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Characterization of GMP-17, a granule membrane protein that moves to the plasma membrane of natural killer cells following target cell recognition

(cytotoxic lymphocytes/TIA-1/GIG-1/NKG7)

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ABSTRACT **Cytotoxic lymphocytes are characterized by their inclusion of cytoplasmic granules that fuse with the plasma membrane following target cell recognition. We previously identified a cytotoxic granule membrane protein designated p15-TIA-1 that is immunochemically related to an RNA-recognition motif (RRM)-type RNA-binding protein designated p40-TIA-1. Although it was suggested that p15-TIA-1 might be derived from p40-TIA-1 by proteolysis, N-terminal amino acid sequencing of p15-TIA-1 immunoaffinity purified from a natural killer (NK) cell line by using monoclonal antibody (mAb) 2G9 revealed that p15-TIA-1 is identical to the deduced amino acid sequence of NKG7 and GIG-1, cDNAs isolated from NK cells and granulocyte-colony-stimulating factor-treated mononuclear cells, respectively. Epitope mapping revealed that mAb 2G9 recognizes the C terminus of p15-TIA-1 and p40-TIA-1. The deduced amino acid sequence of p15-TIA-1/NKG7/GIG-1 predicts that the protein possesses four transmembrane domains, and immuno-electron microscopy localizes the endogenous protein to the membranes of cytotoxic granules in NK cells. Given its subcellular localization, we propose to rename this protein GMP-17, for granule membrane protein of 17 kDa. Immunofluorescence microscopy of freshly isolated NK cells confirms this granular localization. Target cell-induced NK cell degranulation results in translocation of GMP-17 from granules to the plasma membrane, suggesting a possible role for GMP-17 in regulating the effector function of lymphocytes and neutrophils.**

Natural killer (NK) cells and cytotoxic T lymphocytes (CTLs) are characterized by their inclusion of cytoplasmic granules that are released in response to target cell recognition. Cytolytic lymphocyte granules are morphologically complex organelles that can either fuse with the plasma membrane, like secretory granules, or fuse with phagosomes, like primary lysosomes. Although cytotoxic granules are somewhat heterogeneous in structure, they typically contain one or more dense cores which are surrounded by multiple small internal vesicles (1, 2). The dense cores are the major reservoirs of secretory proteins such as perforin, granzymes, and chondroitin sulfate proteoglycans (1). In the multivesicular domain, which resembles endosomally derived multivesicular bodies found in many cell types, lysosomal membrane proteins such as lamp-1, lamp-2, and lgp-120 are abundantly expressed (1). At the C terminus of these proteins is a conserved motif that is required for their delivery to lysosome or granule membranes (3). We have characterized an ≈15-kDa granule membrane protein, designated p15-TIA-1, that is recognized by 2G9, a monoclonal antibody (mAb) that stains the granules of NK cells and CTLs (4, 5). Following lymphocyte activation, proteins migrating with apparent molecular masses of 28 kDa (p28-TIA-1) and 40 kDa (p40-TIA-1) are also recognized by the 2G9 mAb in immunoblots (4). Molecular cloning of p40-TIA-1 revealed it to be a member of the RNA-recognition motif (RRM) family of RNA-binding proteins (5), and *in vitro* studies suggested that p15-TIA-1 might be derived from p40-TIA-1 by proteolysis (5). However, as pulse–chase analysis did not support a precursor–product relationship between p40-TIA-1 and p15-TIA-1 (4), we purified p15-TIA-1 from the granules of a NK cell line (YT) and determined its N-terminal amino acid sequence. This sequence was found to be identical to the predicted N terminus of a cDNA-deduced structure previously isolated from NK cells and granulocyte-colony-stimulating factor (G-CSF)-treated mononuclear cells isolated from a patient with chronic myelogenous leukemia [designated NKG7 (6) and GIG-1 (7), respectively]. The C terminus of p15-TIA-1/NKG7/GIG-1 contains a lysosome targeting motif that forms part of the 2G9 epitope, revealing the basis for the immunological similarity between p15-TIA-1 and p40-TIA-1. Because p15-TIA-1/NKG7/GIG-1 is predicted to contain four transmembrane regions, is localized to the membranes of cytotoxic granules, and has a predicted molecular mass of about 17 kDa, we have redesignated this antigen GMP-17, for granule membrane protein of 17 kDa. Analysis of the subcellular localization of GMP-17 during activation of NK cells reveals that GMP-17 is relocalized from granules to the plasma membrane, suggesting that GMP-17 may contribute to the effector function of cytotoxic lymphocytes.

