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REVIEW ARTICLE

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Granzyme B and natural killer (NK) cell death

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Abstract Granzyme B is a unique serine protease, which plays a crucial role for target cell death. Several mechanisms of delivery of granzyme B to target cells have been recently identified. Granzyme B directly activates Bid, a specific substrate for granzyme B, resulting in caspase activation. Granzyme B efficiently cleaves many prominent autoantigens, and the hypothesis that autoantibodies arise when cryptic determinants are revealed to the immune system has been proposed. Some autoantibodies directed against granzyme B-specific neoepitopes are present in serum from patients with autoimmune diseases. In the tissues from autoimmune diseases, granzyme B might play an important role for disease progression (i.e., rheumatoid arthritis synovium) or inhibition (i.e., regulatory T cells). We have identified a novel type of activation-induced cell death (granzyme B leakage-induced cell death). Activation-induced natural killer (NK) cell death is accompanied by the leakage of granzyme B from intracellular granules into the cytoplasm, and it triggers apoptosis by directing Bid to mitochondrial membranes. An excess of "leaked" granzyme B over its inhibitor, serpin proteinase inhibitor 9, is a major determinant of cell death. The role of granzyme B in autoimmunity and its influence on NK cell death are discussed.

Key words Apoptosis · Autoantibody · Granzyme B · Natural killer (NK) cell

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Introduction

Granzyme B is a 32 kDa serine protease found in the lytic granules of natural killer (NK) cells and cytotoxic T lymphocytes (CTL). When granzyme B and perforin are secreted into the interspace between the NK cell and the target cell, granzyme B finds its way into the cytoplasm of the target cell, where it triggers apoptotic cell death.[1,2] A possible role for granzyme B in initiating and/or propagating autoimmune disease has recently been proposed.[3] The capacity of an antigen to be a granzyme B substrate is a strong predictor of its status as a molecular target for autoantibody production.

Natural killer cells are important effectors of the innate immune response. By monitoring the level of class I major histocompatibility complex (MHC) molecules expressed on the surface of cells, NK cells function as sentinels of virus infection and cellular transformation. Target cells expressing reduced levels of class I MHC molecules trigger NK cells to release cytotoxic granules containing effector molecules (i.e., perforin, granzymes) that bring about target cell death.[4,5] In addition, NK cells can participate in immune regulation by eliminating autoreactive T cells and B cells.[6–8] Reduced NK cell number or impaired NK cell function might permit the persistence of viral infection, malignant disease, or autoimmune disease. In this review, we discuss the role of granzyme B in autoimmunity and in NK cell death.

Granzyme B and its inhibitor, proteinase inhibitor 9

Granzyme B and its receptor

Granzyme B was originally discovered in the cytoplasmic granules of cytotoxic cells including NK cells and CTL.[9] This serine protease is a potent inducer of apoptosis in target cells. For many years, granzyme B had been thought to be expressed in a limited panel of cells; however, it has recently been found in chondrocytes,[10] neutrophils,[11] and some malignant cells.[12,13] Using immunohistochemical, in

situ hybridization, and reverse transcription–polymerase chain reaction analysis, granzyme B and perforin have been shown to exist in chondrocytes.[10] Normal cartilage consists of only one type of cell, the chondrocyte, and the extracellular matrix. Articular cartilage is not supplied with blood, suggesting that this region would be an immunoprivileged site. Several experiments have showed that the mechanism of endogenous cartilage degradation is mediated by chondrocyte apoptosis.[14] The existence of granzyme B and perforin in chondrocytes might be a good explanation for chondrocyte apoptosis, resulting in cartilage degradation. Neutrophils have been known to play a crucial role for inflammation. Many proteases are found in granules from neutrophils, including myeloperoxidase, proteinase 3, and others.[15] In addition to these proteases, it has been recently reported that neutrophils contain granzyme B in the cytoplasm, which induces efficient cellular cytotoxicity when targeted through Fc receptors to appropriate antibody-coated target cells.[11] However, the existence of granzyme B in this cell remains controversial.[16,17]

