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Regulation of smooth muscle cell growth, function and death *in vitro* by activated mast cells—a potential mechanism for the weakening and rupture of atherosclerotic plaques

Markus J. Leskinen, Petri T. Kovanen, Ken A. Lindstedt*

Wihuri Research Institute, Kalliolinnantie 4, FIN-00140 Helsinki, Finland

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Abstract

The fibrous cap of a lipid-containing atherosclerotic plaque consists of collagen produced by arterial smooth muscle cells (SMCs) of synthetic phenotype. A thick cap protects the lipid-rich core, whereas a thin cap predisposes it to rupture, with ensuing acute clinical complications, such as myocardial infarction. Among the pathological mechanisms leading to plaque weakening and rupture, one possibility is loss of the matrix-synthesizing SMCs. Indeed, caps of ruptured coronary plaques contain a reduced number of SMCs. In contrast, in such lesions, the number of activated inflammatory cells, such as mast cells, is increased, suggesting that they may regulate the SMC number. We have shown that heparin proteoglycans secreted by activated mast cells can efficiently inhibit proliferation of SMCs *in vitro* and reduce their ability to produce collagen. Chymase, a neutral serine protease secreted by activated mast cells, can also inhibit SMC-mediated collagen synthesis by a transforming growth factor-β-dependent and -independent mechanism, and moreover, cause degradation of the collagen matrix by activating latent interstitial collagenase (MMP-1). Furthermore, chymase can induce SMC apoptosis by degrading the extracellular matrix component fibronectin necessary for SMC adhesion, with subsequent disruption of focal adhesions and loss of outside-in survival signaling. Thus, activated mast cells may participate in the weakening and rupture of atherosclerotic plaques by secreting mediators, such as heparin proteoglycans and chymase, which affect the growth, function and death of arterial SMCs.

Keywords: Apoptosis; Atherosclerosis; Extracellular matrix; Mast cell; Plaque rupture; Smooth muscle cell

1. Initiation and progression of the atherosclerotic disease

Atherosclerosis is a slowly progressing disease characterized by accumulation of lipids and fibrous elements in the innermost layer of the arterial wall, the intima [1]. The first visible sign of an ongoing process of atherosclerosis is the formation of fatty streaks, i.e. the intracellular accumulation of lipids in phagocytes, mainly monocyte-derived macrophages. Fatty streaks are the cellular hallmark of atherogenesis, but they never obstruct the arterial lumen, and are clinically silent [2]. However, they are precursors of true atherosclerotic lesions, the atheromas or atherosclerotic plaques, in which lipids also accumulate extracellularly deep in the intima, so forming a lipid core [3]. The formation of an atherosclerotic plaque is the first sign of a clinically significant atherosclerotic lesion [4].

2. Coronary plaque rupture—clinical manifestation of the atherosclerotic disease

The most important mechanism of the onset of acute coronary syndromes, including unstable angina, acute myocardial infarction and sudden cardiac death, is the rupture of such a coronary atherosclerotic plaque, with ensuing thrombotic occlusion of the coronary artery [5–7]. The risk of plaque rupture appears to depend critically on both the quantity and quality of the cellular and extracellular matrix components of the fibrous cap of an advanced atherosclerotic plaque [8]. Typically, a stable plaque has a small lipid core and a thick fibrous cap, being rich in SMCs and collagen but poor in inflammatory cells.

* Corresponding author. Tel.: +358-9-681-411; fax: +358-9-637-476.
E-mail address: Ken.Lindstedt@wri.fi (K.A. Lindstedt).
Abbreviations: SMC, smooth muscle cell; M_r, molecular weight; TGF-β, transforming growth factor-β; MMP, matrix metalloproteinase; TNF-α, tumor necrosis factor α.

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In contrast, a lesion with a large lipid core and a thin fibrous cap, containing only a few SMCs and little collagen but many inflammatory cells, is prone to rupture [9]. The central role of inflammation in plaque rupture is supported by the observation that the number of inflammatory cells, such as macrophages, T-lymphocytes and mast cells, is increased at sites of plaque rupture [10–12].

