, Janeway CA Jr. CD8 T cell clones from young nonobese diabetic (NOD) islets can transfer rapid onset of diabetes in NOD mice in the absence of CD4 cells. *J. Exp. Med* 1996;183:67–76. [PubMed: 8551245]
- Wong FS, Karttunen J, Dumont C, Wen L, Visintin I, Pilip IM, Shastri N, Pamer EG, Janeway CA Jr. Identification of an MHC class I-restricted autoantigen in type 1 diabetes by screening an organ-specific cDNA library. *Nat. Med* 1999;5:1026–1031. [PubMed: 10470079]
- Worthylake RA, Burridge K. Leukocyte transendothelial migration: Orchestrating the underlying molecular machinery. *Curr. Opin. Cell Biol* 2001;13:569–577. [PubMed: 11544025]
- Yana I, Weiss SJ. Regulation of membrane type-1 matrix metalloproteinase activation by proprotein convertases. *Mol. Biol. Cell* 2000;11:2387–2401. [PubMed: 10888676]
- Yang XD, Michie SA, Mebius RE, Tisch R, Weissman I, McDevitt HO. The role of cell adhesion molecules in the development of IDDM: Implications for pathogenesis and therapy. *Diabetes* 1996;45:705–710. [PubMed: 8635641]
- Yoshitomi H, Zaret KS. Endothelial cell interactions initiate dorsal pancreas development by selectively inducing the transcription factor Ptf1a. *Development* 2004;131:807–817. [PubMed: 14736742]
- Zhou YP, Madjidi A, Wilson ME, Nothhelfer DA, Johnson JH, Palma JF, Schweitzer A, Burant C, Blume JE, Johnson JD. Matrix metalloproteinases contribute to insulin insufficiency in Zucker diabetic fatty rats. *Diabetes* 2005;54:2612–2619. [PubMed: 16123349]
- Zucker S, Cao J, Chen WT. Critical appraisal of the use of matrix metalloproteinase inhibitors in cancer treatment. *Oncogene* 2000;19:6642–6650. [PubMed: 11426650]
- Zucker S, Pei D, Cao J, Lopez-Otin C. Membrane type-matrix metalloproteinases (MT-MMP). *Curr. Top. Dev. Biol* 2003;54:1–74. [PubMed: 12696745]

Figure 18.1. Domain structure of MMPs

The structure of MMPs is made up of the following homologous domains: (1) a signal peptide; directs MMPs to the secretory or plasma membrane insertion pathway; (2) a prodomain; confers latency to MMPs, (3) a catalytic domain with the active site zinc atom; (4) a hemopexin-like domain; in coordination with the catalytic domain controls the interactions with substrates, and (5) a flexible hinge region; links the catalytic and the hemopexin domain and provides each domain mobility relative to the other. The membrane-type MMPs contain an additional transmembrane domain and a short cytoplasmic tail domain (MMP-14, MMP-15, MMP-16, and MMP-24) or a glycosylphosphatidyl inositol linkage, which attaches MMP-17 and MMP-25 to the cell surface. MMP-2 and MMP-9

contain fibronectin-like type II repeats which assist in collagen substrate binding. A hemopexin domain is absent in MMP-7 and MMP-26. RXKR, furin cleavage motif. Date of discovery of MMP is shown in parentheses. MMP-1/interstitial collagenase (1986); MMP-2/gelatinase A (1988); MMP-3/stromelysin-1 (1985); MMP-7/matrilysin (1988); MMP-8/collagenase-2 (1990); MMP-9/gelatinase B (1989); MMP-10/stromelysin-2 (1988); MMP-11/stromelysin-3 (1990); MMP-12/metalloelastase (1992); MMP-13/collagenase-3 (1994); MMP-14/MT1-MMP (1994); MMP-15/MT2-MMP (1995); MMP-16/MT3-MMP (1999); MMP-17/MT4-MMP (1996); MMP-18/collagenase-4 (1996); MMP-19/RASI-1 (1996); MMP-20/enamelysin (1997); MMP-21/XMMP (1998); MMP-22/CMMP (1998); MMP-23 (1999); MMP-24/MT5-MMP (1999); MMP-25/MT6-MMP (1999); MMP-26/endometase (2001); MMP-27 (2000); MMP-28/epilysin (2001).

Figure 18.2. Schematic representation of the role of T cell MT1-MMP in diabetogenesis
 AG3340, a hydroxamate class inhibitor of matrix metalloproteinases; CAMs, cell adhesion molecules; HA, hyaluronan; T1D, type 1 diabetes; TCR, T cell receptor; MHC, major histocompatibility complex.