Differential Expression of Proteinase Inhibitor-9 and Granzyme B mRNAs in Activated Immunocompetent Cells

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HORIE, O., SAIGO, K., MURAYAMA, T. and RYO, R. Differential Expression of Proteinase Inhibitor-9 and Granzyme B mRNAs in Activated Immunocompetent Cells. Tohoku J. Exp. Med., 2005, 205 (2), 103-113 —— The role of proteinase inhibitor (PI)-9 in hematopoietic cells remains unclear. To clarify the role of PI-9 in these cells, we compared the expressions of PI-9 mRNA and antigen with those of granzyme B (GrB). While the strongest expression of PI-9 mRNA was observed in a NK cell line YT-N10, it was also expressed in a B-acute lymphoblastic leukemia cell line U-Tree02, an Epstein-Barr Virus (EBV)-transformed B cell clone, a CD8+ T lymphocyte clone and a megakaryocytic cell line CMK, but not in a T cell line Jurkat. Phorbol 12-myristate 13 acetate (PMA) enhanced PI-9 mRNA expression in the CD8+ T lymphocyte clone and YT-N10 cells prior to GrB mRNA expression. IL-2 and IL-12 also had similar effects. PMA increased PI-9 mRNA expression in the EBV-transformed B cell clone and CMK cells, but IL-6 showed no effect. No changes were noted in PI-9 and GrB antigens after the addition of these agonists. Patients with graft-versus-host disease (GVHD) may have activated CTLs and NK cells. We therefore examined the expression of PI-9 and GrB mRNAs in eight patients after allogeneic hematopoietic stem cell transplantation with GVHD (n = 4) or without chronic GVHD (n = 4). Expression of GrB mRNA was significantly increased in three patients with GVHD and one patient without GVHD. Surprisingly, PI-9 mRNA expression was decreased in the eight patients. These results indicate that earlier synthesis of PI-9 may be essential for the prevention of autolysis of immunocompetent cells, and that the expression of PI-9 and GrB mRNAs may be controlled through different pathways. ———

PI-9; granzyme B; cytokine; GVHD; real-time quantitative PCR

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Granzyme B (GrB) derived from immunocompetent cells can cause the destruction of many target cells without their autolysis (Sun et al. 1996). On the other hand, some serine proteinase inhibitors (serpins) may serve to protect cytotoxic cells from self-induced apoptosis. Proteinase in-
hibitor (PI)-9, an intracellular serpin belonging to the ovalbumin family, was isolated from placenta cDNA by Sprecher et al. (1995), and was found to be present in both CTLs and NK cell lines (Sun et al. 1996). PI-9 has a reactive center loop resembling the substrate of GrB at its C-terminal, and inhibits the activity of GrB because it functions as a GrB pseudo-substrate (Sun et al. 1996, 2001). In fact, Bird et al. (1998) demonstrated that cells transfected with the PI-9 gene could inhibit apoptosis induced by GrB.

PI-9 expression has been found in immune-privileged sites such as spleen, eyes, testes and placenta (Bladergroen et al. 2001), leading to the suggestion that it may play a part in resisting GrB-induced apoptosis in order to maintain the functions of these sites. Furthermore, PI-9 has also been detected in liver cells (Kanamori et al. 2000), endothelial cells, mesothelial cells and dendritic cells (Buzza et al. 2001; Hirst et al. 2003) and may thus be assumed to help protect those cells from GrB-mediated apoptosis, too (Buzza et al. 2001). Although various functions have been proposed for PI-9, little direct evidence of such functions has been presented. In particular, the role of PI-9 in hematopoietic cells remains unclear. We therefore tried to clarify the role of PI-9 in these cells by comparing the expressions of PI-9 mRNA and antigen with those of GrB.