MATERIAL AND METHODS

GMP-17 Purification. All purification procedures were done at 4°C or on ice. About 6×10^9 YT cells (kindly provided by K. Smith, Cornell University, New York) were sonicated in 40 ml of lysis buffer (50 mM Tris·HCl, pH 7.5/150 mM NaCl/1 mM EDTA/1 mM phenylmethanesulfonyl fluoride containing aprotinin at 10 μg/ml and leupeptin at 50 μg/ml) and centrifuged at 100,000 × *g* for 30 min. The pellet was resuspended in lysis buffer containing 1% (vol/vol) Nonidet P-40 (NP-40) and centrifuged at 3000 × *g* for 10 min. The supernatant was adjusted to 0.1% SDS and centrifuged at 100,000 × *g* for 30 min. The supernatant was precleared for 1 hr with 0.3 ml of Sepharose CL-4B beads (Pharmacia) and passed over ≈0.3 ml of CNBr-activated Sepharose coupled to 2G9 mAb at 5 mg/ml of packed beads. The resin was washed six times in buffer A (1% NP-40/0.5% sodium deoxycholate/0.2% SDS/25 mM Tris·HCl, pH 7.5/0.5 M NaCl) and the bound protein was eluted at 37°C in 2× SDS/PAGE sample buffer containing 0.5

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Abbreviation: CTL, cytotoxic T lymphocyte; NK, natural killer; NP-40, Nonidet P-40; mAb, monoclonal antibody; G-CSF, granulocyte-colony-stimulating factor; HA, hemagglutinin.

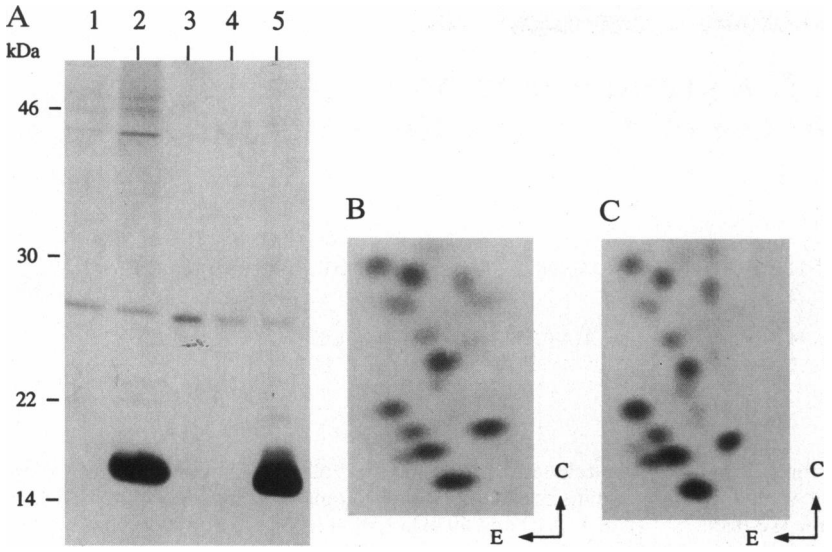

FIG. 1. Comparison of 2G9 mAb-reactive 17-kDa protein derived from YT cells and GMP-17 expressed in COS-7 cells. (*A*) YT cells (lanes 1 and 2) and COS-7 cells transfected with plasmid vector pMT.2 (lane 3) or pMT. GMP-17 (lanes 4 and 5) were metabolically labeled with [^{35}S]methionine and [^{35}S]cysteine, lysates were prepared, and then proteins were immunoprecipitated with 2G9 mAb (lanes 2, 3, and 5) or control IgG mAb (lanes 1 and 4). Immunoprecipitates were separated in an SDS/14% polyacrylamide gel. Molecular mass markers (kDa) are on the left. (*B* and *C*) Metabolically labeled 2G9 mAb-reactive protein from YT cells and recombinant GMP-17 from COS-7 cells were transferred to nitrocellulose and digested with chymotrypsin. Peptides derived from endogenous p17 (*B*) and recombinant p17 (*C*) were dried and separated by electrophoresis (arrow E) at pH 1.9 and ascending chromatography (arrow C).

M 2-mercaptoethanol. The eluted protein was separated by electrophoresis under reducing conditions in an SDS/14% polyacrylamide gel and then blotted onto a poly(vinylidene difluoride) (PVDF) membrane (Applied Biosystems). Following blotting, the membrane was rinsed with water and stained with amido black, and the membrane portion containing the 17-kDa protein was excised for amino acid sequence analysis. N-terminal sequence analysis was performed at the Worcester Microsequencing Facility (Worcester, MA) with an Applied Biosystems model 494 Procise sequencer.

Isolation of GMP-17 cDNA. A GMP-17 cDNA clone was isolated by PCR cloning using appropriate primers derived from the GIG-1 sequence (ref. 7; accession no. JC2081) and a phytohemagglutinin-activated T-cell library DNA (Clontech). GMP-17 cDNA was inserted into plasmid vectors prior to DNA sequence determination by the dideoxy chain-termination method.

