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Granulysin-Mediated Tumor Rejection in Transgenic Mice

Lisa P. Huang, Shu-Chen Lyu, Carol Clayberger, and Alan M. Krensky

Granulysin (GNLY) is a cytolytic molecule expressed by human CTL and NK cells with activity against a variety of tumors and microbes, including Mycobacterium tuberculosis. Although the molecular mechanism of GNLY-induced apoptosis of Jurkat T cells is well defined in vitro, no direct evidence for its in vivo effects has been demonstrated. Because there is no murine homolog of GNLY, we generated mice expressing GNLY using a bacterial artificial chromosome containing the human GNLY gene and its 5′ and 3′ flanking regions. GNLY is expressed in leukocytes from transgenic mice with similar kinetics as in PBMC from humans: GNLY is constitutively expressed in NK cells and, following stimulation through the TCR, appears in T lymphocytes 8–10 days after activation. Both forms of GNLY (9 and 15 kDa) are produced by activated T cells, whereas the 15-kDa form predominates in freshly isolated NK cells from transgenic animals. GNLY mRNA is highest in spleen, with detectable expression in thymus and lungs, and minimal expression in heart, kidney, liver, muscle, intestine, and brain. Allospecific cell lines generated from GNLY transgenic animals showed enhanced killing of target cells. In vivo effects of GNLY were evaluated using the syngeneic T lymphoma C6VL. GNLY transgenic mice survived significantly longer than nontransgenic littermates in response to a lethal tumor challenge. These findings demonstrate for the first time an in vivo effect of GNLY and suggest that GNLY may prove a useful therapeutic modality for the treatment of cancer. The Journal of Immunology, 2007, 178: 77–84.

Materials and Methods

Generation of transgenic mice

The DNA fragment used for the transgene was human bacterial artificial chromosome (BAC) clone RP11-439L14 (Chori Children’s Hospital Oakland Research Institute). This BAC clone is constructed in the BAC vector pBACe3.6 and grows in Luria-Bertani medium supplemented with 20 μg/ml chloramphenicol. The BAC is 186 kb, covering four genes (partial bacterial artificial chromosome (BAC) clone RP11-439L14 (Chori Children’s Hospital Oak-land Research Institute). This BAC clone is constructed in the BAC vector pBACe3.6 and grows in Luria-Bertani medium supplemented with 20 μg/ml chloramphenicol. The BAC is 186 kb, covering four genes (partial small nuclear ribonucleoprotein (SmRNP) assembly defective 1 homologue, surfactant pulmonary-associated protein B (SFTPB), GNLY, and basic helix-loop-helix transcription factor 6). It was streaked to single colonies from a bacterial Luria-Bertani agar stab culture in host Escherichia coli DH10. Ten single colonies were purified and confirmed by two different GNLY PCR sets that cover exons 1–4. BAC DNA was purified to microinjection quality and quantity. Size, concentration, and purity were confirmed by restriction enzyme digestions and clamped homogeneous electric field gel electrophoresis. BAC DNA was microinjected into embryonic stem cells and offspring were produced. DNA was isolated from mouse tails and analyzed by PCR using two pairs of GNLY (CBA × C57BL/6)F1 gene-specific probes: pair 1, GNLY 5′ exon 1 (GCGCCCTCCTGCTC...
C57BL/6 (H-2 b) mice were purchased from Charles River Laboratories. MHC class I protocols approved by the institutional review board. C6VL is an MHC universitу Medical Center. Studies reported in this work were performed using All mice were housed at the Laboratory Animal Facility at Stanford University. NK cell MACS isolation kits (Miltenyi Biotec); 98% pure C57BL/6 or CBA/J backgrounds.