Granzyme B has been thought to enter target cells in concert with the formation of membrane pores that are formed by the protein perforin. Early studies suggested that at the beginning of the interaction between effector cells and target cells, perforin simply induced plasma membrane pore formation to pour granzyme B into the cytoplasm of the target cells. However, recent progress on the mechanisms of granzyme B delivery suggest more promising hypotheses.[18] First, granzyme B receptor, mannose 6-phosphate/insulin-like growth factor II receptor, discovered by Motyka et al., has been shown to play a role in endocytic uptake of granzyme B.[19] Second, granzyme B–serglycin complexes, which also interact with perforin, are incorporated into target cells without plasma membrane pore formation through a dynamin-dependent mechanism.[20,21] Finally, another pathway has been more recently reported in which (i) granzyme B binds to target cells through electrostatic interactions;[22] (ii) granzyme B undergoes electrostatic exchange from serglycin to target cells;[23] (iii) granzyme B induces target cell membrane blebbing via direct cleavage of ROCK II (the serine/threonine kinase Rho-associated coiled coil-containing protein kinase II);[24] and (iv) cell surface-bound heat shock protein 70 (Hsp 70) mediates perforin-independent apoptosis by specific binding and uptake of granzyme B.[25] Further investigation is needed to identify which pathway is dominant in apoptosis triggered by granzyme B. Moreover, the precise role of perforin and its cooperation with granzyme B must be revealed, as it is clear that perforin does more than simply to provide transmembrane pores, in the target cell membrane.

Granzyme B mainly triggers caspase activation indirectly. It directly activates Bid, a BH3-interacting dominant death agonist, which results in the leakage of pro-apoptotic mitochondrial mediators, such as cytochrome *c*, into the cytosol.[26] Cytochrome *c* then forms a complex with apoptosis proteinase-activating factor (Apaf)-1 and caspase 9. Caspase 9 activates caspase 3, the major effector caspase, resulting in apoptosis.[27] Cells isolated from various tissues of Bid-deficient mice are resistant to granzyme B-induced cell death, demonstrating that Bid plays a central role for this granzyme B-mediated apoptosis.[28]

Proteinase inhibitor 9

The serpin, proteinase inhibitor 9 (PI-9), is an endogenous serine proteinase inhibitor which is the only human protein capable of inhibiting granzyme B activity in a variety of cells including NK cells.[29] Proteinase inhibitor 9 has been proposed to play an important role in protecting NK cells from the toxic effects of granzyme B during granule assembly.[30,31] Immunohisto/cytochemistry analysis has shown that PI-9 exists in primary lymphoid organs, inflammatory infiltrates, dendritic cells, endothelial cells, mast cells, and at immune-privileged sites like the placenta, the testis, the ovary, and the eye.[32,33] Overexpression of PI-9 in tumors might be one explanation for the escape from effector cell attack.[34,35] When immature murine dendritic cells (DCs) differentiate to become mature DCs, they become resistant to CTL killing. The mechanism of resistance might be explained by the induction of SPI-6, the mouse homologue of human PI-9.[36]

Another function of PI-9 is as an endogenous inhibitor of interleukin 1β-converting enzyme (ICE, caspase 1) activity.[37] Since ICE can activate the proinflammatory cytokines interleukin (IL)-1β and IL-18, PI-9 may regulate the enzyme involved in inflammatory processes of chronic inflammatory diseases. From these points of view, PI-9 can regulate both apoptosis and inflammation.