Cell Transfection and Labeling. The expression vectors pMT.2 and pMT.HA containing the GMP-17 cDNA were transfected into COS-7 cells essentially as described (8). YT cells and COS-7 cells were metabolically labeled with [^{35}S]methionine and [^{35}S]cysteine, and then solubilized in lysis buffer containing 1% NP-40. Protein was immunoprecipitated with 2G9 mAb, the anti-influenza hemagglutinin (HA) mAb 12CA5, or an isotype-matched control mAb essentially as described (8). The relative amount of GMP-17 protein immunoprecipitated was determined

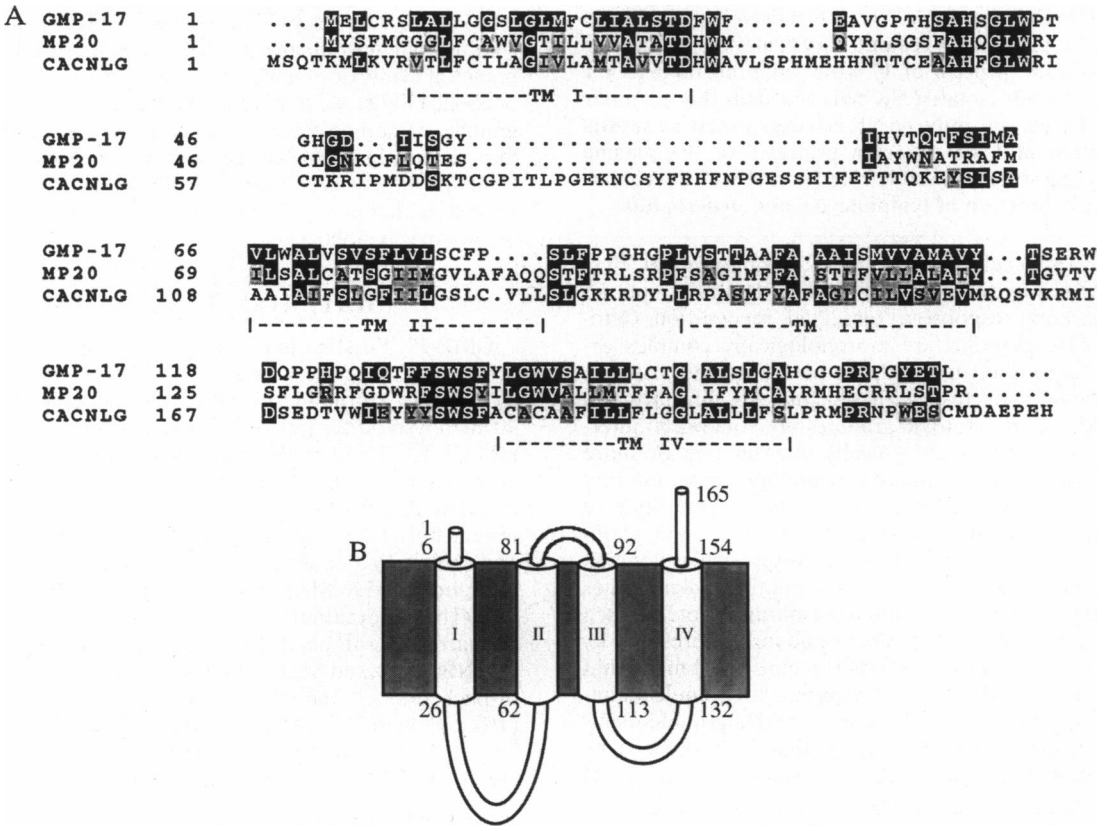

FIG. 2. (*A*) The predicted amino acid sequence of GMP-17, determined from cDNA clones isolated by PCR using primers based on the GIG-1 sequence (7), aligned with the MP20 (14) and CACNLG (13) sequences. (*B*) A schematic model of GMP-17 incorporated into a lipid bilayer.

by measuring the amount of radioactivity present in the excised region of the gel containing GMP-17 following separation of proteins by electrophoresis. For peptide maps, proteins were transferred to nitrocellulose following electrophoresis and visualized by autoradiography for 12 hr. Nitrocellulose containing the 17-kDa 2G9 antigen was excised and the protein was subjected to limit digestion at 37°C with chymotrypsin (0.1 mg/ml; Worthington) in 0.1 M ammonium bicarbonate. Liberated peptides were dried and separated in two dimensions by electrophoresis at pH 1.9 in acetic acid/formic acid/water (8:2:90, vol/vol) for 45 min at 800 V and by ascending chromatography in 1-butanol/pyridine/acetic acid/water (37.5:25:7.5:30, vol/vol).

