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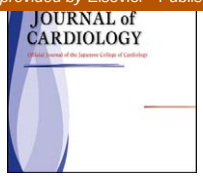

Review

Granzyme B as a novel factor involved in cardiovascular diseases

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Summary Apoptosis plays an important role in cardiovascular diseases such as atherosclerosis, ischemic heart disease, and congestive heart failure. Previous studies have demonstrated that oxidative stress, physiological stress, and inflammatory cytokines such as tumor necrosis factor and Fas ligand are involved in apoptosis of cardiovascular system. We demonstrate that another apoptosis-related pathway, i.e. granzyme B/perforin system is involved in cardiovascular diseases. Expression of granzyme B, a member of serine protease family is increased in acute coronary syndrome, coronary artery disease with end-stage renal disease, and subacute stage of acute myocardial infarction. Although granzyme B is extensively researched in immunological disorders, the role of granzyme B/perforin system was not clear in the cardiovascular field. In addition, little is known regarding the inhibition of granzyme B system in the clinical situation. In this review we demonstrate recent findings of granzyme B in cardiovascular diseases and possible therapeutic applications of inhibiting the granzyme B/perforin system.

Contents

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Introduction

Atherosclerosis has been found to be induced by complex processes including lipid accumulation, cellular migration, inflammation, matrix remodeling, and apoptosis. Apoptosis is supposed to play important roles in cardiovascular diseases [1]. It has been reported that apoptosis is involved in progression of atherosclerosis, plaque rupture, and extracellular matrix remodeling [2—4]. Apoptosis has been found to be induced by a number of mechanisms, including oxidative stress, serum deprivation, chemical inducers, ultraviolet exposure, thermal stress, and cytokines. Several cytokines are found to induce apoptosis by binding to their specific receptors. Most of these ''death-ligands'' belong to the tumor necrosis factor (TNF) family, including TNFα, lymphotoxin α, Fas ligand, and TRAIL/Apo2L.

There has been a report of the critical role of cytotoxic immune responses as a mediator of apoptotic cell death in atherosclerotic diseases [5]. Cytotoxic T lymphocytes (CTLs) exert critical protection against viral infection and tumor cells, however they can also induce harmful reactions such as autoimmune disease, graft rejection, and graft versus host disease (GVHD). Granzyme B/perforin system is mainly exerted by circulating white blood cells such as CTLs (adaptive immune system) and natural killer (NK) cells (innate immune system). Granzyme B and perforin are stored in secretory granules inside the leukocytes. These molecules are released outside of the cells by cell to cell contact, integrin engagement with extracellular matrix proteins [6,7], receptor activation by cytokines (interleukin-2 and TNF) [8], chemokines (macrophage inflammatory protein-1 and monocyte chemoatatic protein-1) [9,10], and stimulation by bacteria or lipopolysaccharide. Perforin polymerizes to form transmembrane channels and allows granzyme B access into target cells (Fig. 1). Granzyme B is a member of the serine protease family and induces cell death by mechanisms such as the activation of caspases, degradation of structural proteins and directing the proapoptotic molecule Bid to the mitochondrial compartment. To date, five members of granzyme have been identified: granzyme A, B, H, K, and M. These granzymes are produced as inactive forms and are activated by cathepsin C-mediated removal of propeptide [11,12]. Among these enzymes, granzyme A and B are well characterized. Granzyme A and B are known to be involved in CTL-mediated target cell apoptosis. Granzyme B cleaves Asp or Glu residue of proscaspases, resulting in activation of caspases cascade. The activated caspases induce DNA fragmentation and cellular apoptosis [13—17]. In normal conditions, granzymes are playing important roles in elimination of abnormal cells. Granzyme B-deficient mice show a normal phenotype with an exception of reduced activity of target cell apoptosis, antiviral activity, and tumor cell elimination [17,18]. Thus granzyme B is playing an important role in CTL-mediated immune response. However, previous studies have clarified excess production of granzyme B is involved in a number of pathological conditions including chronic inflammatory diseases including rheumatoid arthritis, GVHD, as well as cardiovascular diseases [19,20].

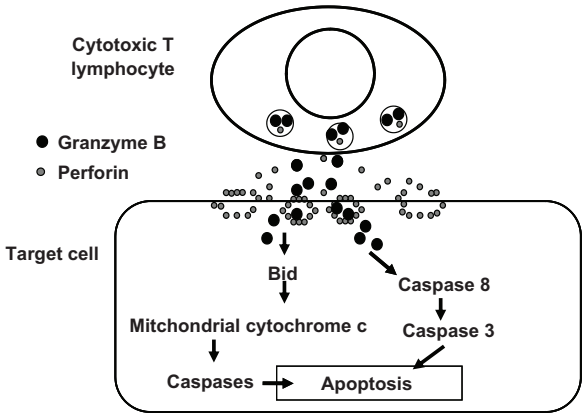

Figure 1 Scheme of granzyme B/perforin system. Perforin polymerizes to form transmembrane channels and allows granzyme B access into target cells. Granzyme B, a member of the serine protease family induces cell death by mechanisms such as the activation of caspases, degradation of structural proteins and directing the proapoptotic molecule Bid to the mitochondrial compartment.