**Mice and cell lines**

CBA/J (H-2 d) mice were purchased from The Jackson Laboratory, and C57BL/6 (H-2 b) mice were purchased from Charles River Laboratories. All mice were housed at the Laboratory Animal Facility at Stanford University Medical Center. Studies reported in this work were performed using protocols approved by the institutional review board. C6VL is an MHC class I, MHC class II T cell lymphoma cell line of C57BL/6 mouse origin (33). The RMA-S cell line, derived from a C57BL/6 mouse, is a T lymphoma devoid of internally derived antigenic peptides and that expresses low levels of MHC class I (34). EL4.F15, a mouse thymoma (H-2 b) defective in Fas signaling, was a gift from M. Simon (Max-Planck-Institut for Immunobiology, Freiburg, Germany) (35). YAC-1, a mouse lymphoma established by inoculation of the Moloney leukemia virus into a newborn A/JSn mouse, is a target for NK cells (36).

**NK cell purification and activation of splenocytes**

Murine NK cells were isolated from GNLY+/− splenocytes by negative selection using NK cell MACS isolation kits (Miltenyi Biotec); >98% stained with the NK cell-specific Ab DX5 (BD Biosciences) by FACS analysis. Human NK cells were purified by PBMC from the human NK cell MACS isolation kits (Miltenyi Biotec), and purity was confirmed by FACS analysis using the human NK-specific Ab CD56 (BD Biosciences). Cytokine/chemokine stimulation and permeabilization (Cytofix/Cytoperm; BD Pharmingen), samples were stained with rabbit anti-GNLY antiserum (1) diluted 1/10,000 in staining buffer, followed by FITC-conjugated goat anti-rabbit secondary Ab diluted 1/2,000, or with PE-granzyme B Ab (BD Pharmingen). Fluorescence was analyzed using a FACSCalibur four-color flow cytometry system (BD Biosciences). All FACs data are representative of three or more independent experiments.

**Quantitative real-time RT-PCR (qRT-PCR)**

Total RNA was isolated from cells (stabilized in RNA solution when later use) using TRIzol reagent (Invitrogen Life Technologies). One million cells were first blocked with unlabeled Abs against CD16 (F(ab′)2; BD Biosciences; clone 3G8) and CD32 (F(ab′)2, Caltag Laboratories) and then labeled with fluorescein-labeled Abs specific for CD4 (F(ab′)2, clone H129.19), CD8 (F(ab′)2, clone 53-6.7), or NK (clone DX5) (all from BD Pharmingen). After fixation and permeabilization (Cytofix/Cytoperm; BD Pharmingen), samples were stained with fluorescein-labeled Abs against NK cell activating receptor NKG2D (clone 1F9), and FITC-labeled Abs against mouse CD8 and mouse CD4 (all from BD Pharmingen). Intracellular staining for human NK cells was performed using A 7900HT Fast Real-Time PCR System (Applied Biosystems): denaturing at 94°C for 1 min, followed by 40 cycles of 30 s at 94°C, 30 s at 65°C, and 1 min at 72°C; final extension at 72°C for 10 min. The 3′-exon 3 (CAGTAACAGATCCAAGAGATGGAAAGAGAGTAGC) and 3′-control-RC (GGCATAAACTATAGCTGAATTATTCCATGCCCCC). Five mice expressing the transgene were backcrossed for more than eight generations with wild-type C57BL/6 mice or six generations with CBA/J mice, producing independent lines of GNLY heterozygous offspring on pure C57BL/6 or CBA/J backgrounds.

**Intracellular GNLY staining and flow cytometric analysis**

One million cells were first blocked with unlabeled Abs against CD16 (F(ab′)2; BD Biosciences; clone 3G8) and CD32 (F(ab′)2, Caltag Laboratories) and then labeled with fluorescein-conjugated Abs specific for CD4 (clone H129.19), CD8 (clone 53-6.7), or NK (clone DX5) (all from BD Pharmingen). Intracellular staining for human NK cells was performed using A 7900HT Fast Real-Time PCR System (Applied Biosystems): denaturing at 94°C for 1 min, followed by 40 cycles of 30 s at 94°C, 30 s at 65°C, and 1 min at 72°C; final extension at 72°C for 10 min. The 3′-exon 3 (CAGTAACAGATCCAAGAGATGGAAAGAGAGTAGC) and 3′-control-RC (GGCATAAACTATAGCTGAATTATTCCATGCCCCC). Five mice expressing the transgene were backcrossed for more than eight generations with wild-type C57BL/6 mice or six generations with CBA/J mice, producing independent lines of GNLY heterozygous offspring on pure C57BL/6 or CBA/J backgrounds.

