Brief report

The serine protease granzyme M is preferentially expressed in NK-cell, γδ T-cell, and intestinal T-cell lymphomas: evidence of origin from lymphocytes involved in innate immunity

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Granzyme M (GM) is a novel serine protease whose expression is highly restricted to natural killer (NK) cells, CD3$^+$CD56$^+$ T cells, and γδ T cells. Using a GM-specific monoclonal antibody, we analyzed the expression of GM in 214 mature T-cell and NK-cell lymphomas. GM was preferentially expressed in nasal NK/T-cell lymphomas (100%), γδ T-cell lymphomas (100%), and intestinal T-cell lymphomas (85%). In contrast, GM expression was present at low prevalence in mycosis fungoides/Sézary syndrome (3%), anaplastic large-cell lymphoma (6%), panniculitis-like T-cell lymphoma (11%), and angioimmunoblastic T-cell lymphoma (0%) cases. Peripheral T-cell lymphomas of unspecified subtype showed an intermediate frequency (37%) of GM expression, consistent with their heterogeneous origin. We conclude that GM expression is a distinctive feature of the nasal NK/T-cell, γδ T-cell, and intestinal T-cell lymphomas, and suggest that these tumors develop from lymphocytes involved in innate immunity. (Blood. 2003;101: 3590-3593)

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Introduction

Target cell death induced by cytotoxic lymphocytes involves several molecular mediators, including membrane-bound proteases known as granzymes.[1] To date, 5 granzymes have been demonstrated in human cells.[1] These enzymes are similar in structure, but differ in their substrate specificity and chromosomal locations.[1]

Granzyme M (GM), a novel member of this family, has an unusual enzyme specificity, preferring cleavage after methionine, leucine, or norleucine.[2] Its expression is restricted to natural killer (NK) cells, CD3$^+$CD56$^+$ T cells, and γδ T cells.[3-4] It has been suggested that this enzyme may play a role in the effector phase of innate immune responses.[4]

Lymphomas arising from NK and cytotoxic T cells have been increasingly recognized over the past few years. All of these diverse lymphomas express various cytotoxic lymphocyte-associated proteins.[5-10] In vivo expression of GM has not yet been reported in human lymphomas.

In order to define the expression pattern of GM and its coexpression with other cytotoxic proteins (CtxPs), we performed an immunohistochemical study in a wide variety of mature human T-cell and NK-cell lymphomas, using a GM-specific monoclonal antibody.

Study design

Formalin-fixed, paraffin-embedded samples from 214 mature T-cell and NK-cell lymphomas were retrieved from the files of the Hematopathology Section, Laboratory of Pathology, National Cancer Institute, Bethesda, MD; the Institute of Pathology, University of Wurzburg, Germany, and the Laboratory of Tumor Pathology and Molecular Diagnostics, Bay Zoltan Foundation for Applied Research, Szeged, Hungary. All cases had been previously immunophenotyped in paraffin or frozen sections and classified according to the World Health Organization classification.[10]

The GM-specific mouse monoclonal antibody 4H10 (1:2000 dilution) was prepared in the laboratory of M.J.S., as previously described.[3] This antibody reacts with most splenic red pulp γδ T cells, with a low but variable number of lymphocytes in nonneoplastic lymph nodes (0%-10%) and weakly with scattered intramucosal and intraepithelial lymphocytes (IELs). It is of interest that the intensity of IEL GM expression is greatly increased in most reactive conditions, including celiac disease (L.K., unpublished observations, June 2001).

Additional immunostains were performed for TIA-1 and granzyme B (GB), using TIA-1/2G9 (Coulter Immunology, Hialeah, FL; 1:2000) and GB/GrB7 (Monosan, Uden, The Netherlands; 1:20).

Immunohistochemistry was performed following heat-induced antigen retrieval. Primary antibodies were applied for 60 minutes at room temperature, and developed as previously described.[8] An immunoreaction was