Previously the granzyme/perforin system has been reported to play an important role in acute myocarditis and immunological rejection of the transplanted heart. Seko et al. demonstrated infiltration of perforin in the hearts of mice with acute viral myocarditis, suggesting an important role in the mechanism of myocardial damage [21]. They provided the evidence that CTLs injure cardiac myocytes by releasing perforin and may play a critical role in the myocardial damage in acute viral myocarditis. Felzen et al. showed that the combination of granzyme A and perforin damages guinea pig ventricular myocytes [22]. As shown by these reports, the granzyme/perforin system is predominantly focused on cardiovascular diseases with immunological disorders. However, recent studies have demonstrated a role of the granzyme/perforin system in other cardiovascular diseases such as chronic angina pectoris, acute coronary syndrome, and acute myocardial infarction.

Atherosclerotic disease and granzyme B

Activation of the immune response is essential to eliminate infected cells and tumor cells in humans. However, excess immune response contributes to various pathological processes, i.e. autoimmune disease, chronic obstructive pulmonary disease, graft versus host reaction, and atherosclerosis. Endothelium has a critical role to prevent atherosclerosis by releasing nitric oxide, producing anticoagulant factors, and regulating permeability of circulating substances into vessel walls. Endothelial apoptosis leads to atherosclerotic plaque formation by increasing smooth muscle cell proliferation, leukocyte migration into subendothelial layers, thrombus formation, and accumulation of circulating lipids to vessel walls. Clément et al. have reported that granzyme B can be a predictive marker for acute GVHD or cardiac rejection after bone marrow or heart transplantation [23]. Acceleration of coronary atherosclerosis is well-known in patients receiving heart transplantation. Choy et al. have shown the critical role of granzyme B in vascular disease in patients receiving heart transplantation [24].

Accumulation of T lymphocytes is reported in atherosclerotic lesions, in particular, shoulder lesion of the plaques

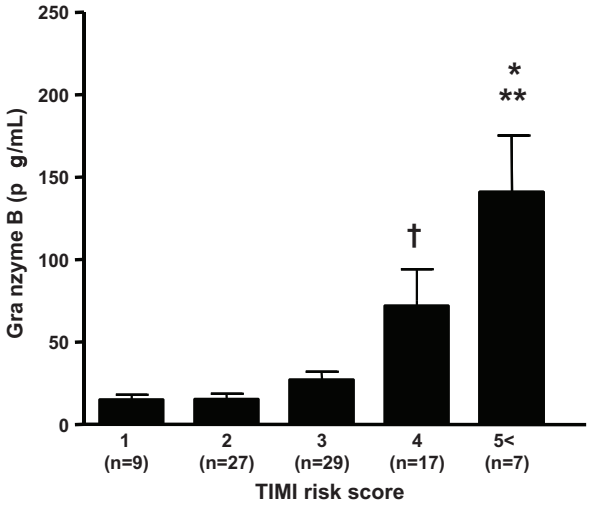

Figure 2 The relationship between levels of granzyme B in the supernatant of cultured peripheral blood mononuclear cells and TIMI risk score in patients with unstable angina pectoris. Patients with unstable angina were classified into five groups according to the Thrombolysis In Myocardial Infarction (TIMI) risk score (TIMI 1, 2, 3, 4, and more than 5). The levels of granzyme B in the supernatant of cultured peripheral blood mononuclear cells were elevated when the TIMI risk score increased (TIMI 1, 14.8 ± 3.1 pg/mL; TIMI 2, 15.2 ± 3.4 pg/mL; TIMI 3, 25.2 ± 5.1 pg/mL; TIMI 4, 71.7 ± 22.3 pg/mL; TIMI 5 and 6, 132 ± 31.0 pg/mL). *$p < 0.05$ versus TIMI1 and 3; **$p < 0.01$ versus TIMI 2; †$p < 0.05$ versus TIMI 2.