**FIGURE 1.** GNLY expression in transgenic mice. A. Schematic representation of the human GNLY gene and its position in BAC RP11-439L14. E = Exon. B, Fluorescent in situ hybridization for transgenic line A (upper panel) and transgenic line B (lower panel). There is a single transgene integration site for each line, at chromosome 14E2 in line A and at chromosome 18E for line B. C, qRT-PCR analysis of tissue distribution of GNLY mRNA in GNLY+/− mice. Each value represents the average ± SEM of three mice.
performed in triplicate. Results are representative of two or more independent experiments.

Flow-based killing assay (FloKA)

For allospecific CTL, spleens were harvested from CBA/J mice that had been primed 4 wk prior by i.p. injection with $10^7$ irradiated (10,000 rad) EL4.F15 cells. Splenocytes were cultured in Medium I in 24-well plates at $2 \times 10^6$ cells/well. Irradiated (10,000 rad) EL4.F15 ($2 \times 10^5$/well) were added on day 0 and every week thereafter. Cultures were supplemented with 50 U/ml rIL-2 beginning on day 7. Immediately before FloKA, cells were purified over Ficoll, washed twice, and resuspended in Medium I and 50 U/ml IL-2.

For NK cells, splenocytes were cultured in 24-well plates in Medium I plus 50 ng/ml IL-15 (R&D Systems) for 8 days. NK cells were isolated by negative selection using NK cell MACS isolation kits (Miltenyi Biotec). Purity was $\geq 98\%$, as determined by staining with the DX5 Ab and FACS analysis immediately before FloKA.

For FloKA, target cells (El4.F15, RMA-S, or YAC-1) were washed three times with PBS and labeled with 1 $\mu$M CFSE (Molecular Probes) for 15 min at 37°C. The reaction was stopped by addition of RPMI 1640 supplemented with 10% FCS. Cells were washed twice with PBS supplemented with 2% FCS, resuspended in Medium I plus 50 U/ml IL-2 (for CTL) or 50 ng/ml IL-15 (for NK cells). Effector cells were mixed with $10^5$ labeled target cells in 50 $\mu$l of medium into 96-well plates. A total of 1 $\mu$g/ml 7-aminoactinomycin D (Calbiochem) was added to each well immediately before FACS analysis.

Tumor challenge

C6VL and RMA-S tumor cell lines used for challenge were expanded in vitro in Medium I and injected within 1 wk of culture. Tumor cells were washed three times and diluted in PBS. Eight- to 10-wk-old mice (C57BL/6 background) were injected i.p. with 5,000 C6VL cells or 70,000 RMA-S cells in 500 $\mu$l of PBS. Survival of mice was monitored daily for at least 60 days after tumor injection. For the RMA-S tumor, mice were
FIGURE 3. GNLY expression in freshly isolated, unstimulated NK cells. A and B, Fold increase in GNLY, granzyme B, and perforin mRNA. Expression in unseparated murine splenocytes (A) or human PBMC (B) was compared with expression in purified NK and non-NK cells. C and D, Western blot of GNLY in the same GNLY/H1 (C) and human (D) cells as in A and B. Jab-1 protein was used as a loading control. Each figure is a representative example of at least two independent experiments with similar results. Each value represents the average ± SD of three or more repeated experiments. Jab-1 designates mouse protein, and JAB-1 designates human protein.