3. The mast cell—an inflammatory cell present in the atherosclerotic plaque

In coronary plaques, mast cells are present in the rupture-prone border region connecting the fibrous cap and the normal intima, i.e. the shoulder region (on average, 6 mast cells/mm^2), as well as in the fibrous cap and in the core regions (on average, 2 mast cells/mm^2) [13]. Interestingly, the activated and degranulated mast cells, as a proportion of all the mast cells in the respective area, is especially high in the shoulder region (85%) as compared with the normal intima (18%). When mast cells are activated *in vivo* or *in vitro* through either IgE-mediated crosslinking of Fc-epsilon-RI [14], by histamine releasing factors secreted by neighboring T lymphocytes [15] or macrophages [16], or by components of the complement system (C3a, C5a) [17], they release the content of their secretory granules in a process of exocytosis called degranulation. The secretory granules, consisting of preformed mediators bound to a network of negatively charged heparin and chondroitin sulfate proteoglycans, become swollen, and their individual membranes fuse to form tubular degranulation channels in which the granules lie in chains [18]. The degranulation channels then open to the extracellular fluid, and the soluble components of the granules, such as histamine, diffuse away. In contrast, the heparin proteoglycans and the mast cell-specific neutral proteases (e.g. chymase and carboxypeptidase A) remain tightly bound to each other, forming proteolytically active extracellular ''granule remnants'' [19]. The exocytosed remnants are eventually ingested by adjacent phagocytes, such as macrophages and smooth muscle cells [20,21]. The activated and degranulated mast cells reconstitute their lost preformed mediators through rapid onset of *de novo* synthesis, so allowing formation of new secretory granules. Finally, the recovered mast cells are ready to participate in a new process of activation and degranulation [22].

It is noteworthy that patients who died of acute myocardial infarction had, at the sites of plaque erosion or rupture, on average 25 mast cells/mm^2 (6% of all nucleated cells), of which 86% were activated [12]. In both the adjacent atheromatous area and the unaffected intimal area, mast cells were less abundant (1 and 0.1% of all nucleated cells). Interestingly, the degree of degranulation was found to be higher in intimal areas with increased numbers of macrophages and T-lymphocytes, suggesting that the factors responsible for mast cell degranulation *in vivo* may be of inflammatory origin. Since the complement cascade is activated in the advanced human coronary plaque [23], some of its active compounds, notably the anaphylatoxins C5a and C3a, may locally stimulate the coronary mast cells to degranulate.

4. The activated mast cell—a potential regulator of SMC growth, function and death

The role of mast cells in the destabilization of coronary atheromas have been further studied in coronary atherectomy specimens from patients with chronic stable angina, unstable angina, and severe unstable angina [24]. The study shows that the numbers of mast cells correlate with the clinical severity of the syndrome. Thus, the average densities of mast cells in the lesions were 1, 2.5 and 5 mast cells/mm^2 in chronic stable, unstable, and severe unstable angina, respectively. Similarly, the numbers of T-lymphocytes and macrophages also gradually increased, whereas, the number of SMCs decreased as the severity of the clinical presentation of coronary artery disease increased. The above findings are consistent with the hypothesis that the infiltrates of mast cells, macrophages and T-lymphocytes may affect SMC growth and death. Furthermore, this suggests that these inflammatory cells are present in the lesions prior to erosion or rupture, rather than being mere participants in the inflammatory response subsequent to the symptom-causing ulceration.

4.1. Inhibition of SMC proliferation

When sensitized (IgE-bearing) rat serosal mast cells were stimulated (with antigen) to degranulate in the presence of rat aortic smooth muscle cells, the rate of proliferation of the cocultured SMCs decreased sharply [25]. The inhibitory effect was found to depend on the very high molecular weight heparin proteoglycans (average M_r 750,000) released from the stimulated mast cells. Addition of such purified heparin proteoglycans to SMCs in culture efficiently blocked their cell cycle at the transition from G0 to S phase and the exit from the G2/M phase. The inhibitory effect of the mast cell-derived heparin proteoglycans resembled that of commercial heparin, but was more efficient than commercial heparin of high (average M_r 15,000) or low (average M_r 5000) molecular weight. Heparin glycosaminoglycans (average M_r 75,000) purified from the mast cell-derived heparin proteoglycans also inhibited SMC growth efficiently, although less strongly than their parent heparin proteoglycans. Kazi *et al.* [26] has recently shown that heparin functions by inhibiting the activation of the extracellular signal-regulated kinase, ERK 1 and 2. Thus, activated coronary mast cells, by releasing heparin proteoglycans, could participate in the local regulation of SMC growth in the plaque [25] (see Fig. 1, step A).