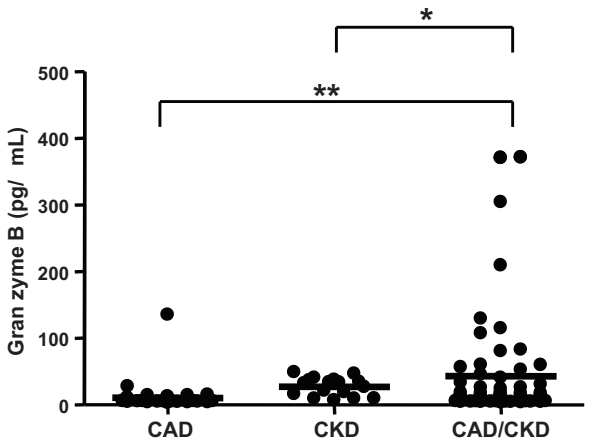

Figure 3 Levels of plasma granzyme B in patients with coronary artery and chronic kidney disease. The Kruskal–Wallis test revealed that plasma levels of granzyme B in CAD complicated CKD patients were significantly higher than those in patients with CAD or patients with CKD (CAD, 10.6 ± 2.8; CKD, 27.3 ± 3.3; CAD/CKD, 35.5 ± 8.1 pg/mL, $p < 0.05$). *$p < 0.05$; **$p < 0.001$. CAD, coronary artery disease; CKD, chronic kidney disease; CAD/CKD, patients with both coronary artery and chronic kidney disease.

[25–27]. Activated T lymphocytes are supposed to play an important role in vulnerability of plaques. It has been reported that expression of matrix degrading enzymes was increased both in systemic circulation and in vulnerable atheromatous plaques in patients with acute coronary syndrome. We have observed the increased production of granzyme B from peripheral blood mononuclear cells (PBMCs) in patients with unstable angina pectoris [28] (Fig. 2). We have also found granzyme B expression in mononuclear cells in ruptured coronary plaques. Importantly, the granzyme B production from PBMCs was related to Thrombolysis in Myocardial Infarction (TIMI) risk score of acute coronary syndrome. Interestingly, studies have clarified that granzyme B can cleave extracellular matrix proteins including fibronectin [29], vitronectin, and laminin [30]. The primary cleavage site of vitronectin was demonstrated as the Arg-Gly-Asp (RGD) integrin site, which is consistent with Asp-ase activity of granzyme B. Thus granzyme B not only induces apoptotic cell death but also degrades extracellular matrix, leading to weakening of the fibrous cap of atheromatous plaques. Previous studies have reported that a number of proteases such as matrix metalloproteinases (MMPs), elastases, myeloperoxidase, neutrophil proteinase 3, and neutral serine proteases (chymase and tryptase) are supposed to play important roles in degradation of the extracellular matrix, leading to the thinning and weakening of the fibrous cap [27,31,32]. Among these enzymes, MMPs have been extensively investigated. Our results suggest that extracellular granzyme B released from PBMCs might be another candidate that induces atherosclerotic plaque vulnerability. Recently, Chamberlain et al. have reported a role of granzyme B in vascular remodeling using ApoE and granzyme B double-deficient mice [33]. This study has shown the evidence that granzyme B directly degrades extracellular matrix such as fibrillin-1, leading to an aortic aneurysmal formation.

Normally plasma levels of granzyme B in healthy subjects is up to 15 pg/mL, which is almost below the detection limit of enzyme-linked immunosorbent assays. Expression of granzyme B is increased in patients with acute inflammatory diseases, malignancy, and GVHD. As shown in Fig. 3, we have found that patients with angina pectoris complicated with chronic kidney disease (CKD) showed increases in plasma granzyme B levels compared with CKD alone or angina pectoris alone [34]. In particular, plasma granzyme B level was markedly increased in coronary artery disease patients with end-stage renal disease (ESRD). These results might indicate that elevation of plasma granzyme B is one of the factors that promotes coronary atherosclerosis in patients with ESRD. A number of epidemiological studies have reported that CKD is an independent risk factor for cardiovascular diseases [35–37]. Although a number of reports have demonstrated increased cardiovascular mortality in CKD, the precise mechanisms of how CKD takes part in the onset of cardiovascular diseases are still not fully understood. Various cytokines might be involved in plaque formation in patients with CKD. Our data suggest that granzyme B can be one of the atherogenic factors in subjects such as patients with CKD.

Acute myocardial infarction and granzyme B

Left ventricular (LV) remodeling after acute myocardial infarction (AMI) is a critical complication that leads to thrombosis, heart failure, and ventricular arrhythmia, lead-