Immuno-Electron Microscopy. Peripheral blood mononuclear cells were enriched for NK cells by negative selection using antibody-coated magnetic beads (9). Cells were prepared for immuno-cryo-electron microscopy and developed with the 2G9 mAb (IgG1) followed by goat anti-mouse IgG directly conjugated to 5-nm gold particles (4).

Immunofluorescence. Freshly isolated resting NK cells were cytospun (500 rpm for 3 min), air dried, and processed for immunofluorescence as described below. NK cells were cultured with human diploid fibroblasts (ATCC CRL 1475) precoated with anti-major histocompatibility complex antibody (W6/32, murine IgG2a) for 10–30 min prior to fixation. Samples were fixed with 2.0% freshly prepared paraformaldehyde in phosphate-buffered saline (PBS) for 15 min at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 10 min, and blocked in PBS containing 2.5% normal goat serum. Cells were then incubated with a mixture of 2G9 mAb (5 μg/ml in blocking buffer) and anti-PEN5 mAb 5H10 [2.5 μg/ml (ref. 10)] or with isotype-matched control mAbs for 1 hr at room temperature. Cells were then washed in PBS and incubated with isotype-specific secondary antibodies (Southern Biotechnology Associates) and Hoechst dye 33258 (bisbenzimide, 0.5 μg/ml; Sigma) in blocking buffer for 1 hr. Cells were washed thoroughly in PBS and mounted in a polyvinol-based mounting medium (11). Specimens were viewed and photographed on a Nikon FXA fluorescence microscope using Fujichrome 400 film.

RESULTS

Characterization of the 2G9 mAb Antigen p15-TIA-1. To characterize the structure of the cytotoxic granule-associated protein p15-TIA-1 (4), N-terminal sequence analysis was performed with protein purified by immunoaffinity chromatography using the 2G9 mAb. The resulting 25-aa N-terminal sequence was 100% identical to the deduced N-terminal amino acid sequence of NKG7 (6) and GIG-1 (7). NKG7 and GIG-1 cDNAs were isolated by differential screening of NK cell and G-CSF-stimulated mononuclear cell cDNA libraries, respectively, and encode a 165-aa protein. To confirm that recombinant NKG7/GIG-1 is recognized by the 2G9 mAb, the NKG7/GIG-1 cDNA was isolated by PCR cloning and the recombinant protein was transiently expressed in COS-7 cells. Immunoprecipitations of solubilized transfectants with the 2G9 mAb yielded a protein with similar mobility (≈17 kDa) in SDS/polyacrylamide gels as endogenous YT-derived 2G9 antigen (Fig. 1*A*). Two-dimensional chymotryptic peptide maps of recombinant NKG7/GIG.1 protein (Fig. 1*B*) and YT-derived 2G9 antigen (Fig. 1*C*) were essentially identical. Moreover, all peptides comigrated when the samples were mixed prior to mapping (data not shown). Thus, p15-TIA-1 is identical to NKG7/GIG-1. Because p15-TIA-1/NKG7/GIG-1 localizes to cytotoxic granules and because this protein is likely to be a membrane-spanning protein (see below), we have redesignated it GMP-17, for granule membrane protein of 17 kDa.

Within the 165-aa sequence of GMP-17 are four hydrophobic domains located at residues 7–25, 63–80, 93–112, and 133–153 (underlined in Fig. 2*A*) that are predicted to be membrane-spanning peptides (12). A possible model for

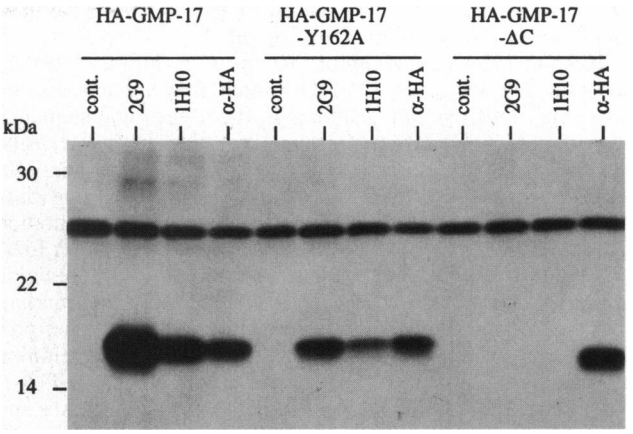

FIG. 3. Epitope mapping of 2G9 mAb. HA–GMP-17, HA–GMP-17-Y162A, and HA–GMP-17-ΔC were expressed and metabolically labeled in COS-7 cells and immunoprecipitated with either isotype-matched control IgG, 2G9 mAb, 1H10 mAb, or the anti-HA tag mAb 12CA5. Molecular mass standards (kDa) are indicated on the left.