**Results**

**GNLY transgenic mice**

Transgenic mice were generated to investigate the in vivo function of GNLY. Initial attempts used cDNA encoding the 9- or 15-kDa forms of GNLY driven by either the mouse TCR promoter (a gift from M. Davis, Stanford University, Stanford, CA) or the granzyme B promoter (a gift from T. Ley, Washington University, St. Louis, MO). Although in both cases the cDNA was incorporated into the mouse DNA, neither mRNA nor protein could be detected, suggesting that tissue-specific promoter elements were lacking in these constructs or that certain intron(s) or more distant gene regions were required for the expression of this molecule. Therefore, we obtained a human BAC (RP11-439L14) that contains the complete GNLY gene and 3’ and 5’ flanking regions (Fig. 1A). Eight GNLY transgenic (GNLY+/−) mice were derived. Seven of eight mice gave germline transmission, and four of these seven lines express GNLY protein. Lines A and B express GNLY at the highest levels and appear indistinguishable in degree of transgene expression and phenotype. They are both from parental strain B6CBAF/J (strain details: F1 hybrid from C57BL/6J female × CBA/J male). Both lines were used in the experiments reported with similar results. The two selected lines were used separately in individual experiments, and results obtained were identical. GNLY+/− mice display normal development, fertility, and no abnormal phenotype when housed in a specifically pathogen-free environment. Fluorescent in situ hybridization indicates a single transgene integration site for each line, at chromosome 14E2 in mouse and 18E in line B (Fig. 1A, upper panel) and at chromosome 18E in line B (Fig. 1B, lower panel).

mRNA tissue distribution of the GNLY transgene was assessed by qRT-PCR (Fig. 1C). The highest expression was observed in spleen, with detectable expression in the thymus and lung, and minimal expression in heart, kidney, liver, muscle, intestine, and brain. These findings demonstrate that GNLY is largely expressed in immune organs. No GNLY mRNA was detected in any of these organs in nontransgenic littermates using the same qRT-PCR probe (data not shown).

**GNLY expression in activated splenocytes**

GNLY is expressed late after activation of human PBMC (Fig. 2A) (1). To characterize GNLY protein expression in GNLY+/− animals, splenocytes were activated with anti-CD3 and anti-CD28 Abs, and aliquots were removed on days 0–12 for assessment of GNLY mRNA (by qRT-PCR) and protein (by Western blot) expression (Fig. 2, B–D). On day 8, 86% of cells were CD8+, and this increased to 95% CD8+ at day 12. In GNLY+/− cultures, GNLY mRNA was expressed with nearly identical kinetics to that in activated human PBMC (Fig. 2, A and B), and expression was significantly delayed compared with that of perforin and granzyme B. Similar to activated human PBMC, two forms of GNLY, 9 and 15 kDa, are detected (Fig. 2E) (1). Granzyme B expression is detected early (by 2 days after activation), and expression increases dramatically at days 10–14. Glycosylation of granzyme B results in a number of larger species detected from days 10 to 14 after activation (37). Using intracellular staining and FACS analysis of anti-CD3/anti-CD28-activated splenocytes on day 12 of culture, 22% of the cells express GNLY and essentially all of these coexpress granzyme B (Fig. 2F).

**GNLY expression in NK cells**

Because NK cells express perforin and granzymes (38), we reasoned that the low level of GNLY expression in freshly isolated splenocytes (Fig. 1C) might be attributed to NK cells. To investigate this, NK and non-NK cells were isolated from GNLY+/− splenocytes and from human PBMC (Fig. 3, A and B). For both human and mouse cells, essentially all of the mRNA encoding GNLY, granzyme B, and perforin was found in the NK population, and the fold increase of each of these genes in NK cells relative to splenocytes or PBMC was similar for mouse and human NK cells, respectively. Lysates from these cells were analyzed by Western blot for GNLY protein (Fig. 3, C and D). Densitometry of the Western blots revealed that in human NK cells, expression of the 9- and 15-kDa forms is similar: 15 kDa/JAB-1 = 0.8; 9 kDa/JAB-1 = 0.46. In contrast, in NK cells isolated from GNLY+/− spleens, only the 15-kDa form is detectable and, by densitometry, the amount is less than in human NK cells (15 kDa/JAB-1 = 0.26). GNLY was not detected in non-NK cells from either human PBMC or spleens from GNLY+/− mice.