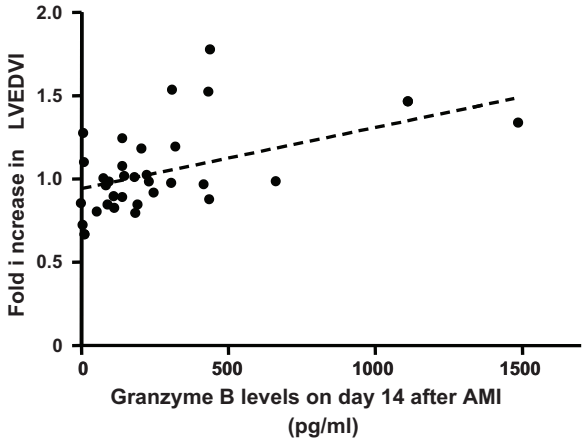

Figure 4 Correlation between plasma levels of granzyme B and left ventricular remodeling after acute myocardial infarction (AMI). The figure shows simple correlation between plasma levels of granzyme B on day 14 and the fold-increase in left ventricular end-diastolic volume index (LVEDVI) over six months after onset of AMI. There was a significant positive correlation between plasma levels of granzyme B on day 14 and the fold-increase in LVEDVI six months after onset of AMI ($r = +0.45$, $p < 0.01$).

ing to an unfavorable clinical outcome [38]. The size of infarction determined within several hours after the attack is the most critical determinant of subsequent heart failure. However, mechanical factors, medical interventions, and humoral factors such as vasoactive substances, growth factors, and cytokines have been reported to be associated with LV remodeling in the chronic phase after AMI [39—42]. In particular, apoptotic cell death in infarcted tissue is reported to play an important role in the progression of LV remodeling after AMI [43,44]. Hayakawa et al. reported that inhibition of granulation tissue cell apoptosis by a pancaspase inhibitor significantly improved LV remodeling and heart failure in the chronic stage after myocardial infarction [45].

We hypothesized that granzyme B is involved in LV remodeling through induction of apoptotic cell death and extracellular matrix degradation activity. We have measured plasma levels of granzyme B in patients with AMI and found increased plasma levels of granzyme B after AMI [46]. The plasma levels of granzyme B peaked on day 7 after AMI with a 4.6-fold increase compared to that on the day of the admission. Interestingly, plasma granzyme B level on day 14 after AMI was correlated with increase of LV end-diastolic volume 6 months after the onset (Fig. 4). TNFα and Fas ligand also increased after AMI, however we did not observe a significant relationship between these markers and LV remodeling. In the rat model of AMI, we found that protein expression of granzyme B was increased at the infarcted myocardium from day 3 to 17 after AMI (unpublished observation). The finding is quite interesting to prove increased local expression of granzyme B in infarcted myocardium.

Inhibition of granzyme B

Normally, activity of proteases is tightly regulated to avoid unfavorable damage of tissues *in vivo*. For example, MMPs are inhibited by binding of their internal inhibitors: tissue inhibitors of MMPs (TIMPs) in a 1 to 1 molar stoichiometric fashion. It has been reported that granzyme B is efficiently inhibited by α1-antitrypsin and to a lesser extent by α2-macrogloblin [47]. However, other reports showed that the inhibitory action of α1-antitrypsin for granzyme B is still controversial [48,49].

Serpins are a group of proteins with similar structures that inhibit proteases by forming complexes with their proteases. The first members of the serpin superfamily extensively studied were antithrombin and antitrypsin, which play key roles in controlling blood coagulation and inflammation [50]. Nowadays, more than 1000 serpins have now been identified. Human protease inhibitor-9 (PI-9, serpinB9) is a member of the serpin family that has been found to be an intrinsic inhibitor for granzyme B. PI-9 was originally found in T cells to protect themselves against apoptosis by secreted granzyme B. It has been reported that PI-9 forms a complex with granzyme B and inhibits its activity [51,52]. PI-9 is predominately distributed in cytosol and nucleus because it lacks a peptide sequence for secretion. The presence of circulating PI-9 was proved by Rowshani et al. [53], however it is still not clear that circulating PI-9 can inhibit extracellular granzyme B *in vivo*. Kurschus et al. have shown activity of granzyme B is not inhibited by co-incubation with human plasma *in vitro* [49]. However, a protective role of PI-9 and SPI-6, a murine orthologue of human PI-9 against granzyme B-induced apoptosis has been reported in both *in vitro* and *in vivo* models [54—56]. Sipione et al. reported that Sertoli cells secret another serpin, serpina3n that can bind and inhibit activity of granzyme B [57]. The regulation mechanism of PI-9 is still not clear. Lazarczyk et al. have reported that pentoxifylline upregulated PI-9 mRNA expression in human erythroleukemia cell lines [58]. Jiang et al. have demonstrated that estrogen induces PI-9 expression in hepatoblastoma cells, HEPG2ER7 cells [59]. Induction of PI-9 by estrogen might account for a novel mechanism for estrogen-mediated increase in tumor incidence and gender difference of prevalence in cardiovascular diseases. Interestingly, Young et al. have demonstrated increased expression of interleukin-1β and decreased expression of PI-9 in unstable human atherosclerotic plaques [60]. They have demonstrated that PI-9 was an endogenous inhibitor for interleukin-1β (IL-1β)-converting enzyme. Thus administration of PI-9 inhibits chronic inflammation and could be a novel therapeutic tool to treat and prevent cardiovascular diseases.