**MATERIALS AND METHODS**

**Cells**

B cells from a normal individual were transformed with the Epstein-Barr virus (EBV). The EBV-transformed B cell clone, a new B-acute lymphoblastic leukemia (ALL) cell line known as U-Tree02 (Saigo et al. 2003), a B cell line Raji (Nyormoi et al. 1973), a myeloid cell line HL-60 (Collins et al. 1979), a T cell line Jurkat (Gillis and Watson 1980), a NK cell line YT-N10 (Yodoi et al. 1985) and a megakaryocytic cell line CMK (Sato et al. 1989; Takada et al. 1996) were cultured in RPMI1640 medium (GIBCO-BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; HyClone Laboratories, Inc., Logan, UT, USA) and incubated at 37°C in a 5%CO₂ atmosphere. A CD8⁺ T lymphocyte clone, which was immortalized with the Herpesvirus saimili (HVS) (Biesinger et al. 1992; Meinl et al. 1995), was cultured in RPMI1640 medium (GIBCO-BRL) supplemented with 10% heat-inactivated human AB-type serum and 50 U/ml recombinant human IL-2 (Pepro Teck Ec Ltd., London, UK) at 37°C in a 5%CO₂ atmosphere. The CD8⁺ T lymphocyte clone was used as positive control for the mRNA and antigen expressions of PI-9 and GrB.

**Cell stimulation**

Fifty U/ml IL-2 combined with 2 U/ml IL-12, 20 U/ml IL-6 (all Pepro Teck Ec Ltd.) and 10 ng/ml phorbol 12-myristate 13 acetate (PMA; Sigma Chemical Co., St.Louis, MO, USA), were used as agonists. The CD8⁺ T lymphocyte clone, YT-N10 cells, the EBV-transformed B cell clone and CMK cells were suspended at concentration of 5.0 × 10⁵ cells/ml, cultured and collected 0, 3, 6, 12 and 24 hours after the addition of the agonists.

**Blood samples**

Blood samples were obtained from five healthy individuals, four patients after allogeneic hematopoietic stem cell transplantation (HSCT) with chronic graft-versus-host disease (GVHD) and four patients after HSCT without chronic GVHD. Patients with acute GVHD have various complications such as severe infections and microangiopathic tissue damage. Furthermore, high doses of immuno-suppressive agents are generally administered to patients with acute GVHD (Ryo et al. 1999). For these reasons, we examined the mRNA and antigen of PI-9 and of GrB in patients who had undergone allogeneic HSCT more than six months previously. The eight patients had undergone HSCT at the Hyogo Medical Center for Adults. The research was carried out according to the principles of the Declaration of Helsinki, and the study was approved by the review board of the Hyogo Medical Center for Adults and the Medical Ethics Review Committee of Kobe University Graduate School of Medicine. All samples were obtained with informed consent. Blood samples (5 ml) were collected with ethylenediaminetetraacetic acid (EDTA)-2Na and kept at 4°C before analysis. The nucleated cells were isolated after removal of erythrocytes with lysis buffer (155 mM NH₄Cl, 10 mM KHCO₃ and 0.1 mM EDTA-2Na). It was not possible to isolate perfectly immunopotent cells from the nucleated cells in 5 ml collected blood samples, so that total RNA from the nucleated cells was used for the real-time quantitative PCR. The numbers of T cells, B cells, CTLs and NK cells in the samples were not counted.
**Plasmid construction of PI-9**

The control plasmid DNA was constructed by using amplified PI-9 cDNA derived from peripheral blood mononuclear cells (PBMCs) of a healthy individual. The PI-9 cDNA was amplified by PCR using the primers 5'-T CCGATCCCATATGGAAACTTCTTCT-3' (forward) and 5'-GGAATTCAGATTCGAGCAGCA-3' (reverse). Amplification conditions consisted of a 2 min denaturing step at 94°C, followed by 27 cycles of incubation at 94°C for 1 min, at 58°C for 1 min, and at 72°C for 45 sec. The PI-9 cDNA, digested by BamH I and EcoR I, was cloned into the pUC19 vector (all from Takara Biomedicals Co., Shiga). The vector was then transfected into *Esherichia coli* strain DH5α using a DNA ligation kit (both Takara Biomedicals Co.). The plasmid DNA from cultured bacteria was extracted and purified with the aid of the QIAGEN plasmid Midi kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer’s directions. The original numbers of PI-9 transcript copies were determined by comparing optical density values against the molecular weight of different dilutions of the plasmid DNA.