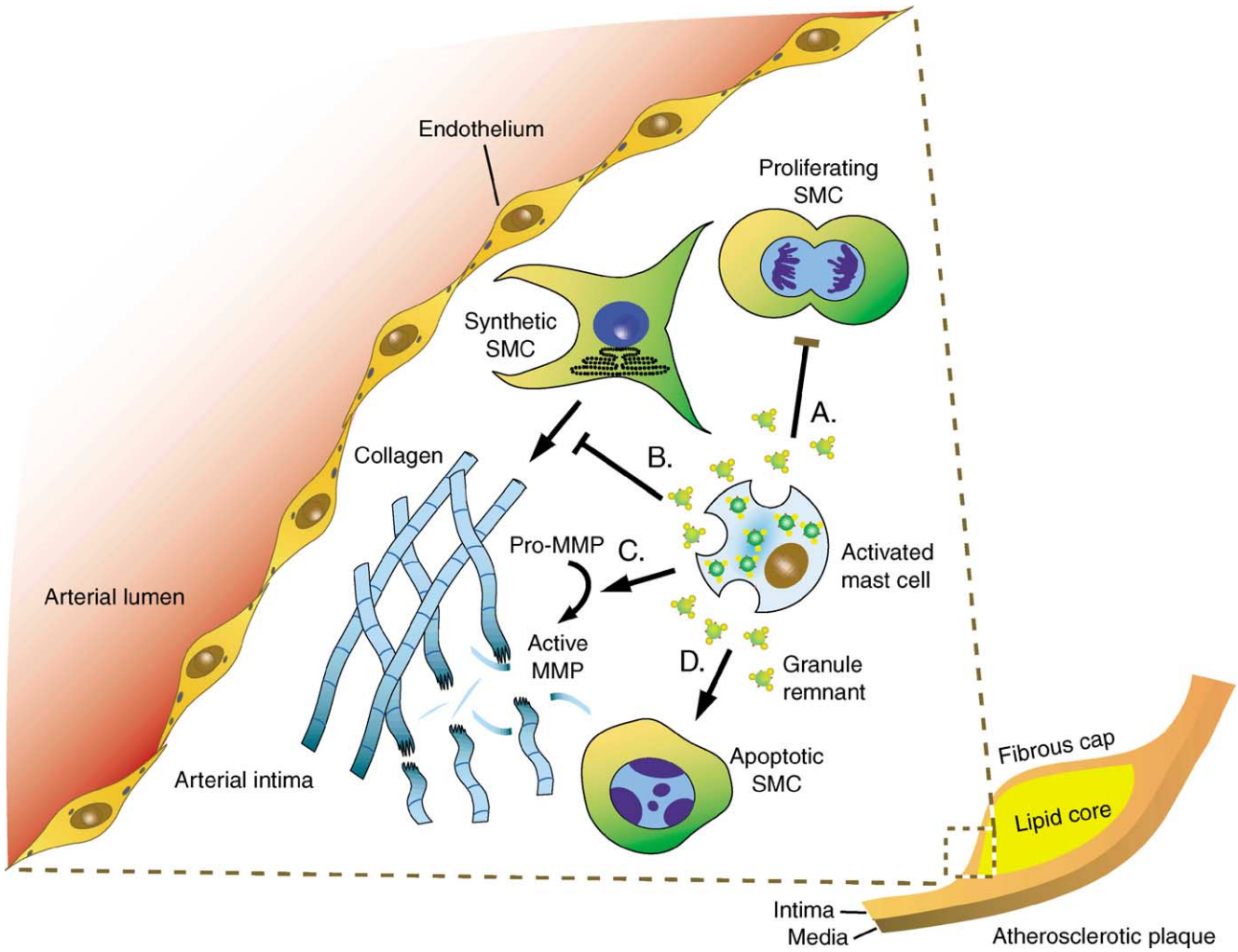

Fig. 1. A schematic model illustrating the various effects of activated intimal mast cells on intimal SMC growth, function and death, in the pathological process of weakening and rupture of an atherosclerotic plaque. The model depicts how activated mast cells in the shoulder region (the magnified area) of an atherosclerotic plaque may (A) inhibit SMC proliferation, (B) inhibit SMC-mediated collagen synthesis, (C) induce the secretion and activation of MMPs, leading to collagen degradation, and (D) induce SMC apoptosis. The mediators involved in each effect are described in more detail in the corresponding text.

4.2. Inhibition of SMC-mediated collagen synthesis

Mast cell chymase can also inhibit SMC growth and collagen expression *in vitro* through either a TGF-β-dependent or a TGF-β-independent mechanism [27]. Thus, we found that addition of purified chymase to cultured rat aortic SMCs of the synthetic phenotype (s-SMCs) inhibited their proliferation by blocking the G0/G1 → S transition in the cell cycle. Furthermore, purified rat chymase and recombinant human chymase inhibited the mRNA expression of type I and type III collagen in rat aortic s-SMCs and in human coronary arterial SMCs, respectively [27]. Since SMCs are the sole producers of the tensile components of the extracellular matrix (notably of collagen type I), a decrease in their numbers would lead to diminished production of the plaque-stabilizing molecules and, therefore, to reduced plaque stability (see Fig. 1, step B).

4.3. Induction of MMP synthesis and MMP-mediated collagen degradation

Mast cells in rupture-prone areas of human coronary atheromas also contain the potent proinflammatory cytokine TNF-α [28], which *in vitro* can induce both paracrine and autocrine synthesis and release of the 92 kDa gelatinase or matrix metalloproteinase 9 (MMP-9) from macrophages [29] and from mast cells [30], respectively. Indeed, in coronary atherectomy specimens from patients with chronic stable angina, unstable angina and severe unstable angina, the highest numbers of TNF-α-positive mast cells and of MMP-9-positive macrophages were found in the patients with the most severe symptoms [23]. These observations suggest that, in unstable angina, coronary mast cells release TNF-α, which, via activation of nuclear factor-kappa-B, induces the production of MMP-9 in the neighboring macrophages.