In contrast to the later expression of GNLY in GNLY+/− splenocytes activated by anti-CD3 and anti-CD28 Abs, IL-15 induces expression of both forms of GNLY within 4 days (Fig. 4A). After 8 days of culture with IL-15, approximately half of the cells are weighed daily and sacrificed when their body weight increased by 25%. Survival statistical analysis was performed using the LogRank method in GraphPad Prism software.

**Immunofluorescence cell staining and microscopy**

Cells were immobilized on poly(lysine)-coated slides, and exocytosis was conducted, as previously described (1).

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**FIGURE 2.** Immunofluorescence analysis of anti-CD3/anti-CD28-activated splenocytes. A, Fold increase of GNLY, granzyme B, and perforin expression (Fig. 2, A and B). B, Similar to activated human PBMC, two forms of GNLY, 9 and 15 kDa, are detected (Fig. 2E) (1). Granzyme B expression is detected early (by 2 days after activation), and expression increases dramatically at days 10–14. Glycosylation of granzyme B results in a number of larger species detected from days 10 to 14 after activation (37). Using intracellular staining and FACS analysis of anti-CD3/anti-CD28-activated splenocytes on day 12 of culture, 22% of the cells express GNLY and essentially all of these coexpress granzyme B (Fig. 2F).

**FIGURE 3.** GNLY expression in freshly isolated, unstimulated NK cells. A and B, Fold increase in GNLY, granzyme B, and perforin mRNA. Expression in unseparated murine splenocytes (A) or human PBMC (B) was compared with expression in purified NK and non-NK cells. C and D, Western blot of GNLY in the same GNLY+/− (C) and human (D) cells as in A and B. Jab-1 protein was used as a loading control. Each figure is a representative example of at least two independent experiments with similar results. Each value represents the average ± SD of three or more repeated experiments. Jab-1 designates mouse protein, and JAB-1 designates human protein.
CD8⁺, half are NK cells, and a small minority are CD4⁺ (Fig. 4B). One-third of NK cells, but only 5% of CD8⁺ cells, express GNLY (Fig. 4B). GNLY and perforin levels decreased by day 12 because some cells were undergoing apoptosis. Thus, NK cell expression of GNLY is both constitutive and inducible, but T cells express GNLY only after activation through the TCR.

**GNLY is exocytosed upon stimulation of the TCR**

We previously showed that stimulation of activated human CD8⁺ cells with anti-CD3 Ab results in granule exocytosis, releasing intracellular GNLY stores into the extracellular environment (1). Similar exocytosis of GNLY is observed after anti-CD3 mAb treatment of activated cells from GNLY⁻/⁻ mice (Fig. 4D, upper and lower left panels). In contrast, NK cells do not release GNLY after stimulation with anti-CD3 Ab (Fig. 4D, upper and lower right panels). FACS analysis of these cells revealed that only NKT cells (among NK cells) released GNLY in response to anti-CD3 (data not shown).