**Real-time quantitative PCR**

Northern blot analysis has shown that the PI-9 gene can generate four different transcripts in leukemic cell lines (Sun et al. 1996). The primers and probe were designed to detect, with the aid of the Primer Express software program (Applied Biosystems, Foster City, CA, USA), the transcripts encoding a reactive center loop capable of producing a GrB complex. The selected forward (F) primer, reverse (R) primer and probe were

PI-9 F: 5’-ATTGAAGAGTTGTTGCAGCTGTA-3’,
PI-9 R: 5’-CCTGGTCCAGGCTGTGAGTTTC-3’ and
PI-9 probe: 5’FAM-CCAGGAGGACAAAGGCTAGTGC-TAMRA3’.

The probe was labeled with a fluorescent FAM (6-carboxy-fluorescein) at the 5’ end and a fluorescent TAMRA (6-carboxy-tetramethylrodamine) at the 3’ end. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control to minimize variability in the results caused by differences in the reverse transcriptase efficiency or RNA integrity among the samples. The GAPDH forward (F) primer, reverse (R) primer and probe were

GAPDH F: 5’-GAAGGTAAAGTCGGAGTTC-3’,
GAPDH R: 5’-GAAGATGGTGATGGGATTTCC-3’ and
GAPDH probe: 5’VIC-CAAGCTTCCGTCTCAGCC-TAMRA3’ (GAPDH primers and probe; all from Applied Biosystems). PI-9 gene control plasmids, serially diluted from $10^2$ to $10^5$ copies, were used as standards for drawing the standard curves in the real-time quantitative PCR. A reaction mixture without the template was used as negative control. The standard curve was drawn by plotting the logarithm of the known numbers of transcript copies of the standards versus the threshold cycle (C$_T$) values. The GAPDH standard curve was drawn in the same manner. Using these standard curves, C$_T$ values for each cDNA of interest were used to calculate the initial number of target transcript copies in the samples. The normalized value of the number of PI-9 transcript copies in each of the samples was expressed as the ratio of the number of PI-9 transcript copies to the number of GAPDH transcript copies. The primers and probe for quantification of GrB mRNA were used as described previously (Takahashi et al. 2003). Total RNA was extracted from cultured cells or nucleated cells obtained from blood samples with the aid of Isogen (Nippon Gene Co., Tokyo). Total RNA at a concentration of 0.0125 μg/μl was reverse transcribed using Random Primers and Rever Tra Ace-α(TM) (Toyobo Co., Osaka) in a total volume of 20 μl. A portion (2.5 μl) of the cDNA was used for real-time quantitative PCR, which was carried out with an ABI/PRISM 7700 Sequence Detector System (Applied Biosystems) with slight modifications as described previously (Das et al. 2000; Takahashi et al. 2001). For each 27.5 μl TaqMan reaction, 2.5 μl cDNA was mixed with 11.7 μl distilled water, 0.15 μl designed sense primer (100 μM), 0.15 μl designed anti-sense primer (100 μM), 0.5 μl TaqMan probe (5.0 μM) and 12.5 μl 2 x TaqMan universal PCR Master Mix (Applied Biosystems), which was composed of a PCR buffer, MgCl$_2$, deoxyadenosine triphosphate (dATP), deoxyctydine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), deoxyuridine triphosphate (dUTP), AmpErase UNG and Ampli Taq Gold DNA polymerase. The conditions used for all real-time quantitative PCRs were 95°C for 10 min, 50 cycles at 95°C for 30 sec, at 60°C for 30 sec and at 72°C for 30 sec, and again at 72°C for 5 min.