Mast cells may also directly synthesize and release MMPs, such as interstitial collagenase (MMP-1) [31] and 92-kDa gelatinase (MMP-9), both of which have been found in atherosclerotic lesions [29,32]. In addition, chymase and tryptase, the two major neutral proteases of human mast cells, are both capable of degrading the pericellular matrix components, fibronectin and vitronectin [33,34]. They may also trigger a more extensive degradation of the surrounding extracellular matrix, by effectively activating locally secreted MMPs. MMPs are synthesized and secreted as zymogens, i.e. as inactive proenzymes (proMMPs), and, consequently, have to be activated upon secretion [35]. Interestingly, human skin chymase can effectively activate proMMP-1 by cleaving the proenzyme at the Leu83–Thr84 position [36]. Moreover, tryptase can activate prostromelysin (proMMP-3) [37], which, in addition to being a powerful matrix-degrading enzyme, can activate other proMMPs.

Thus, novel functions for activated intimal mast cells are emerging. These functions relate to the hypothesis that coronary atheromas may rupture because of a locally increased activity of the enzymes involved in digestion of the extracellular matrix [8,38]. Indeed, in the human arterial intima, mast cells are the major local source of active neutral proteases, suggesting that they may contribute to the weakening and rupture of the atherosclerotic plaques either by directly affecting plaque remodeling, or by activating MMPs capable of degrading most of the extracellular matrix components (see Fig. 1, step C).

4.4. Induction of SMC apoptosis

Activated mast cells secrete large amounts of pre and newly formed mediators, which participate in the inflammatory reactions. Several of these mast cell-derived mediators, such as TNF-α and chymase, have proapoptotic properties [39–41]. Indeed, we have recently shown that chymase released from activated rat serosal mast cells is able to induce apoptosis in rat vascular SMCs *in vitro* [42]. The relevance of this finding was further confirmed in a human cell culture system, using recombinant human chymase and human coronary artery SMCs.