Our data suggest that PI-9 can be applicable for prevention of acute coronary syndrome and atherosclerotic plaque formation in patients with CKD and left ventricular remodeling after AMI. In our preliminary experiments, marked elevation of granzyme B was found, however no apparent expression of PI-9 was observed in the infarcted myocardium of the rat model of AMI. Thus inhibition of granzyme B might prevent cardiac rupture and progression of ventricular remodeling after AMI. Because PI-9 is predominantly expressed in cytosol, administration of PI-9 can block extracellular granzyme B more efficiently. Interestingly, a recent study demonstrated inhibition of IL-1 by a recombinant human IL-1 receptor antagonist (anakinra) reduced left ventricular remodeling [61]. It is possible that PI-9 prevents ventricular remodeling after AMI by inhibit-

ing IL-1 mediated pathway. To our knowledge, there are few studies regarding application of granzyme B inhibitor in the treatment of cardiovascular diseases. Rapamycin treatment prevents rejection of cardiac allografts via an inhibition of local granzyme B expression in cardiac allografts in a mouse model [62]. Suzuki et al. have demonstrated that plasminogen activator inhibitor-1, of the serpin family improved the LV function in a porcine model of autoimmune myocarditis [63]. These reports support the possibility of the PL-9 application in various cardiovascular diseases such as acute coronary syndrome, chronic atherosclerotic disease, and myocarditis. We also have to be very careful about side effects of granzyme B inhibition such as disturbance of immune systems and increasing the prevalence of malignancy. Several inhibitors for granzyme B are already available for experiments including Z-AAD-CMK, Ac-IEPD-CHO, and acethyl-Ile-Glu-Thr-Asp-aldehyde. So far, these granzyme B inhibitors cannot be applicable for humans. However, inhibition of granzyme B/perforin system by another chemical compound or recombinant protein could be a new approach to treat human cardiovascular diseases.

Conclusions

Granzyme B could be a novel molecule that plays an important role in cardiovascular diseases. It is suggested that granzyme B is involved in chronic as well as acute inflammation in atherosclerotic coronary artery diseases. Inhibition of granzyme B could be a novel therapeutic approach to treating cardiovascular diseases such as prevention of progression of atherosclerosis, plaque rupture, and ventricular remodeling after AMI.

References

[1] Clarke M, Bennett M, Littlewood T. Cell death in the cardiovascular system. Heart 2007;93:659—64.

[2] Halvorsen B, Otterdal K, Dahl T, Skjelland M, Gullestad L, Øie E, Aukrust P. Atherosclerotic plaque stability — what determines the fate of a plaque? Prog Cardiovasc Dis 2008;51:183—94.

[3] Zernecke A, Bernhagen J, Weber C. Macrophage migration inhibitory factor in cardiovascular disease. Circulation 2008;117:1594—602.

[4] Björkerud S, Björkerud B. Apoptosis is abundant in human atherosclerotic lesions, especially in inflammatory cells (macrophages and T cells), and may contribute to the accumulation of gruel and plaque instability. Am J Pathol 1996;149:367—80.

[5] Choy J, Podor T, Yanagawa B, Lai J, Granville D, Walker D, McManus B. The regulation and consequences of immune-mediated cell death in atheromatous diseases. Cardiovasc Toxicol 2003;3:269—82.

[6] Vacca A, Ria R, Presta M, Ribatti D, Iurlaro M, Merchionne F, Tanghetti E, Dammacco F. Alpha(v)beta(3) integrin engagement modulates cell adhesion, proliferation, and protease secretion in human lymphoid tumor cells. Exp Hematol 2001;29:993—1003.

[7] Doucey M, Legler D, Faroudi M, Boucheron N, Baumgaertner P, Naeher D, Cebecauer M, Hudrisier D, Rüegg C, Palmer E, Valitutti S, Bron C, Luescher I. The beta1 and beta3 integrins promote T cell receptor-mediated cytotoxic T lymphocyte activation. J Biol Chem 2003;278:26983—91.

[8] Isaaz S, Baetz K, Olsen K, Podack E, Griffiths G. Serial killing by cytotoxic T lymphocytes: T cell receptor triggers degranulation, re-filling of the lytic granules and secretion of lytic proteins via a non-granule pathway. Eur J Immunol 1995;25:1071—9.

[9] Taub D, Sayers T, Carter C, Ortaldo J. Alpha and beta chemokines induce NK cell migration and enhance NK-mediated cytolysis. J Immunol 1995;155:3877—88.

[10] Loetscher P, Seitz M, Clark-Lewis I, Baggiolini M, Moser B. Activation of NK cells by CC chemokines. Chemotaxis, Ca2+ mobilization, and enzyme release. J Immunol 1996;156:322—7.