Table 1. Expression of granzyme M and other cytotoxic proteins in cases studied

	TIA-1 (%)	Granzyme B (%)	Granzyme M (%)	Overall* (%)
GM⁺CtxP⁺ lymphomas				
Nasal NK/TCL	25/25 (100)	25/25 (100)	25/25 (100)	25/25 (100)
γδ TCL	5/5 (100)	5/5 (100)	5/5 (100)	5/5 (100)
Hepatosplenic	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)
Other locations	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)
Intestinal TCL	26/27 (96)	21/27 (78)	22/27 (85)	26/27 (96)
CD56⁻	20/21 (95)	19/21 (90)	20/21 (95)	20/21 (95)
CD56⁺	6/6 (100)	2/6 (33)	2/6 (33)	6/6 (100)
GM⁻CtxP⁺ lymphomas				
ALCL	32/49 (65)	32/49 (65)	3/49 (6)	39/49 (80)
Cutaneous	3/10 (30)	3/10 (30)	2/10 (20)	6/10 (60)
Systemic	29/39 (74)	29/39 (74)	1/39 (3)	33/39 (85)
Panniculitis-like TCL	18/18 (100)	18/18 (100)	2/18 (11)	18/18 (100)
CD56⁻	17/17 (100)	17/17 (100)	1/17 (6)	17/17 (100)
CD56⁺	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)
GM⁻CtxP⁻ lymphomas				
AILT	1/23 (4)	1/23 (4)	0/23 (0)	1/23 (4)
MF/SS	2/31 (6)	1/31 (3)	1/31 (3)	2/31 (6)
"Mixed" lymphomas				
TCL unspecified	12/36 (33)	13/36 (36)	14/36 (39)	15/36 (42)
Nodal	8/27 (30)	9/27 (33)	9/27 (33)	9/27 (33)
Extranodal	4/9 (44)	4/9 (44)	5/9 (56)	6/9 (67)
Total	121/214 (57)	116/214 (54)	72/214 (34)	131/214 (61)

*Positive for at least one cytotoxic cell-associated protein.

scored positive if cytoplasmic granular immunostaining occurred in at least 20% of the tumor cells, after excluding GM⁺ reactive small lymphocytes.

Results and discussion

GM expression was identified in 25 of 25 nasal NK/T-cell lymphomas (NK/TCLs), 5 of 5 γδ T-cell lymphomas (γδTCLs), 22 of 27 intestinal T-cell lymphomas (ITCLs), 2 of 10 primary cutaneous anaplastic large-cell lymphomas (PC-ALCLs), 1 of 39 systemic ALCLs (S-ALCLs), 2 of 18 subcutaneous panniculitic T-cell lymphomas (SPTCLs), 1 of 31 mycosis fungoides/Sézary syndrome (MF/SS) cases, 0 of 23 angioimmunoblastic T-cell lymphomas (AILTs), and in 14 of 36 peripheral T-cell lymphomas of unspecified subtype (PTCLs-NOS) (Table 1). GM expression roughly segregated mature T-cell and NK-cell lymphomas into 2 broad groups, representing tumors derived from T- or NK-cells linked to innate immunity (GM⁺ cases), and tumors derived from lymphocytes involved in adaptive immunity (GM⁻ cases). When evaluated with 2 other CtxPs (TIA-1 and GB), 4 categories of mature T-cell and NK-cell lymphomas could be distinguished: (1) GM⁺CtxP⁺ lymphomas; (2) GM⁻CtxP⁺ lymphomas; (3) GM⁻CtxP⁻ lymphomas; and (4) lymphomas with mixed characteristics.

GM⁺CtxP⁺ lymphomas (NK/TCLs, γδ TCLs, ITCLs)

All 25 NK/TCLs were GM⁺ (Figure 1A) and CtxP⁺. NK/TCLs comprise a distinct lymphoma entity characterized by CD56 and CtxP expression, presence of Epstein-Barr virus (EBV), and lack of rearranged T-cell receptor genes.[6,7,10] Accordingly, 24 of our 25 cases were CD56⁺ and 17 of 18 were EBV⁺. Most cases of nasal NK/TCL are proposed to arise from NK cells.[6,7,10] NK cells are crucial components of the innate immune system, representing the first line of defense. Sayers et al[4] described high constitutive GM expression in NK cells, but not in purified or activated conventional αβ T cells. The uniformly high GM expression in our cases further strengthens the NK-cell origin of this lymphoma entity.

GM and CtxP expression was found in all 5 γδTCLs (Figure 1B). This is consistent with the identification of GM in peripheral blood γδ T cells.[4] γδ T cells also participate in innate immune responses,[11] and therefore in γδTCLs as well as in normal γδ T cells, GM expression correlates with innate immune cell derivation.