**Detection of PI-9 and GrB antigens**

Flow cytometric analysis of expressions of PI-9 and GrB antigens in cultured samples or nucleated cells obtained from blood samples was performed with a BD Biosciences FACSCalibur flow cytometer and CellQuest software (BD Biosciences, San Jose, CA, USA) (Kook et
The PI-9 antibody was produced from a rabbit immunized with recombinant histidine-tagged PI-9, which was purified from transformed yeast lysates by Ni-NTA resin column (QIAGEN Inc.) chromatography as described elsewhere (Sprecher et al. 1995; Heim et al. 1998). The IgG fraction was purified by Protein G-Sepharose column chromatography (Amersham Biosciences Corp., Piscataway, NJ, USA). The specificity of the polyclonal antibody against PI-9 was confirmed by Western blotting. Fluorescence-labeled anti-rabbit goat immunoglobulin (BD Biosciences) was used as a second antibody. GrB-7 (Sanbio B.V., Uden, The Netherlands) was used as the first antibody and FITC-conjugated anti-mouse IgG2a (CALTAG Laboratories, Burlingame, CA, USA) as the second antibody for the detection of GrB antigen. Approximately $1.0 \times 10^6$ cultured cells or $3.0 \times 10^6$ nucleated cells were fixed in 4% paraformaldehyde in PBS for 10 min. The samples were centrifuged, resuspended in PBS containing 0.3% saponin for 10 min, centrifuged and resuspended in PBS containing 20% FBS for 20 min. The cells were centrifuged and incubated with anti-PI-9 polyclonal antibody or normal rabbit IgG for 25 min, washed, and then incubated with the second antibody. After two more washes the cells were analyzed. The samples for GrB were incubated with GrB-7 or isotype control Ab (IgG2a; CALTAG Laboratories) for 25 min. All procedures were performed at room temperature. The protocol of animal experiment was approved by the Committee on Animal Experimentation of Kobe University School of Medicine.

**Statistical analysis**

The Student $t$-test was used for all statistical analyses. $P$ values less than 0.05 were considered significant. The data showed a high degree of reproducibility among the samples.

**RESULTS**

**Levels of PI-9 mRNA expression in various hematopoietic cells**

As shown in Fig. 1, the strongest expression of PI-9 mRNA was observed in the NK cell line YT-N10. YT-N10 cells expressed at a ratio of approximately 0.197 of PI-9 transcript copies to GAPDH transcript copies, whereas the CD8$^+$ T lymphocyte clone expressed at a ratio of approximately 0.111. The expression of PI-9 transcript

![Fig. 1. PI-9 mRNA levels in various hematopoietic cells.](image)

A real-time quantitative PCR assay was used to quantify the values of PI-9 mRNA. The values were expressed as the ratio as described in Materials and Methods. Total RNA was extracted from the cultured cells. The y-axis indicates the ratio of the number of PI-9 transcript copies to the number of GAPDH transcript copies. The numbers represent average values of duplicates in two separate experiments.
copies by U-Tree02 cells was 0.031 times higher than that of GAPDH, while the expression of PI-9 transcript copies by the \textit{EBV}-transformed B cell clone was 0.012 times higher. Raji cells expressed at a ratio of approximately 0.009 of PI-9 transcript copies, while the PI-9 mRNA level in HL-60 cells was lower (ratio: 0.007). PI-9 mRNA expression was also detected in CMK cells (ratio: 0.001), but interestingly, no such expression was detected in Jurkat cells.

\textbf{Effects of PMA and cytokines on PI-9 and granzyme B mRNAs in hematopoietic cells}

PI-9 and GrB mRNA expressions in YT-N10 cells and in the CD8\(^+\) T lymphocyte clone after the addition of PMA are shown in Fig. 2A and Fig. 2B, respectively. The strongest expression of PI-9 mRNA in YT-N10 cells was observed 3 hours after the addition of PMA \((p < 0.05)\), whereas GrB mRNA peaked 12 hours after PMA addition \((p < 0.05; \text{Fig. 2A})\). A similar result was obtained with the CD8\(^+\) T lymphocyte clone, as

![Diagram](image)

\textbf{Fig. 2.} Effect of PMA on PI-9 and granzyme B mRNAs in YT-N10 cells and the CD8\(^+\) T lymphocyte clone. Values of PI-9 and GrB mRNA expressions in YT-N10 cells and the CD8\(^+\) T lymphocyte clone after the addition of PMA were quantified. The values as the ratio are presented as the means ± s.d. of four different cultures. Values of PI-9 and GrB mRNA expressions over time in untreated cells are shown as (△) and (▲), respectively.

\* significantly greater than control culture \((p < 0.05)\).

A: YT-N10 cells were stimulated with 10 ng/ml PMA for 0, 3, 6, 12 and 24 hours. After the stimulation, total RNA was extracted from YT-N10 cells. The left y-axis shows the ratio of the number of PI-9 transcript copies to the number of GAPDH transcript copies, and the right y-axis that of granzyme B transcript copies.