[11] Brown G, McGuire M, Thiele D. Dipeptidyl peptidase I is enriched in granules of in vitro- and in vivo-activated cytotoxic T lymphocytes. J Immunol 1993;150:4733—42.

[12] Smyth M, McGuire M, Thia K. Expression of recombinant human granzyme B. A processing and activation role for dipeptidyl peptidase I. J Immunol 1995;154:6299—305.

[13] Caputo A, James M, Powers J, Hudig D, Bleackley R. Conversion of the substrate specificity of mouse proteinase granzyme B. Nat Struct Biol 1994;1:364—7.

[14] Darmon A, Nicholson D, Bleackley R. Activation of the apoptotic protease CPP32 by cytotoxic T-cell-derived granzyme B. Nature 1995;377:446—8.

[15] Shi L, Kraut R, Aebersold R, Greenberg A. A natural killer cell granule protein that induces DNA fragmentation and apoptosis. J Exp Med 1992;175:553—66.

[16] Nakajima H, Park H, Henkart P. Synergistic roles of granzymes A and B in mediating target cell death by rat basophilic leukemia mast cell tumors also expressing cytolysin/perforin. J Exp Med 1995;181:1037—46.

[17] Heusel J, Wesselschmidt R, Shresta S, Russell J, Ley T. Cytotoxic lymphocytes require granzyme B for the rapid induction of DNA fragmentation and apoptosis in allogeneic target cells. Cell 1994;76:977—87.

[18] Revell P, Grossman W, Thomas D, Cao X, Behl R, Ratner J, Lu Z, Ley T. Granzyme B and the downstream granzymes C and/or F are important for cytotoxic lymphocyte functions. J Immunol 2005;174:2124—31.

[19] Buzza M, Bird P. Extracellular granzymes: current perspectives. Biol Chem 2006;387:827—37.

[20] Chamberlain C, Granville D. The role of Granzyme B in atheromatous diseases. Can J Physiol Pharmacol 2007;85:89—95.

[21] Seko Y, Shinkai Y, Kawasaki A, Yagita H, Okumura K, Yazaki Y. Evidence of perforin-mediated cardiac myocyte injury in acute murine myocarditis caused by Coxsackie virus B3. J Pathol 1993;170:53—8.

[22] Felzen B, Berke G, Rosen D, Coleman R, Tschopp J, Young J, Binah O. Effects of purified perforin and granzyme A from cytotoxic T lymphocytes on guinea pig ventricular myocytes. Cardiovasc Res 1994;28:643—9.

[23] Clément M, Soulié A, Legros-Maida S, Guillet J, Gluckman E, Sigaux N, Sasportes M. Perforin and granzyme B: predictive markers for acute GVHD or cardiac rejection after bone marrow or heart transplantation. Nouv Rev Fr Hematol 1991;33:465—70.

[24] Choy J, McDonald P, Suarez A, Hung V, Wilson J, McManus B, Granville D. Granzyme B in atherosclerosis and transplant vascular disease: association with cell death and atherosclerotic disease severity. Mod Pathol 2003;6:460—70.

[25] van der Wal A, Becker A, van der Loos C, Das P. 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.

[26] Lendon C, Davies M, Born G, Richardson P. Atherosclerotic plaque caps are locally weakened when macrophages density is increased. Atherosclerosis 1991;87:87—90.