The mechanism of chymase-induced apoptosis of SMCs involves proteolytic degradation of the extracellular matrix component, fibronectin, with subsequent disruption of focal adhesions in the SMCs [43]. Focal adhesion kinase (FAK), one of the key mediators of focal adhesions and SMC survival, was rapidly degraded in the presence of chymase and/or fibronectin degradation products. Subsequently, downstream mediators of the focal adhesion kinase-dependent survival-signaling pathway, such as Akt, were inactivated by dephosphorylation [43]. Chymase-induced SMC apoptosis could also be inhibited by sodium orthovanadate, a wide spectrum inhibitor of tyrosine phosphatases, confirming the critical role of dephosphorylation in chymase-mediated SMC apoptosis. Interestingly, a recent study indicates that chymase has a biological function in fibronectin turnover [44]. Briefly, a mouse strain with a defect in its heparin biosynthesis showed significantly reduced levels of chymase, with an altered processing of fibronectin, i.e. loss of fibronectin degradation, suggesting that chymase is the major fibronectin-degrading enzyme *in vivo*.

In contrast to other neutral proteases, chymase in its natural form, i.e. bound to heparin proteoglycans is also capable of inducing SMC apoptosis in the presence of natural protease inhibitors [43]. This confirms the finding that chymase, when bound to the heparin proteoglycan chains of the exocytosed mast cell granules, is capable of exerting proteolytic activities even in the presence of physiological inhibitors, such as α_1-antitrypsin, α_2-macroglobulin, and α_1-antichymotrypsin [45]. Interestingly, the mast cell-mediated proapoptotic effect seems to be purely paracrine, since the activated mast cells themselves survive the process of degranulation by overexpressing the prosurvival bcl-2 homologue A1 [46] (see Fig. 1, step D).

5. Conclusions

Taken together, these *in vitro* and *in vivo* findings point to the participation of a new plaque-destabilizing factor in acute coronary syndromes, i.e. the presence of activated mast cells in the vulnerable coronary plaques. Thus, by releasing neutral proteases, heparin proteoglycans and proinflammatory cytokines, activated mast cells may affect the growth, function, and death of intimal SMCs that are critical for the stability of the atherosclerotic plaque. The above experimental studies also suggest the important function of extracellular matrix components and integrin-mediated ‘‘outside-in signaling’’ in determining the life and death of these intimal cells.

Although, activated intimal mast cells appear to be a highly potential source of molecules capable of affecting plaque remodeling, the role of other inflammatory cells in the process of plaque weakening and rupture should not be underscored. In this context, the mast cell-mediated remodeling of the plaque, as described above, serves as a model for the overall role of inflammatory cells in the pathological process of plaque rupture. Future experiments will be necessary to determine the importance of the individual types of inflammatory cells in the onset, progression and execution of the pathological processes ultimately culminating in the rupture of an atherosclerotic plaque.

References

[1] Stary HC, Chandler AB, Glagov S, Guyton JR, Insull Jr W, Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissler RW. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on