In the ITCLs, 96% expressed CtxPs and 85% were GM⁺ (Figure 1C). Besides lymphoma cells, IELs were also GM⁺. ITCL
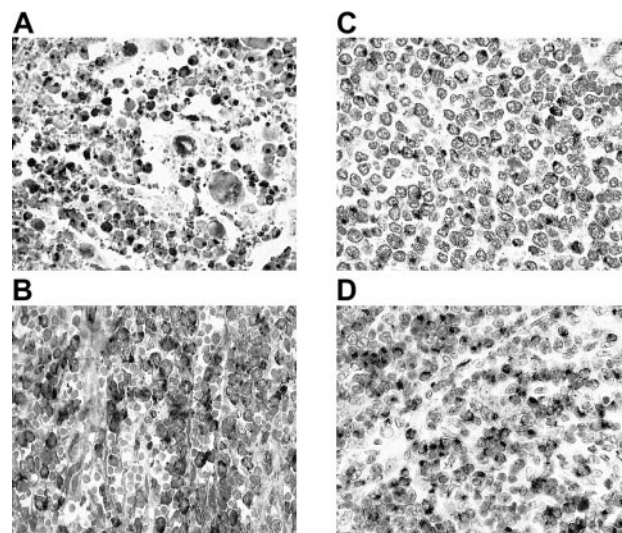

Figure 1. Granzyme M (GM) expression in T-cell and NK-cell lymphomas. (A) Nasal NK/T-cell lymphoma (nasal biopsy) with exuberant apoptotic necrosis. Apoptotic and viable tumor cells are strongly GM⁺. (B) Hepatosplenic γδ T-cell lymphoma (splenectomy specimen). Cordal and intrasinusoidal lymphomatous cells show intense GM positivity. (C) Intestinal T-cell lymphoma with medium-sized cells (resection specimen). Lymphoma cells reveal GM expression in the Golgi region. (D) Peripheral T-cell lymphoma of unspecified subtype (lymph node biopsy). There is an infiltrate predominantly composed of medium-sized cells with strong GM positivity in most cells.

is proposed to arise from IEL. The majority of intestinal IELs are TCRαβ CD8$^+$ cells possessing CtxPs, which suggests a cytotoxic T-cell function.[12,13] A smaller number have a γδ T-cell phenotype[14] and a minor subset have properties consistent with NK-cell derivation.[13] Intestinal αβ CD8$^+$ IELs are distinct from conventional T cells, sharing common developmental and functional features with NK cells,[13,15,16] including the expression of NK-cell receptors CD160/BY55[17] and CD94.[18] In addition, intestinal IEL populations represent oligoclonally or monoclonally expanded T cells[19] similar to the NK-cell receptor–positive peripheral blood T cells.[20] These unique features suggest that human αβ IEL cells are an integral component of the innate immune system. This idea is also supported by their GM expression, a feature shared with other effector cells of the innate immune system.[4] The current study, indicating that GM is expressed in most ITCLs, suggests that a majority of these cases are tumors of intestinal αβ T cells involved in innate immunity. The expression of NK-cell receptors in some ITCL cases[21,22] supports this hypothesis. One case lacking CtxP expression was CD4$^+$ and probably represented a tumor derived from lamina propria T cells. Contrary to most ITCLs and other CD56$^+$ lymphomas in our series, only 2 of 6 CD56$^+$ ITCLs expressed GM. This finding corresponds with the observation that these lymphomas are morphologically and phenotypically distinct from other ITCLs,[23] suggesting a derivation from a unique IEL population.

GM$^-$CtxP$^+$ lymphomas (PC-ALCLs, S-ALCLs, SPTCLs)

In our series, 2 PC-ALCLs and 1 S-ALCL were GM$^+$ (6%). The latter was CD56$^+$ and showed "null-cell" phenotype, suggesting a possible NK-cell origin of this otherwise typical ALK$^+$ case. The 2 GM$^+$ PC-ALCLs exhibited CD4$^+$ and double-negative phenotypes, respectively, but were CD56$^-$. Some ITCLs can have anaplastic features and show strong CD30 expression, mimicking S-ALCL. However, the rarity of GM expression in S-ALCL supports the concept that S-ALCL and ITCL with anaplastic features should be distinguished. This distinction has clinical implications as well, since ITCL has a much poorer prognosis than S-ALCL.[10]

In SPTCLs, 2 of 18 (11%) were GM$^+$. Both were CD8$^+$ and one coexpressed CD56. The latter case is likely a subcutaneous γδ T-cell lymphoma, but no frozen material was available to stain for the TCRδ chain. The remaining cases revealed the activated cytotoxic T-lymphocyte phenotype (CD8$^+$).[9,10]

GM$^-$CtxP$^-$ lymphomas (MF/SS cases, AILTs)