GMP-17 is shown in Fig. 2*B*. Sequence comparison with available protein database sequences revealed that GMP-17 is most similar to several proteins which are also predicted to be four-transmembrane-region proteins. In particular, GMP-17 is 30% identical to the γ subunit of the human skeletal muscle 1,4-dihydropyridine-sensitive calcium channel (CACNLG,

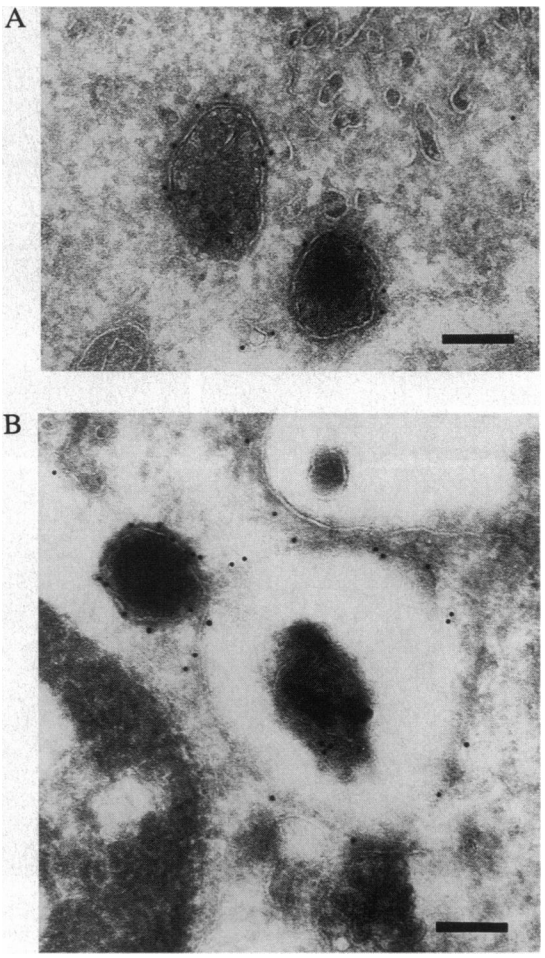

FIG. 4. Immunolocalization of GMP-17 in NK granules. Frozen sections prepared from peripheral blood NK cells were labeled with 2G9 mAb and developed with colloidal gold-conjugated rabbit anti-mouse Ig. (Bar = 0.5 μm.)

Fig. 2*A*; ref. 13) and 23% identical to the major rat lens membrane protein MP20 (Fig. 2*A*; ref. 14).

2G9 mAb Epitope Mapping. At the C terminus, GMP-17 contains the sequence GYETL which may be a lysosome targeting motif (3) and is similar to the C-terminal sequence of p40-TIA-1 (GYETQ). Because mutation of the tyrosine residue within the putative lysosome targeting motif in p40-TIA-1 (GYETQ → GAETQ) abrogated 2G9 mAb binding (data not shown), we introduced a similar tyrosine-to-alanine mutation in GMP-17 (GYETL → GAETL). GMP-17 and GMP-17-Y162A protein were expressed in COS cells as fusion proteins containing at their N termini a 16-aa HA tag sequence. Following transient transfection and metabolic labeling with [^{35}S]methionine, proteins were solubilized and subjected to immunoprecipitation analysis using 2G9, 1H10 (raised against recombinant p40-TIA-1, but crossreacts with GMP-17), and 12CA5 (anti-HA) mAbs and an isotype-matched control mAb (Fig. 3). The GMP-17 Y162A mutation decreased 2G9 and 1H10 mAb binding 10-fold and 6-fold, respectively. In contrast, anti-HA precipitated similar amounts of HA–GMP-17 and HA–GMP-17-Y162A (Fig. 3). Thus, GMP-17 tyrosine-162 is likely to be involved in binding to the 2G9 and 1H10 mAbs. To confirm that the C terminus of GMP-17 is essential for 2G9 and 1H10 binding, a HA-tagged GMP-17 deletion mutant lacking the last 8 aa (HA–GMP-17-ΔC) was subjected to a similar analysis. Whereas anti-HA mAb 12CA5 immunoprecipitated HA–GMP-17-ΔC, neither 2G9 nor 1H10 immunoprecipitated this protein (Fig. 3), demonstrating that the C-terminal 8 aa are essential for 2G9 and 1H10 binding.