Arteriosclerosis, American Heart Association. Arterioscler Thromb 1994;14:840–56.
[2] McGill Jr HC. Fatty streaks in the coronary arteries and aorta. Lab Invest 1968;18:560–4.
[3] Kovanen PT. Atheroma formation: defective control in the intimal round-trip of cholesterol. Eur Heart J 1990;11:238–46.
[4] Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull Jr W, Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation 1995;92:1355–74.
[5] Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes. N Engl J Med 1992;326:242–50.
[6] Falk E. Why do plaques rupture? Circulation 1992;86(Suppl III): 30–42.
[7] Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. Arterioscler Thromb Vasc Biol 2000;20:1262–75.
[8] Libby P. Inflammation in atherosclerosis. Nature 2002;420:868–74.
[9] Richardson PD, Davies MJ, Born GVR. Influence of plaque configuration and stress distribution on fissuring of coronary atherosclerotic plaque. Lancet 1989;2:941–4.
[10] Moreno PR, Falk E, Palacios IF, Newell JB, Fuster V, Fallon JT. Macrophage infiltration in acute coronary syndromes. Implications for plaque rupture. Circulation 1994;90:775–8.
[11] van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. Circulation 1994;89:36–44.
[12] Kovanen PT, Kaartinen M, Paavonen T. Infiltrates of activated mast cells at the site of coronary atheromatous erosion or rupture in myocardial infarction. Circulation 1995;92:1084–8.
[13] Kaartinen M, Penttilä A, Kovanen PT. Accumulation of activated mast cells in the shoulder region of human coronary atheroma, the predilection site of atheromatous rupture. Circulation 1994;90:1669–78.
[14] Ishizaka T, Ishizaka K. Activation of mast cells for mediator release through IgE receptors. Prog Allergy 1984;34:188–235.
[15] Sedgwick JD, Holt PG, Turner KJ. Production of a histamine-releasing lymphokine by antigen- or mitogen-stimulated human peripheral T cells. Clin Exp Immunol 1981;45:409–18.
[16] Liu MC, Proud D, Lichtenstein LM, MacGlashan DW, Schleimer RP, Adkinson NF, Kagey-Sobotka A, Schulman ES, Plaut M. Human lung macrophage-derived histamine-releasing activity is due to IgE-dependent factors. J Immunol 1986;136:2588–95.
[17] Metcalfe DD, Kaliner M, Donlon MA. The mast cell. Crit Rev Immunol 1981;3:23–74.
[18] Röhlich P, Anderson P, Uvnäs B. Electron microscope observations on compounds 48–80-induced degranulation in rat mast cells. Evidence for sequential exocytosis of storage granules. J Cell Biol 1971;51: 465–83.
[19] Kovanen PT. The mast cell–a potential link between inflammation and cellular cholesterol deposition in atherogenesis. Eur Heart J 1993;14(Suppl K):105–17.
[20] Kokkonen JO, Kovanen PT. Stimulation of mast cells leads to cholesterol accumulation in macrophages *in vitro* by a mast cell granule-mediated uptake of low density lipoprotein. Proc Natl Acad Sci USA 1987;84:2287–91.
[21] Wang Y, Lindstedt KA, Kovanen PT. Mast cell granule remnants carry LDL into smooth muscle cells of synthetic phenotype and induce their conversion into foam cells. Arterioscler Thromb Vasc Biol 1995;15: 801–10.
[22] Dvorak AM, Schleimer RP, Schulman ES, Lichtenstein LM. Human mast cells use conservation and condensation mechanisms during recovery from degranulation. *In vitro* studies with mast cells purified from human lungs. Lab Invest 1986;54:663–78.
[23] Laine P, Pentikäinen MO, Wurzner R, Penttilä A, Paavonen T, Meri S, Kovanen PT. Evidence for complement activation in ruptured coronary plaques in acute myocardial infarction. Am J Cardiol 2002; 90:404–8.
[24] Kaartinen M, van der Wal AC, van der Loos CM, Piek JJ, Koch KT, Becker AE, Kovanen PT. Mast cell infiltration in acute coronary syndromes: implications for plaque rupture. J Am Coll Cardiol 1998; 32:606–12.
[25] Wang Y, Kovanen PT. Heparin proteoglycans released from rat serosal mast cells inhibit proliferation of rat aortic smooth muscle cells in culture. Circ Res 1999;84:74–83.