In our MF/SS cases (7 patch, 18 plaque, and 6 tumor stage), 2 of 31 (6%) were CtxP$^+$ and 1 (3%) was GM$^+$. Both CtxP$^+$ cases were plaque stage and neither was CD8$^+$, CD56$^+$, or of γδ T-cell origin. Of interest, most CD4$^+$ cutaneous T-cell lymphomas have a mature αβ memory T-cell phenotype,[24] consistent with our own results. Contrary to a recent report by Vermeer et al,[25] we did not find a correlation between disease stage and CtxP expression. The lower percentage of CtxP$^+$ cases in our study (6% vs 45%), and the lack of correlation between CtxP$^+$ and disease stage may be due to our more stringent positive inclusion criterion (20% vs 10%), and/or our small number of tumor stage cases.[6]

Although cases of AILT showed many small nonneoplastic lymphocytes with CtxP expression, CtxP (with one exception) and GM expression were not observed in the atypical cells of these cases. This finding concurs with results from Sayers et al,[4] who demonstrated no detectable GM in highly purified CD4$^+$ T cells, which are believed to be the precursors of AILT.[26]

Lymphomas with mixed characteristics (PTCLs-NOS)

PTCLs-NOS displayed an intermediate prevalence of GM expression (39%), somewhat higher in extranodal (56%) than in nodal (33%) cases (Figure 1D). Most GM$^+$ nodal cases (67%) were CD8$^+$, whereas the majority of the extranodal cases (60%) possessed a double-negative phenotype. Only one nodal case was CD56$^+$ and it expressed GM. This lymphoma category represents a diverse group of T-cell neoplasms that do not correspond to any of the well-defined entities.[10] Its diversity is underscored by our results, showing no consistent pattern of GM expression.

Summary

Our results suggest that GM expression distinguishes 2 broad groups of lymphomas. The lymphoma entities that comprise each of these groups have characteristics of cells that belong to either the innate immune system (GM$^+$ group), or the adaptive immune system (GM$^-$ group), respectively. Therefore, we postulate that GM$^+$ mature T-cell and NK-cell lymphomas derive from lymphocytes involved in the innate immune system.

References

1. Smyth MJ, Kelly JM, Sutton VR, et al. Unlocking the secrets of cytotoxic granule proteins. J Leukoc Biol. 2001;70:18-29.

2. Smyth MJ, O'Connor MD, Trapani JA, Kershaw MH, Brinkworth RI. A novel substrate-binding pocket interaction restricts the specificity of the human NK cell-specific serine protease, Metase-1. J Immunol. 1996;156:4174-4181.

3. Smyth MJ, O'Connor MD, Kelly JM, Ganesvaran P, Thia KY, Trapani JA. Expression of recombinant human Met-ase-1: a NK cell-specific granzyme. Biochem Biophys Res Commun. 1995; 217:675-683.

4. Sayers TJ, Brooks AD, Ward JM, et al. The restricted expression of granzyme M in human lymphocytes. J Immunol. 2001;166:765-771.

5. Cooke CB, Krenacs L, Stetler-Stevenson M, et al. Hepatosplenic T-cell lymphoma: distinct clinico-

pathologic entity of cytotoxic γδ origin. Blood. 1996;88:4265-4274.

6. Jaffe ES, Chan JK, Su IJ, et al. Report of the Workshop on Nasal and Related Extranodal Angiocentric T/Natural Killer Cell Lymphomas: definitions, differential diagnosis, and epidemiology. Am J Surg Pathol. 1996;20:103-111.

7. Chiang AKS, Chan ACL, Srivastava G, Ho FCS. Nasal T/natural killer (NK)-cell lymphomas are derived from Epstein-Barr virus-infected cytotoxic lymphocytes of both NK- and T-cell lineage. Int J Cancer. 1997;73:332-338.

8. Krenacs L, Wellmann A, Sorbara L, et al. Cytotoxic cell antigen expression in anaplastic large cell lymphomas of T- and null-cell type and Hodgkin's disease: evidence for distinct cellular origin. Blood. 1997;89:980-989.

9. Kumar S, Krenacs L, Elenitoba-Johnson KSJ, et al. Subcutaneous panniculitic T-cell lymphoma is

a tumor of cytotoxic T lymphocytes. Human Pathol. 1998;29:397-403.

10. Jaffe ES, Harris NL, Stein H, Vardiman JW. Tumours of Haematopoietic and Lymphoid Tissues: Pathology and Genetics: World Health Organization of Tumours. Lyon, France: IARC Press; 2001.