**Effects of GNLY expression on in vitro cytotoxicity**

To assess the role of GNLY in cytotoxicity in vitro, we adapted the FloKA described by Ley and coworkers (39). Four weeks after immunization with allogeneic cells, splenocytes from GNLY⁻/⁻ animals and from nontransgenic littermates were cultured with irradiated EL4.F15 target cells. On day 14 of culture, these cells were assayed for cytotoxicity against CFSE-labeled EL4.F15 target cells.
ANTITUMOR EFFECT OF HUMAN GNLY IN TRANSGENIC MICE

for incorporation of 7-aminoactinomycin D, which measures apoptosis/late cell death (Fig. 5). EL4.F15 cells are unable to signal through Fas, so lysis is mediated mainly by the granule exocytosis pathway (35, 39). At early time points (1 and 2 h), GNLY+/− effector cells show significantly enhanced killing of the targets over a wide range of E:T ratios. However, by 3–4 h, the difference between the cells expressing GNLY and the nontransgenic littermates controls is much less, especially at higher E:T ratios. Thus, GNLY plays a role in CTL-mediated lysis.

Similar experiments were conducted to assess the role of GNLY in NK cell-mediated cytotoxicity. Splenocytes from GNLY+/− and nontransgenic littermates were cultured for 8 days with IL-15, at which time NK cells were isolated using magnetic beads. There was no difference in cytotoxicity using YAC-1 (Fig. 6) or RMA-S cells (data not shown) as targets in the FloKA assay.

**FIGURE 6.** GNLY expression in activated NK cells does not increase apoptosis. Purified NK cells from GNLY+/− and GNLY−/− splenocytes incubated with IL-15 for 8 days lyse YAC-1 target cells at various E:T ratios (2-h incubation), as measured by FloKA. 7-AAD, 7-aminoactinomycin D; FSC, forward light scatter.

**FIGURE 7.** GNLY protects mice from C6VL tumor, but not RMA-S tumor challenge. **A,** Nontransgenic littermates (n = 12) and GNLY−/− (n = 11) mice were challenged with i.p. injection of 5,000 C6VL tumor cells (median survival: nontransgenic littermates = 27.7 days, GNLY−/− = 32 days, p = 0.03). **B,** Nontransgenic littermates (n = 9) and GNLY+/− (n = 8) mice were challenged with i.p. injection of 70,000 RMA-S tumor cells. The mean survival: nontransgenic littermates = 29.5 days, GNLY+/− = 31.2 days, p = 0.4. Each figure is a representative example of at least three independent experiments with similar results.

**GNLY and tumors in vivo**

The effects of GNLY expression on tumor rejection in vivo were evaluated in two lymphoma models. CD8+ T cells are necessary and sufficient for protection against the C6VL T cell lymphoma (40), whereas NK cells are central to rejection of the MHC-deficient RMA-S tumor (34). GNLY−/− mice showed significant protection against the C6VL tumor compared with nontransgenic littermates (p = 0.03) (Fig. 7A). Both GNLY+/− and nontransgenic littermates began to die by day 24 after injection, but the rate of death was slower in the GNLY+/− group, with 20% of the animals surviving >80 days. However, nontransgenic littermates died rapidly, with 100% mortality by 40 days. In contrast, GNLY−/− and nontransgenic littermates injected with the RMA-S tumor showed similar survival, suggesting that GNLY plays little or no role in rejection of this tumor (Fig. 6).

**Discussion**

To date, the in vivo function of GNLY has been inferred from in vitro assays (5, 9, 13, 30, 41–48) and clinical correlates (6, 17, 26, 28, 49–51). rGNLY is cytotoxic against a variety of microbes (13, 42, 52) and tumor cells (5, 52); GNLY is coexpressed with perforin and granzymes in cytotoxic granules in CTL and NK cells (13, 53); and increased GNLY expression is associated with improved prognosis in cancer patients (6, 31, 32). This study is the first direct demonstration that GNLY functions in vivo to enhance tumor-specific immunity.

GNLY homologues have been identified for pig (NK-lysin) (54), cow (Bo-lysin) (55), and horse (56), but not for rodents (10). Although gene disruption in mice has proven highly informative in defining the function of perforin (57) and granzymes (58), such experiments are not possible for GNLY. Therefore, we engineered a transgenic mouse to assess the in vivo effects of GNLY. Initial efforts used a TCR promoter and the human granzyme B promoter, both of which have been used previously to produce transgenic animals expressing proteins of interest (59, 60). However, no mRNA or protein was detected in mice using these promoters for either the 9- or 15-kDa forms of GNLY, suggesting that flanking and/or intronic sequences are required for expression.