Granzyme B substrates and the production of autoantibodies

Recently the hypothesis that autoimmunity arises when cryptic determinants are revealed to the immune system has been proposed.[38–40] Granzyme B is a unique protease that efficiently cleaves many prominent autoantigens, summarized in Table 1. Some of these autoantigens are substrates for granzyme B but not caspase 3 (e.g., CENP-B, fibrillarin, B23, PMS1, and M3R), while other autoantigens are cleaved by both proteases (e.g., La, PARP, NuMA, and fodrin). Importantly, the cleavage sites for granzyme B and caspase 3 are distinct.[3,39] Since caspase 3 is expressed ubiquitously in cells and is activated by a variety of apoptotic stimuli, and since thymocytes are exposed to these fragments during development, it is likely that tolerance to caspase 3 fragments exists in vivo. On the other hand, granzyme B is mainly expressed in cytotoxic cells, and granzyme B only encounters substrate in target cells when they encounter cytotoxic T cells, which release granule contents into the cytoplasm of target cells. It is unclear if granzyme B cleavage fragments are present in the thymus during the education of T cells. We have recently identified novel apoptosis-specific autoantibodies directed against granzyme B-induced cleavage fragments (27 kDa) of the SS-B (La) autoantigen in sera from patients with primary Sjögren's syndrome (SS).[41] A 27 kDa fragment was detected by Western blotting using sera from 13 of 74 primary SS patients (16.9%). This fragment was recognized by anti-La

Table 1. Granzyme B substrates

Substrate	Function	Cleavage site	First author[Ref.]
Alanyl tRNA synthetase	Translation	VAPD[632]	Casciola-Rosen[3]
Bid	Pro-apoptotic	IEAD[75]	Sutton[81]
B23	Nucleolar phosphoprotein	LAAD[161]	Ulanet[82]
Caspase 3	Effector caspase	IETD[175]	Andrade[83]
Caspase 7	Effector caspase	IQAD[198]	Andrade[83]
Caspase 10	Initiator caspase	IEAD[372]	Sun[84]
CD3ζ	Signal transducer	Many	Wieckowski[67]
CENP-B	Centromere protein	VDSD[457]	Casciola-Rosen[3]
DNA-PKcs	DNA repair	VGPD[2698]	Andrade[83]
Fibrillarin	snoRNP protein	VGPD[184]	Casciola-Rosen[3]
Filamin	Cytoskeletal protein	Many	Browne[85]
Fodrin	Unknown	IVTD[1554] AEID[1961]	Nagaraju[86], Kuwana[87]
Histidyl tRNA synthetase	Translation	LGPD[48]	Casciola-Rosen[3]
ICAD/DFF45	Nuclease	DETD[117] VTGD[6]	Thomas[88], Sharif-Askari[89]
Isoleucyl tRNA synthetase	Translation	VTPD[983]	Casciola-Rosen[3]
Ki-67	Cell proliferation	VCTD[1481]	Casciola-Rosen[3]
Ku-70	DNA replication, repair	ISSD[79]	Casciola-Rosen[3]
La	Pol III transcription	LEED[220]	Casciola-Rosen[3]
Lamin B	Nuclear membrane protein	VEVD[231]	Zhang[90]
Mi-2	DNA methylation, chromatin remodeling	VDPD[1312]	Casciola-Rosen[3]
Neuronal glutamate receptor (GluR3B)	Autoantigen	ISND[388]	Gahring[91]
NF90	Transcription	Unknown	Graham[92]
NuMA	Mitosis	VATD[1705]	Casciola-Rosen[3]
PARP	DNA binding protein	VDPD[536]	Andrade[83]
PM-Scl	Exoribonuclease	VEQD[252]	Casciola-Rosen[3]
PMS1	DNA repair	ISAD[496]	Casciola-Rosen[3]
PMS2	DNA repair	VEKD[493]	Casciola-Rosen[3]
RNA polymerase I	RNA synthesis	ICPD[448]	Casciola-Rosen[3]
RNA polymerase II	RNA synthesis	ITPD[370]	Casciola-Rosen[3]
SRP-72	Protein translation, ER localization	VTPD[373]	Casciola-Rosen[3]
Topoisomerase I	DNA unwinding, SR protein kinase	IEAD[15]	Casciola-Rosen[3]
Type 3 muscarinic acetylcholine receptor (M3R)	Acetylcholine signal transduction	MDQD[330] PSSD[387]	Nagaraju[86]
UBF/NOR-90	Nucleolar transcription factor	VRPD[220]	Casciola-Rosen[3]
U1–70 kDa	RNA splicing	LGND[409]	Casciola-Rosen [3]