[27] Lee R, Schoen F, Loree H, Lark M, Libby P. Circumferential stress and matrix metalloproteinase 1 in human coronary atherosclerosis. Implications for plaque rupture. Arterioscler Thromb Vasc Biol 1996;16:1070—3.
[28] Tsuru R, Kondo H, Hojo Y, Gama M, Mizuno O, Katsuki T, Shimada K, Kikuchi M, Yashiro T. Increased granzyme B production from peripheral blood mononuclear cells in patients with acute coronary syndrome. Heart 2008;94:305—10.
[29] Choy J, Hung V, Hunter A, Cheung P, Motyka B, Goping I, Sawchuk T, Bleackley R, Podor T, McManus B, Granville D. Granzyme B induces smooth muscle cell apoptosis in the absence of perforin: involvement of extracellular matrix degradation. Arterioscler Thromb Vasc Biol 2004;24: 2245—50.
[30] Buzza M, Zamurs L, Sun J, Bird C, Smith A, Trapani J, Froelich C, Nice E, Bird P. Extracellular matrix remodeling by human granzyme B via cleavage of vitronectin, fibronectin, and laminin. J Biol Chem 2005;280:23549—58.
[31] Kaartinen M, Penttilä A, Kovanen P. Accumulation of activated mast cells in the shoulder region of human coronary atheroma, the predilection site of atheromatous rupture. Circulation 1994;90:1669—78.
[32] Baldus S, Heeschen C, Meinertz T, Zeiher A, Eiserich J, Münzel T, Simoons M, Hamm C, CAPTURE Investigators. Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes. Circulation 2003;108:1440—5.
[33] Chamberlain C, Ang L, Boivin W, Cooper D, Williams S, Zhao H, Hendel A, Folkesson M, Swedenborg J, Allard M, McManus B, Granville D. Perforin-independent extracellular granzyme B activity contributes to abdominal aortic aneurysm. Am J Pathol 2010;176:1038—49.
[34] Ikemoto T, Hojo Y, Kondo K, Takahashi N, Hirose M, Nishimura Y, Katsuki T, Shimada K. Plasma granzyme B as a predicting factor of coronary artery disease — clinical significance in patients with chronic renal failure. J Cardiol 2009;54:409—15.
[35] Ninomiya T, Kiyohara Y, Kubo M, Tanizaki Y, Doi Y, Okubo K, Wakugawa Y, Hata J, Oishi Y, Shikata K, Yonemoto K, Hirakata H, Iida M. Chronic kidney disease and cardiovascular disease in a general Japanese population: the Hisayama Study. Kidney Int 2005;68:228—36.
[36] Go A, Chertow G, Fan D, McCulloch C, Hsu C. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med 2004;351:1296—305.
[37] Irie F, Iso H, Sairenchi T, Fukasawa N, Yamagishi K, Ikehara S, Kanashiki M, Saito Y, Ota H, Nose T. The relationships of proteinuria, serum creatinine, glomerular filtration rate with cardiovascular disease mortality in Japanese general population. Kidney Int 2006;69:1264—71.
[38] Suzuki H, Geshi E, Nanjyo S, Nakano H, Yamazaki J, Sato N, Tanaka K, Takano T, Yagi H, Shibata T, Mochizuki S, Katagiri T. Inhibitory effect of valsartan against progression of left ventricular dysfunction after myocardial infarction: T-VENTURE study. Circ J 2009;73:918—24.
[39] Nagaya N, Uematsu M, Kojima M, Ikeda Y, Yoshihara F, Shimizu W, Hosoda H, Hirota Y, Ishida H, Mori H, Kangawa K. Chronic administration of ghrelin improves left ventricular dysfunction and attenuates development of cardiac cachexia in rats with heart failure. Circulation 2001;104:1430—5.
[40] Nakamura T, Matsumoto K, Mizuno S, Sawa Y, Matsuda H, Nakamura T. Hepatocyte growth factor prevents tissue fibrosis, remodeling, and dysfunction in cardiomyopathic hamster hearts. Am J Physiol Heart Circ Physiol 2005;288:H2131—9.
[41] Lee S, Wolf P, Escudero R, Deutsch R, Jamieson S, Thistlethwaite P. Early expression of angiogenesis factors in acute myocardial ischemia and infarction. N Engl J Med 2000;342:626—33.
[42] Fuchs M, Hilfiker A, Kaminski K, Hilfiker-Kleiner D, Guener Z, Klein G, Podewski E, Schieffer B, Rose-John S, Drexler H. Role of interleukin-6 for LV remodeling and survival after experimental myocardial infarction. FASEB J 2003;17:2118—20.
[43] Takemura G, Ohno M, Hayakawa Y, Misao J, Kanoh M, Ohno A, Uno Y, Minatoguchi S, Fujiwara T, Fujiwara H. Role of apoptosis in the disappearance of infiltrated and proliferated interstitial cells after myocardial infarction. Circ Res 1998;82:1130—8.
[44] Sam F, Sawyer D, Chang D, Eberli F, Ngoy S, Jain M, Amin J, Apstein C, Colucci W. Progressive left ventricular remodeling and apoptosis late after myocardial infarction in mouse heart. Am J Physiol Heart Circ Physiol 2000;279:H422—8.
[45] Hayakawa K, Takemura G, Kanoh M, Li Y, Koda M, Kawase Y, Maruyama R, Okada H, Minatoguchi S, Fujiwara T, Fujiwara H. Inhibition of granulation tissue cell apoptosis during the subacute stage of myocardial infarction improves cardiac remodeling and dysfunction at the chronic stage. Circulation 2003;108:104—9.
[46] Kondo H, Hojo Y, Tsuru R, Nishimura Y, Shimizu H, Takahashi N, Hirose M, Ikemoto T, Ohya K, Katsuki T, Yashiro T, Shimada K. Elevation of plasma granzyme B levels after acute myocardial infarction. Circ J 2009;73:503—7.
[47] Poe M, Blake J, Boulton D, Gammon M, Sigal N, Wu J, Zweerink H. Human cytotoxic lymphocyte granzyme B. Its purification from granules and the characterization of substrate and inhibitor specificity. J Biol Chem 1991;266:98—103.
[48] Tremblay G, Wolbink A, Cormier Y, Hack C. Granzyme activity in the inflamed lung is not controlled by endogenous serine proteinase inhibitors. J Immunol 2000;165:3966—9.
[49] Kurschus F, Kleinschmidt M, Fellows E, Dornmair K, Rudolph R, Lilie H, Jenne D. Killing of target cells by redirected granzyme B in the absence of perforin. FEBS Lett 2004;562:87—92.
[50] Carrell R. Alpha 1-antitrypsin: molecular pathology, leukocytes, and tissue damage. J Clin Invest 1986;78:1427—31.
[51] Hirst C, Buzza M, Bird C, Warren H, Cameron P, Zhang M, Ashton-Rickardt P, Bird P. The intracellular granzyme B inhibitor, proteinase inhibitor 9, is up-regulated during accessory cell maturation and effector cell degranulation, and its overexpression enhances CTL potency. J Immunol 2003;170:805—15.
[52] Bots M, Van Bostelen L, Rademaker M, Offringa R, Medema J. Serpins prevent granzyme-induced death in a species-specific manner. Immunol Cell Biol 2006;84:79—86.
[53] Rowshani A, Strik M, Molenaar R, Yong S, Wolbink A, Bemelman F, Hack C, Ten Berge I. The granzyme B inhibitor SERPINB9 (protease inhibitor 9) circulates in blood and increases on primary cytomegalovirus infection after renal transplantation. J Infect Dis 2005;192:1908—11.
[54] Stout-Delgado H, Getachew Y, Rogers T, Miller B, Thiele D. The role of serpinb9/serine protease inhibitor 6 in preventing granzyme B-dependent hepatotoxicity. Hepatology 2007;46:1530—40.
[55] Bird C, Blink E, Hirst C, Buzza M, Steele P, Sun J, Jans D, Bird P. Nucleocytoplasmic distribution of the ovalbumin serpin PI-9 requires a nonconventional nuclear import pathway and the export factor Crm1. Mol Cell Biol 2001;21:5396—407.
[56] Sun J, Bird C, Sutton V, McDonald L, Coughlin P, De Jong T, Trapani J, Bird P. A cytosolic granzyme B inhibitor related to the viral apoptotic regulator cytokine response modifier A is present in cytotoxic lymphocytes. J Biol Chem 1996;271:27802—9.
[57] Sipione S, Simmen K, Lord S, Motyka B, Ewen C, Shostak I, Rayat G, Dufour J, Korbutt G, Rajotte R, Bleackley R. Identification of a novel human granzyme B inhibitor secreted by cultured sertoli cells. J Immunol 2006;177:5051—8.
[58] Lazarczyk M, Dziunycz P, Niderla J, Milewski L, Lazarczyk M, Boszczyk A, Samaha R, Grzela T. Influence of pentoxifylline on natural cytotoxicity and expression of granzymes and PI-9, a specific granzyme B inhibitor. Int J Mol Med 2006;17:135—9.
[59] Jiang X, Orr B, Kranz D, Shapiro D. Estrogen induction of the granzyme B inhibitor, proteinase inhibitor 9, protects cells