GNLY is constitutively expressed in NK cells isolated from human PBMC or from GNLY+/− spleens. Although the relative amounts of GNLY mRNA in human and GNLY+/− NK cells are similar, there is substantially more GNLY protein in human than GNLY+/− NK cells, suggesting that GNLY protein expression is controlled in part at the level of translation. Moreover, only the 15-kDa form of GNLY is detectable in GNLY+/− mouse NK cells, whereas both the 9- and 15-kDa forms are present in nearly equal amounts in human NK cells. Nevertheless, NK cells from GNLY+/− mice are capable of expressing high levels of both forms of GNLY when activated by IL-15 (Fig. 4A). This suggests that mice maintained in a relatively clean facility are not exposed to environmental Ags that induce NK cell activation, altering the pattern of expression of GNLY.
For humans and GNLY+/− mice, GNLY is constitutively expressed by NK cells and inducible in T cells. We observed that splenocytes from GNLY+/− mice activated with anti-CD3/CD28 show enhanced lysis of targets at early times and at low E:T ratios when assayed by FloKA. Additionally, GNLY+/− mice survived longer than wild-type mice after challenge with the C6VL tumor. In contrast, splenocytes from GNLY+/− mice activated with IL-15 did not show enhanced lysis of RMA-S tumor cells, and there was no difference in survival of GNLY+/− mice challenged with this tumor in vivo. Thus, transgenic human GNLY plays a role in elimination of tumors by CD8+ T cells, but not by NK cells. This is especially interesting in light of previous in vitro data indicating that, although both human CD8+ T cells and NK cells express GNLY and kill Cryptococcus neoformans, only the CD8+ T cells, and not the NK cells, use GNLY to kill this fungus (11).

The BAC clone contains one partial gene (SnRNP assembly defective 1 homologue) and three complete genes SFTPБ, GNLY, and basic helix-loop-helix transcription factor 6 (also known as HATH6 or ATOH8). The SnRNP assembly defective 1 homologue gene is truncated at the 5’ terminus, and therefore is not expressed in the transgenic mice. Using RT-PCR, we detected human SFTPБ mRNA expression in the transgenic mice at a level similar to that of murine SFTPБ. The main function of SFTPБ is to lower the surface tension at the air-liquid interface in the alveoli of lung, mediated chiefly by the granule exocytosis pathway (35). Allostimulatory cultures from GNLY+/− mice showed significantly enhanced cytolysis at early time points and low E:T ratios compared with nontransgenic littermates. In contrast, purified NK cells from GNLY+/− and nontransgenic littermates show equivalent lysis of YAC-1 or RMA-S targets in the FloKA. These studies indicate that GNLY can play a role in allostimulatory cytolysis in vitro. The relatively minor effect of GNLY is most likely due to high expression of other cytolytic molecules such as perforin and granymes. These molecules most likely overwhelm the measurements at higher E:T ratios and longer times in culture.

The identification and characterization of GNLY as an antimicrobial and tumoricidal product of T lymphocytes and NK cells suggest a broader, and perhaps more important, role for these cell types in the ongoing war against microbes and provide an additional effector mechanism against tumors (3, 26, 43, 45, 62). Phagocytes, not lymphocytes, have generally been implicated as the important lines of defense against bacteria (21). The roles of CTL and NK cells have been more narrowly confined to tumor and antiviral immunity and certain specific bacterial and parasitic infections (46, 63). GNLY has broad-based clinical relevance as a viral immunity and certain specific bacterial and parasitic inclusions (46, 63). GNLY has broad-based clinical relevance as an antimi-

References