monoclonal antibodies. Blocking studies using recombinant La protein revealed that an apoptosis-specific B-cell epitope was revealed and detected by serum antibodies from 4 of 13 primary SS patients. This represents the first report of detection of autoantibodies directed specifically against a granzyme B-induced cleaved form of the La autoantigen in sera from primary SS patients.[41] In addition to our results, autoantibodies directed against granzyme B-specific neoepitopes were reported for the U1–70 kDa protein[42] and for centromere protein[43] in sera from patients with SLE and scleroderma, respectively.

Role of granzyme B in autoimmunity

In rheumatoid arthritis (RA) synovium, many granzyme B-positive cells can be seen infiltrating the synovium, especially in early-stage RA.[44] The presence of this population of cells is associated with joint damage in early onset RA.[45] Moreover, the levels of soluble granzyme B are elevated in serum and synovial fluid derived from patients with RA, suggesting that CTL and NK cells play an important role for the pathogenesis of this disease.[46] Indeed, NK cells accumulate in the synovial fluid of RA patients.[47,48] Recently, Goldbach-Mansky et al. reported that a high serum level of granzyme B was an independent predictor of early erosions in patients with rheumatoid factor positive RA, demonstrating that granzyme B may be a useful prognostic marker in early RA and may provide important clues to the pathogenesis of this disease.[49] Future investigations directed at elucidating the crucial roles of granzyme B-positive cells, including NK cells, in RA are clearly indicated.

CD4+CD25+ regulatory T (T_{reg}) cells, established as major controllers of immune responses to self and other antigens, express high levels of FOXP3 (encoding a transcription factor of the forkhead family) gene and play a pivotal role in peripheral tolerance and the prevention of autoimmunity.[50,51] T_{reg} cells can directly regulate the function of T cells, antigen-presenting cells (APC), and B cells.[52,53] The suppressive function of T_{reg} cells is known to depend on direct cellular contact with the target cells.[54] Gondek et al. recently reported that granzyme B is functionally involved in contact-mediated suppression by T_{reg} cells. Evidence for this includes the reduced ability of T_{reg} cells from granzyme $B^{-/-}$ mice to suppress as efficiently as T_{reg} cells from WT mice.[55] In human T_{reg} cells, granzyme A rather than granzyme B appears to be dominant.[56]

Table 2. Kinetics of cell death

Type of cells	Stimulation	Kinetics of death	First author[Ref.]
NK cell	CD16	Very rapid (1 h)	Ortaldo[62]
	CD2	Very rapid (1.5 h)	Ida[61]
	CD94	Rapid (2 h)	Ida[58]
	Fas	Rapid (2 h)	Ortaldo[93]
T cell	Fas	Rapid (2 h)	Nagata[94]
	CD3	Slow (18–24 h)	Dhein[95], Brunner[96], Ju[97]
($CD8^+CD57^+$)	CD2	Very rapid (1–2 h)	Rouleau[98]
B cell	Ig	Slow (12–24 h)	Hasbold[99], Ishigami[100]