Subcellular Localization of GMP-17. Immuno-electron microscopy of NK cells using 2G9 mAb localized GMP-17 to cytotoxic granule membranes in unactivated NK cells (Fig. 4*A*). While the 2G9 mAb recognizes both GMP-17 and p40-TIA-1 by immunoblotting, neither immuno-electron microscopy nor immunofluorescence analysis detected 2G9 crossreactive protein in the nucleus, where p40-TIA-1 resides, strongly suggesting that 2G9 is specific for GMP-17 in these assays (data not shown). The 2G9 epitope, which requires the C-terminal 8 aa, appears to reside on the cytoplasmic face of the outer granule membrane and within the intragranular vesicles (Fig. 4*B*). This result is consistent with the observation that these inner vesicles form by budding inward from the outer granule membrane (15). Immunofluorescence analysis of GMP-17 in resting NK cells [identified by the NK-specific plasma membrane protein PEN5 (10) labeled with anti-5H10 rhodamine] confirms this localization (Fig. 5*A*, green). PEN5 is highly localized (Fig. 5*A*, red) to the plasma membrane of the uropod of most resting NK cells. Following target cell recognition and binding, PEN5 distribution becomes depolarized throughout the plasma membrane of the adhering

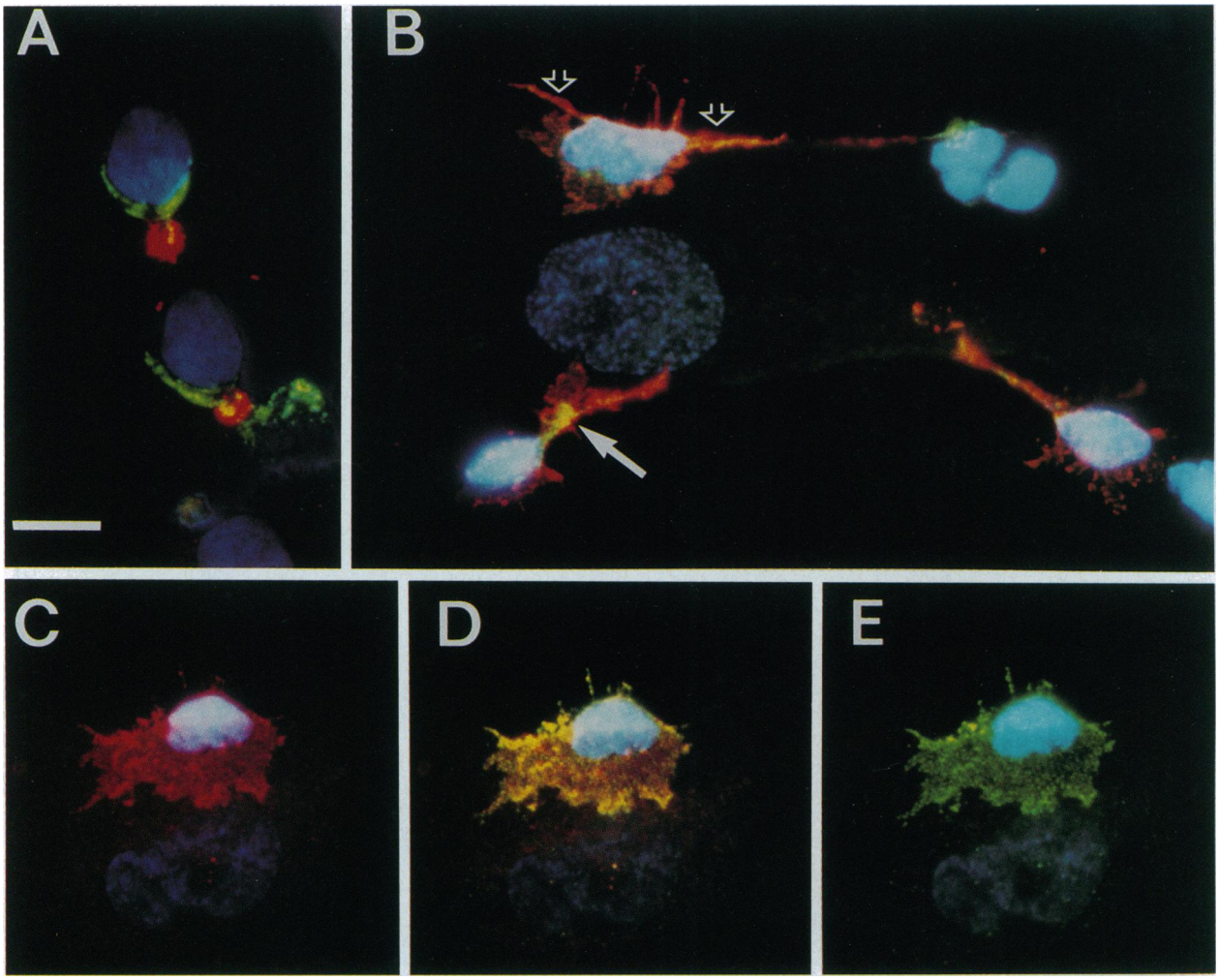

FIG. 5. Localization of GMP-17 and PEN5 in resting versus attacking NK cells. Immunofluorescent staining was performed on cytospin preparations of resting NK cells (*A*) or on attacking NK cells adhered to target fibroblasts (*B–E*). In resting NK cells (*A*), the distribution of GMP-17 (green) is exclusively granular, in marked contrast to the polarized surface distribution of the NK-specific antigen PEN5 (red). *B* displays several NK cells in various stages of degranulation attacking a single fibroblast. GMP-17 (green) is still largely granular in one NK cell (solid arrow) and distinct from the surface PEN5 (red). An adjacent NK cell (open arrows) appears uniformly orange, exhibiting coincident surface distribution of GMP-17 and PEN5. The nucleus of the target cell (large dark blue nucleus, center) exhibits DNA condensation typical of the early stages of apoptosis. In *C–E*, a fully degranulated NK cell displays plasma membrane distribution of GMP-17 in red (*C*) and PEN5 in green (*E*). A superimposed view is shown in *D*. (Bar = 10 nm.)

NK cells (Fig. 5 *B*, *D*, and *E*). Degranulation of adherent NK cells appears to progress as GMP-17-containing granules (Fig. 5*B*, solid arrow, green) move toward the target cell (large dark blue nucleus, center). In fully degranulated NK cells, both GMP-17 (Fig. 5*C*) and PEN5 (Fig. 5*E*) are dispersed throughout the plasma membrane and appear orange when superimposed (Fig. 5*D*; see also open arrows in Fig. 5*B*). Thus, GMP-17 appears to translocate to the cell surface following fusion of the granule membrane, as has been suggested to occur with CD63 during platelet and neutrophil activation and degranulation (16, 17).