11. Boismenu R, Havran WL. An innate view of γδ T cells. Curr Opin Immunol. 1997;9:57-63.

12. Guy-Grand D, Malassis-Seris M, Briottet C, Vassalli P. Cytotoxic differentiation of mouse gut thymodependent and independent intraepithelial T lymphocytes is induced locally: correlation between functional assays, presence of perforin and granzyme transcripts, and cytoplasmic granules. J Exp Med. 1991;173:1549-1552.

13. Lundqvist C, Vladimir B, Hammarstrom S, Athlin L, Hammarstrom ML. Intraepithelial lymphocytes:

evidence for regional specialization and extrathymic T-cell maturation in the human gut epithelium. Int Immunol. 1995;7:1473-1487.

14. Spencer J, Isaacson PG, Diss TC, MacDonald TT. Expression of disulfide-linked and non-disulfide-linked forms of the T-cell receptor γ/δ heterodimer in human intestinal intraepithelial lymphocytes. Eur J Immunol. 1989;19:1335-1338.

15. Suzuki H, Duncan GS, Takimoto H, Mak TW. Abnormal development of intestinal intraepithelial lymphocytes and peripheral natural killer cells in mice lacking the IL-2 receptor β chain. J Exp Med. 1997;185:499-505.

16. Ohteki T, Yoshida H, Matsuyama T, Duncan GS, Mak TW, Ohashi PS. The transcription factor interferon regulatory factor 1 (IRF-1) is important during the maturation of natural killer 1.1[+] T cell receptor-α/β[+] (NK1^{+} T) cells, natural killer cells, and intestinal intraepithelial T cells. J Exp Med. 1998;187:967-972.

17. Anumanthan A, Bensussan A, Boumsell L, et al. Cloning of BY55, a novel Ig superfamily member expressed on NK cells, CTL, and intestinal intraepithelial lymphocytes. J Immunol. 1998;161: 2780-2790.

18. Jabri B, Patey-Mariaud De Serre N, Cellier C, et al. Selective expansion of intraepithelial lymphocytes expressing the HLA-E-specific natural killer receptor CD94 in celiac disease. Gastroenterology. 2000;118:867-879.

19. Blumberg RS, Yockey CE, Gross GG, Ebert EC, Balk SP. Human intestinal intraepithelial lymphocytes are derived from a limited number of T cell clones that utilize multiple V beta T cell receptor genes. J Immunol. 1993;150:5144-5153.

20. Mingari MC, Schiavetti F, Ponte M, et al. Human CD8^{+} T lymphocyte subsets that express HLA class I-specific inhibitory receptors represent oligoclonally or monoclonally expanded cell populations. Proc Natl Acad Sci U S A. 1996;93:12433-12438.

21. Haedicke W, Faith CSH, Chott A, et al. Expression of CD94/NKG2A and killer immunoglobulin-like receptors in NK cells and a subset of extranodal cytotoxic T-cell lymphomas. Blood. 2000;95: 3628-3630.

22. Dukers DF, Vermeer MH, Jaspars LH, et al. Expression of killer cell inhibitory receptors is restricted to true NK cell lymphomas and a subset of intestinal enteropathy-type T cell lymphomas

with a cytotoxic phenotype. J Clin Pathol. 2001; 54:224-228.

23. Chott A, Haedicke W, Mosberger I, et al. Most CD56^{+} intestinal lymphomas are CD8^{+} CD5^{−} T-cell lymphomas of monomorphic small to medium size histology. Am J Pathol. 1998;153:1483-1490.

24. Reinhold U, Liu L, Sesterhenn J, Abken H. CD7-negative T cells represent a separate differentiation pathway in a subset of post-thymic helper T cells. Immunology. 1996;89:391-396.

25. Vermeer MH, Geelen FAMJ, Kummer JA, Meijer CJLM, Willemze C. Expression of cytotoxic proteins by neoplastic T cells in mycosis fungoides increases with progression from plaque stage to tumor stage. Am J Pathol. 1999;154:1203-1210.

26. Willenbrock K, Roers A, Seidl C, Wacker HH, Kuppers R, Hansmann ML. Analysis of T-cell subpopulations in T-cell non-Hodgkin's lymphoma of angioimmunoblastic lymphadenopathy with dysproteinemia type by single target gene amplification of T cell receptor-beta gene rearrangements. Am J Pathol. 2001;158:1851-1857.