against apoptosis mediated by cytotoxic T lymphocytes and natural killer cells. Endocrinology 2006;147:419—26.
[60] Young J, Sukhova G, Foster D, Kisiel W, Libby P, Schönbeck U. The serpin proteinase inhibitor 9 is an endogenous inhibitor of interleukin 1beta-converting enzyme (caspase-1) activity in human vascular smooth muscle cells. J Exp Med 2000;191:1535—44.
[61] Abbate A, Kontos M, Grizzard J, Biondi-Zoccai G, Van Tassell B, Robati R, Roach L, Arena R, Roberts C, Varma A, Gelwix C, Salloum F, Hastillo A, Dinarello C, Vetrovec G, et al. Interleukin-1 blockade with anakinra to prevent adverse cardiac remodeling after acute myocardial infarction (Virginia Commonwealth University Anakinra Remodeling Trial [VCU-ART] Pilot study). Am J Cardiol 2010;105:1371—7.
[62] Wieder K, Hancock W, Schmidbauer G, Corpier C, Wieder I, Kobzik L, Strom T, Kupiec-Weglinski J. Rapamycin treatment depresses intragraft expression of KC/MIP-2, granzyme B, and IFN-gamma in rat recipients of cardiac allografts. J Immunol 1993;151:1158—66.
[63] Suzuki J, Ogawa M, Muto S, Itai A, Isobe M. A specific inhibitor of plasminogen activator inhibitor-1 suppresses rat autoimmune myocarditis. Expert Opin Ther Targets 2008;12:313—20.