DISCUSSION

GMP-17 is an integral membrane protein residing in the granules of NK cells, CTLs, and neutrophils. GMP-17 (previously known as p15-TIA-1) is recognized by the 2G9 mAb, which has proven to be a useful reagent for the identification of cytotoxic lymphocytes infiltrating into sites of tissue destruction during graft-versus-host disease (18) and renal allograft rejection (19). Our results reveal that the primary structure of GMP-17 is encoded by cDNAs previously isolated by differential screening of NK cell and G-CSF-stimulated mononuclear cell cDNA libraries [clones NKG7 (6) and GIG-1 (7), respectively]. There is a strong correlation between the expression of GMP-17 and the expression of perforin and granzyme B in NK cells and CTLs (19, 20), suggesting that GMP-17 may play a role in lymphocyte-mediated cytotoxicity. However, as GMP-17 mRNA was induced in G-CSF-stimulated mononuclear cells and GMP-17 protein was detected by immunoblotting of neutrophil extracts using the 2G9 mAb (data not shown), it is possible that GMP-17 may serve a more general function in granule targeting or signaling.

Although the deduced amino acid sequence of GMP-17 predicts the presence of four transmembrane domains, it does not appear to be a typical member of the four-transmembrane protein superfamily (4TMSF) (21). GMP-17 lacks (*i*) the highly conserved glutamic and glutamine residues found in the transmembrane domains of 4TMSF members, (*ii*) the highly conserved cysteine cluster following transmembrane domain 4, and (*iii*) the 75- to 120-aa loop joining transmembrane domains 3 and 4 (21). Comparison of the deduced amino acid sequence of GMP-17 with proteins in the GenBank database revealed similarities with the major lens membrane protein MP20 (ref. 14; 45% amino acid similarity, 23% identity), and the γ subunit of L-type voltage-gated calcium channels (ref. 13; 50% amino acid similarity, 30% identity), each of which is predicted to span the membrane four times. MP20 has been localized to lens fiber cell junctions, where it is thought to be involved in organizing junctional plaques between fiber cells (22). The γ subunit of the voltage-gated calcium channel has been shown to regulate the voltage dependence and the rate of calcium flux in reconstitution experiments when coexpressed with the β and α_1 subunits (23). By analogy, it is possible that GMP-17 contributes to the formation of junctions between effector cells and target cells following granule exocytosis or that it regulates ion channels required for cytotoxic effector function. The observation that GMP-17 is a component of the small intragranular vesicles that are released into the cleft between effector cells and target cells allows for the possibility of transfer to the target cell plasma membrane. It will be important to test whether GMP-17 can interact with perforin to regulate the lysis of target cells.