Apoptosis in NK cells

Activation-induced NK cell death

We screened the NK cell panel of Workshop (6th International Workshop and Conference, Leucocyte Typing VI, Kobe, Japan) monoclonal antibodies (mAb) for their ability to induce apoptosis in resting and interleukin-2 (IL-2)-activated NK cells. We found that anti-CD94 mAb (NK28, NKH3) consistently triggered apoptosis in IL-2-activated NK cells in addition to anti-CD16 mAb (NK9, 214.1 and 3G8) and anti-Fas mAb (7C11) that had been previously reported to induce cell death.[57] Evidence for CD94-induced apoptosis includes chromatin condensation as measured by increased fluorescence of Hoechst dye, induction of DNA fragmentation, and characteristic morphology by transmission electron microscopy.[58] CD94/NKG2 receptors are lectin-like, disulfide-linked heterodimers expressed as type II membrane proteins on human NK cells and a subset of T cells.[4,5,59] Ligands for CD94/NKG2 are the nonclasssical MHC-I molecules, HLA-E.[60] While the CD94 subunit of the receptor is encoded by a single gene, NKG2 constitutes a multigenic family of five proteins, designated NKG2A, NKG2B (a splice variant of NKG2A), NKG2C, NKG2D, and NKG2E.[59] The cytoplasmic domains are either long (NKG2A and B) or short (NKG2C and E), corresponding to inhibitory or activating CD94/NKG2 isoforms, respectively. As the cytoplasmic tail of CD94 is very short, the signal triggered by CD94 stimulation is transduced via NKG2 molecules. NKG2A stimulation (using Z199 monoclonal antibody) could not induce apoptosis in IL-2-primed NK cells (unpublished data), suggesting that activation-induced cell death requires NKG2C or E (activating receptors) after CD94 stimulation in IL-2-activated NK cells. The ability of CD94 and CD16 to trigger activation-induced NK cell death led us to investigate the potential for CD2, a classical activating molecule, to trigger activation-induced cell death in NK cells. Anti-CD2 monoclonal antibodies also induce apoptosis in IL-2-primed NK cells monitored by chromatin condensation, DNA fragmentation, and cleavage of caspase 3.[61]

As NK cells participate in the regulation of infectious responses, removal of malignant cells, and autoimmunity, it is very important to identify the mechanisms of NK cell death and NK cell dysfunction. Table 2 shows the kinetics of apoptosis in NK cells, T cells, and B cells. Activation-induced NK cell death triggered by CD2, CD16, or CD94 is extraordinarily rapid (e.g., DNA ladders can be demonstrated within 1.5, 1, or 2 h, respectively) compared to the activation-induced death of T or B cells.[58,61,62] In contrast to activation-induced T-cell death, neutralizing antibodies reactive with Fas ligand or tumor necrosis factor (TNF)-α do not inhibit activation-induced NK cell death, suggesting that CD2, CD16, and CD94 are coupled to signaling cascades that directly trigger the core death pathway.[58,61] As the caspase-specific peptide inhibitor (Z-VAD-FMK) could not inhibit activation-induced NK cell death, this process is caspase independent.[63] The rapid kinetics of apoptosis in IL-2-primed NK cells led us to focus on granzyme B, since activated NK cells express large of granzyme B, a potent serine protease that promptly cleaves many substrates and induces apoptosis. We found that granzyme B rapidly leaks from the cytotoxic granules of NK cells following activation via CD2.[63] Granzyme B leakage was confirmed by the formation of sodium dodecyl sulfate-resistant granzyme B/PI-9 complexes and by the colocalization of granzyme B and PI-9, as revealed by immunofluorescent analysis. The proapoptotic Bcl-2 family member Bid, a known substrate for granzyme B, is cleaved during activation-induced NK cell death, suggesting that apoptosis is initiated when truncated Bid is targeted to mitochondrial membranes. The rapid leakage of granzyme B in response to activation stimuli may therefore explain the rapid kinetics of NK cell death reported previously by our group.[58,61] After truncated Bid translocates from the cytosol to mitochondrial membranes, cytochrome *c* is then released from mitochondria, allowing the assembly of the Apaf-1-containing "apoptosome." Procaspase 9 is recruited to the apoptosome to initiate caspase activation. This proposed death cascade correlates with the time course revealed in our Western blotting experiments (i.e., granzyme B leakage, processing of Bid, caspase 9, and caspase 3) (Fig. 1).[63] Activation-induced NK cell death is dependent upon IL-2 priming, suggesting that IL-2 alters some aspect of cellular physiology in a way that promotes apoptosis. We have discovered that IL-2 priming induces the expression of granzyme B without inducing the expression of PI-9, a granzyme B inhibitor that normally protects cytotoxic lymphocytes from inappropriate activation of granzyme B.[63] The ability of IL-2 to prime NK cells for activation-induced death was found to correlate with the altered ratio of granzyme B to PI-9.[63] When granzyme B leaks into the cytosol following CD2-

induced activation, the levels of PI-9 may be insufficient to protect the cell from granzyme B-induced death. We named this novel type of activating-induced cell death GLCD (granzyme B leakage-induced cell death) (Fig. 1).