[26] Kazi M, Lundmark K, Religa P, Gouda I, Larm O, Ray A, Swedenborg J, Hedin U. Inhibition of rat smooth muscle cell adhesion and proliferation by non-anticoagulant heparins. J Cell Physiol 2002; 193:365–72.
[27] Wang Y, Shiota N, Leskinen MJ, Lindstedt KA, Kovanen PT. Mast cell chymase inhibits smooth muscle cell growth and collagen expression *in vitro*: transforming growth factor-beta1-dependent and -independent effects. Arterioscler Thromb Vasc Biol 2001;21:1928–33.
[28] Kaartinen M, Penttilä A, Kovanen PT. Mast cells in rupture-prone areas of human coronary atheromas produce and store TNF-α. Circulation 1996;94:2787–92.
[29] Saren P, Welgus HG, Kovanen PT. TNF-alpha and IL-1beta selectively induce expression of 92-kDa gelatinase by human macrophages. J Immunol 1996;157:4159–65.
[30] Baram D, Vaday GG, Salamon P, Drucker I, Hershkoviz R, Mekori YA. Human mast cells release metalloproteinase-9 on contact with activated T cells: juxtacrine regulation by TNF-alpha. J Immunol 2001;167:4008–16.
[31] Di Girolamo N, Wakefield D. *In vitro* and *in vivo* expression of interstitial collagenase/MMP-1 by human mast cells. Dev Immunol 2000;7:131–42.
[32] Nikkari ST, O'Brien KD, Ferguson M, Hatsukami T, Welgus HG, Alpers CE, Clowes AW. Interstitial collagenase (MMP-1) expression in human carotid atherosclerosis. Circulation 1995;92:1393–8.
[33] Vartio T, Seppä H, Vaheri A. Susceptibility of soluble and matrix fibronectins to degradation by tissue proteinases, mast cells chymase and cathepsin G. J Biol Chem 1981;256:471–7.
[34] Lohi J, Harvima I, Keski-Oja J. Pericellular substrates of human mast cell tryptase: 72,000 dalton gelatinase and fibronectin. J Cell Biochem 1992;50:337–49.
[35] Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, Engler JA. Matrix metalloproteinases: a review. Crit Rev Oral Biol Med 1993;4:197–250.
[36] Saarinen J, Kalkkinen N, Welgus HG, Kovanen PT. Activation of human interstitial procollagenase through direct cleavage of the Leu^{83}–Thr^{84} bond by mast cell chymase. J Biol Chem 1994;269: 18134–40.
[37] Gruber BL, Marchese MJ, Suzuki K, Schwartz LB, Okada Y, Nagase H, Ramamurthy NS. Synovial procollagenase activation by human mast cell tryptase dependence upon matrix metalloproteinase 3 activation. J Clin Invest 1989;84:1657–62.
[38] Libby P. Molecular bases of the acute coronary syndromes. Circulation 1995;91:2844–50.
[39] Lätti S, Leskinen MJ, Wang Y, Kovanen PT, Lindstedt KA. Mast cell-mediated apoptosis of endothelial cells *in vitro*: a paracrine mechanism involving TNF-alpha-mediated down-regulation of bcl-2 expression. J Cell Physiol 2003;195:130–8.
[40] Wallach D. Cell death induction by TNF: a matter of self control. Trends Biochem Sci 1997;22:107–9.
[41] Hara M, Matsumori A, Ono K, Kido H, Hwang MW, Miyamoto T, Iwasaki A, Okada M, Nakatani K, Sasayama S. Mast cells cause apoptosis of cardiomyocytes and proliferation of other intramyocardial cells *in vitro*. Circulation 1999;100:1443–9.

[42] Leskinen M, Wang Y, Leszczynski D, Lindstedt KA, Kovanen PT. Mast cell chymase induces apoptosis of vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 2001;21:516–22.

[43] Leskinen MJ, Lindstedt KA, Wang Y, Kovanen PT. Mast cell chymase induces smooth muscle cell apoptosis by a mechanism involving fibronectin degradation and disruption of focal adhesions. Arterioscler Thromb Vasc Biol 2003;23:238–43.

[44] Tchougounova E, Forsberg E, Angelborg G, Kjellen L, Pejler G. Altered processing of fibronectin in mice lacking heparin. A role for heparin-dependent mast cell chymase in fibronectin degradation. J Biol Chem 2001;276:3772–7.

[45] Lindstedt L, Lee M, Kovanen PT. Chymase bound to heparin is resistant to its natural inhibitors and capable of proteolyzing high density lipoproteins in aortic intimal fluid. Atherosclerosis 2001;155: 87–97.

[46] Karsan A, Yee E, Harlan JM. Endothelial cell death induced by tumor necrosis factor-alpha is inhibited by the Bcl-2 family member, A1. J Biol Chem 1996;271:27201–4.