We have shown that GMP-17 moves to the plasma membrane of NK cells following target cell-induced degranulation. The delivery of granule-associated membrane proteins to the plasma membrane following target cell recognition may be important in regulating interactions between effector cells and target cells, protecting the effector cell, or promoting cytolytic effector function. CD63 is a 4TMSF member associated with the cytoplasmic granules of platelets, lymphocytes, and neutrophils which also moves to the plasma membrane following degranulation (16, 17, 24). A recent report showing that CD63 associates with $\alpha^3\beta_1$ and/or $\alpha^6\beta_1$ integrins suggests a possible role in facilitating interactions between effector cells and target cells (25). The contribution of GMP-17 to the elimination of target cells, whether in cell recognition or by involvement in effector cell signaling, remains to be determined.

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1. Burkhardt, J. K., Hester, S., Lapham, C. K. & Argon, Y. (1990) *J. Cell Biol.* **111,** 1227–1240.
2. Peters, P. J., Borst, J., Oorschot, V., Fukuda, M., Krahenbuhl, O., Tschopp, J., Slot, J. W. & Geuze, H. J. (1991) *J. Exp. Med.* **173,** 1099–1109.
3. Fukuda, M. (1991) *J. Biol. Chem.* **266,** 21327–21330.
4. Anderson, P., Nagler-Anderson, C., O'Brien, C., Levine, H., Watkins, S., Slayter, H. S., Blue, M.-L. & Schlossman, S. F. (1990) *J. Immunol.* **144,** 574–582.
5. Tian, Q., Streuli, M., Saito, H., Schlossman, S. F. & Anderson, P. (1991) *Cell* **67,** 629–639.
6. Turman, M. A., Yabe, T., McSherry, C., Bach, F. H. & Houchins, J. P. (1993) *Hum. Immunol.* **36,** 34–40.
7. Shimane, M., Tani, K., Maruyama, K., Takahashi, S., Ozawa, K. & Asano, S. (1994) *Biochem. Biophy. Res. Commun.* **199,** 26–32.
8. Serra-Pages, C., Kedersha, N. L., Fazikas, L., Medley, Q., Debant, A. & Streuli, M. (1995) *EMBO J.* **14,** 2827–2838.
9. Vivier, E., Morin, P., O'Brien, C., Druker, B., Schlossman, S. F. & Anderson, P. (1991) *J. Immunol.* **146,** 206–210.
10. Vivier, E., Sorrell, J., Ackerly, M., Robertson, M., Rasmussen, R., Levine, H. & Anderson, P. (1993) *J. Exp. Med.* **178,** 2023–2033.
11. Fukui, Y., Yumura, S. & Yumur, T. (1987) *Methods Cell Biol.* **28,** 347–356.
12. Hofmann, K. & Stoffel, W. (1993) *Biol. Chem. Hoppe-Seyler* **374,** 166.
13. Powers, P. A., Liu, S., Hogan, K. & Gregg, R. G. (1993) *J. Biol. Chem.* **268,** 9275–9279.
14. Kumar, K. M., Jarvis, L. J., Tenbroek, E. & Louis, C. F. (1993) *Exp. Eye Res.* **56,** 35–43.
15. van Deurs, B., Holm, P. K., Kayser, L., Sandvig, K. & Hansen, S. H. (1993) *Eur. J. Cell Biol.* **61,** 208–224.
16. Cham, B. P., Gerrard, J. M. & Bainton, D. F. (1994) *Am. J. Pathol.* **144,** 1369–1380.
17. Hamamoto, K., Ohga, S., Nomura, S. & Yasunaga, K. (1994) *Histochem. J.* **26,** 367–375.
18. Sale, G., Anderson, P., Browne, M. & Myerson, D. (1992) *Arch. Pathol. Lab. Med.* **116,** 622–625.
19. Lipman, M., Stevens, A. & Strom, T. (1994) *J. Immunol.* **152,** 5120–5127.
20. Cesano, A., Visonneau, S., Clark, S. C. & Santoli, D. (1993) *J. Immunol.* **151,** 2943–2957.
21. Wright, M. D. & Tomlinson, M. G. (1994) *Immunol. Today* **15,** 588–594.
22. Tenbroek, E., Arneson, M., Jarvis, L. & Louis, C. (1992) *J. Cell Sci.* **103,** 245–257.
23. Wei, X., Perez-Reyes, E., Lacerda, A. E., Schuster, G., Brown, A. M. & Birnbaumer, L. (1991) *J. Biol. Chem.* **266,** 21943–21947.
24. Metzelaar, M. J., Wijngaard, P. L. J., Peters, P. J., Sixma, J. J., Nieuwenhuis, H. K. & Clevers, H. C. (1991) *J. Biol. Chem.* **266,** 3239–3245.
25. Berditchevski, F., Bazzoni, G. & Hemler, M. E. (1995) *J. Biol. Chem.* **270,** 17784–17790.