The survival of virus-infected cells and tumor cells is dependent upon evasion of the host immune response. Downregulation of HLA class I expression is a common strategy used to evade cytotoxic T-lymphocyte recognition. The ability of NK cells to detect cells expressing reduced levels of class I MHC allows immune recognition of these cells.[4,5] A main CD2 ligand, lymphocyte-function-associated antigen-3 (LFA-3) is required for susceptibility to NK cell killing.[64] When the level of class I MHC is recovered and sufficient for recognition by antigen-specific T cells, activated NK cells may undergo apoptosis via LFA-3–CD2 interactions. Such a mechanism might prevent nonspecific killing by NK cells that could result in autoimmune tissue damage. Upregulation of LFA-3 has been reported in both virus-infected cells and malignant cells.[64–66] It is therefore possible that LFA-3/CD2 interactions promote the death of activated NK cells in a way that allows virus-infected or transformed cells to evade this "fail-safe" mechanism.

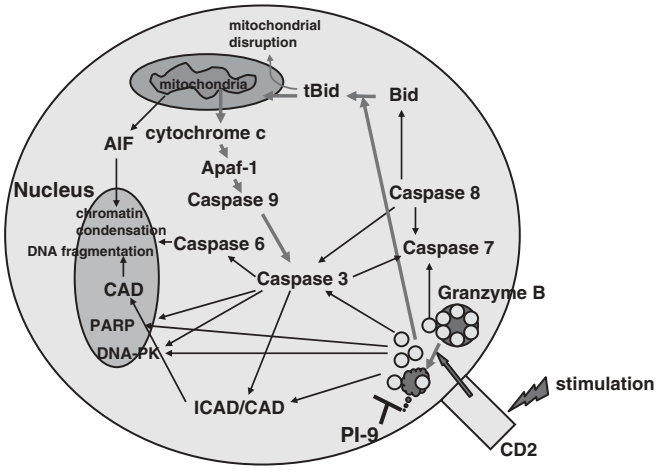

Fig. 1. Mechanisms of granzyme B leakage-induced cell death. *Bold line* shows the pathway of granzyme B leakage-induced cell death. *AIF*, apoptosis-inducing factor; *Apaf-1*, apoptosis proteinase-activating factor-1; *CAD*, caspase-activated deoxyribonuclease; *ICAD*, inhibitor of CAD; *PARP*, polyADP-ribose polymerase; *PK*, protein kinase; *PI*, proteinase inhibitor

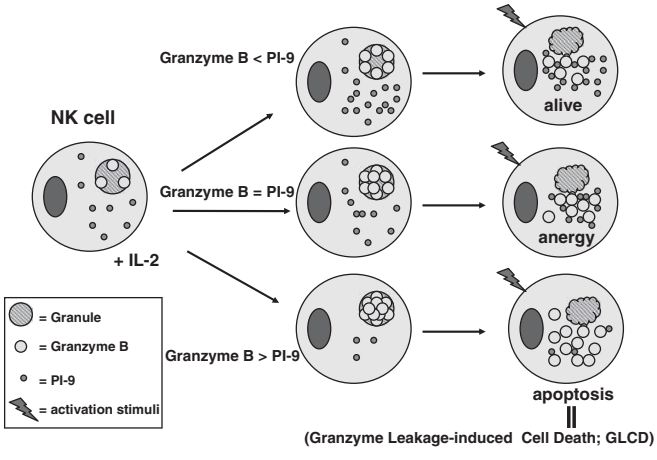

Fig. 2. Balance between granzyme B and proteinase inhibitor 9 (*PI-9*) might determine the fate of natural killer (*NK*) cells. *IL*, interleukin

Once NK cells are activated by cytokines (i.e., IL-2), the selective induction of granzyme B expression alters the ratio of granzyme B:PI-9. As PI-9 is a strong and specific inhibitor of granzyme B, the relative difference between these two molecules in NK cells may be a crucial determinant of the NK cell response to activating stimuli. The ratio between perforin and β-actin was less but significantly correlated with the percentage of $\Delta\Psi m$ compared to granzyme B/PI-9 or granzyme B/β-actin ratio. Although perforin itself cannot induce apoptosis, it may play a role in NK cell death, together with granzyme B. It has been reported that granzyme B and caspase 3 directly cleave the ζ chain,[67,68] leading to defective activation. As granzyme B also directly cleaves caspase 3,[69] it is possible that IL-2-primed NK cells may be rendered anergic before they undergo apoptosis (Fig. 2). This might explain the reduced expression of ζ chain in tumor-infiltrating cytotoxic lymphocytes (including NK cells).[70]

Cytokine-induced NK cell death

Patients with systemic autoimmune diseases have been reported to have reduced numbers of peripheral blood NK cells and impaired NK cell function compared with healthy subjects.[71–78] Cytokines are known to regulate the function of NK cells.[79] A variety of cytokines are found in the sera of patients with systemic autoimmune diseases. Cytokine-induced NK cell death is one of the explanations for low NK cell number in these patients. The ability of selected cytokines to trigger NK cell death prompted us to compare the levels of peripheral blood cytokines with the numbers of NK cells in patients with various systemic autoimmune diseases. In our study, the number of NK cells was significantly decreased in the peripheral blood of patients with systemic autoimmune diseases compared with normal controls.[80] Serum concentrations of IL-18, IL-15, and TNF-α were inversely related to the number of NK cells in both patients and healthy controls.[80] Moreover, IL-18+IL-15, IL-18+IL-12, or TNF-α induced NK cell death in vitro, suggesting that high levels of IL-18, IL-15, and TNF-α are associated with the decreased number of NK cells in patients with systemic autoimmune diseases.[80] The kinetics of cytokine-induced NK cell death are very slow compared with GLCD. The possibility that the slow kinetics of cytokine-induced NK cell death reflect a requirement for autocrine release of Fas ligand or TNF-α was examined by cytokine stimulation in the presence of neutralizing antibodies against Fas ligand or TNF-α. Cytokine-induced NK cell death was partially, but weakly, blocked by neutralizing antibodies against TNF-α but not by neutralizing antibodies against Fas ligand, suggesting that secondary production of TNF-α is required for this functional response.[80] This conclusion is supported by experiments showing that TNF-α induces NK cell death in a dose-dependent manner. Interleukin-18 in combination

with IL-15 resulted in the release of TNF-α into culture supernatants. Less disruption of ΔΨm was observed in NK cells cultured with higher concentration of TNF-α than in the supernatant of medium after IL-18+IL-15 stimulation, suggesting that one or more mechanisms of NK cell death aside from TNF-α still remains. In addition to low NK cell number, NK cell dysfunction is well known in autoimmune diseases. However, the molecular mechanism of the NK dysfunction in patients with autoimmune disease is still poorly understood. Mechanisms of cytokine-induced NK cell death and NK cell dysfunction deserve further investigation.

Conclusion

Granzyme B plays a crucial role for target cell death, and it may play a significant role in autoantibody production and autoimmune progression or regulation. Granzyme B leakage-induced cell death is an important determinant of activation-induced NK cell death, and this process may be important for the fate of NK cells. As NK cells can participate in immune regulation by eliminating autoreactive T cells and B cells, reduced NK cell number or impaired NK cell function might permit the persistence of autoimmune disease.

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Erratum

Mod Rheumatol (2005) 15:4–8

An error appeared in the article cited above. On page 4, the title of the article is incorrectly shown as "Official Japanese guidelines for the use of infliximab for rheumatoid arthritis". It should read "Proposed Japanese guidelines for the use of infliximab for rheumatoid arthritis". The guidelines were given final approval by Japan College of Rheumatology on July 1, 2005.