B: The CD8\(^+\) T lymphocyte clone was stimulated with 10 ng/ml PMA for 0, 3, 6, 12 and 24 hours.
Fig. 3. Effect of IL-2 combined with IL-12 on PI-9 and granzyme B mRNAs in the CD8\(^+\)T lymphocyte clone.

Values of PI-9 (○) and GrB (●) mRNA expressions in the CD8\(^+\)T lymphocyte clone were quantified, and the clone was stimulated with 50 U/ml IL-2 combined with 2 U/ml IL-12 for 0, 3, 6, 12 and 24 hours. Hardly any changes in PI-9 or GrB mRNA expressions in untreated cells were noted over time. After the stimulation, total RNA was extracted from the clone, and the values were expressed as the ratio. The left y-axis shows the ratio of the number of PI-9 transcript copies to the number of GAPDH transcript copies, and the right y-axis that of granzyme B transcript copies. Data are presented as the means ± s.d. of four different cultures.

* significantly greater than control culture (\(p < 0.05\)).

Fig. 4. Effect of PMA and IL-6 on PI-9 mRNA in the EBV-transformed B cell clone.

Values of PI-9 mRNA expression in the EBV-transformed B cell clone were quantified after the addition of PMA (○) or IL-6 (●). The B cell clone was stimulated with 10 ng/ml PMA or with 20 U/ml IL-6 for 0, 1, 3, 6, 12 and 24 hours. Hardly any changes in PI-9 mRNA in untreated cells were noted over time. Total RNA was extracted from the clone, and the values were expressed as the ratio. The y-axis shows the ratio of the number of PI-9 transcript copies to the number of GAPDH transcript copies. Data are presented as the means ± s.d. of four different cultures.

* significantly greater than control culture (\(p < 0.05\)).
seen in Fig. 2B. The combination of IL-2 and IL-12 augmented PI-9 mRNA expression in the CD8\(^+\) T lymphocyte clone prior to that of GrB mRNA (Fig. 3), and the result was similar to that obtained with YT-N10 cells (data not shown). The levels of PI-9 mRNA expression in the EBV-transformed B cell clone reached their peak 12 hours after the addition of PMA (\(p < 0.05\); Fig. 4). IL-6 had no effect on PI-9 mRNA expression as shown in Fig. 4, while slight elevation of PI-9 mRNA was observed in CMK cells 12 hours after the addition of PMA (data not shown).

**Effects of PMA and cytokines on PI-9 and granzyme B antigens in immunocompetent cells**

PI-9 antigen expression in the CD8\(^+\) T lymphocyte clone 6, 12 and 24 hours after the addition of 10 ng/ml PMA or 50 U/ml IL-2 combined with 2 U/ml IL-12 did not change, nor did that of the GrB antigen in the CD8\(^+\) T lymphocyte clone 6, 12 and 24 hours after the addition of these agonists (Fig. 5). A similar result was obtained with YT-N10 cells (data not shown). Western blot analysis also showed no changes of PI-9 antigen in these samples (data not shown).

**Levels of PI-9 and GrB mRNA expressions after allogeneic HSCT**

The expressions of PI-9 and GrB mRNA in the patients with and without GVHD were observed. As shown in Fig. 6, the mean\(\pm\)s.d. of the ratio of GrB transcript copies of healthy individuals (control) was 0.029 \(\pm\) 0.049 and that of the ratio of PI-9 transcript copies of healthy individuals (control) was 0.068 \(\pm\) 0.048. Chronic GVHD was not diagnosed in four of the eight patients who had undergone allogeneic HSCT. One of these patients (Case 3) showed a significantly increased GrB transcript copy values with a ratio of 0.15, while the other three showed normal GrB transcript copy values. All four patients without...
chronic GVHD showed lower levels of PI-9 mRNA, and the ratio in Case 3 was significantly lower than the normal ratio of 0.011. Chronic GVHD was diagnosed in four of the eight patients, and in three of them (Cases 6, 7 and 8) GrB transcript copy values were significantly higher with respective ratios of 0.090, 0.087 and 0.184. Case 5, on the other hand, showed normal values. All four patients with chronic GVHD showed lower levels of PI-9 mRNA. Especially, the ratios of PI-9 transcript copies of cases 5 and 8 were at 0.005 and 0.016, respectively. These values were significantly lower than those of normal individuals.

**DISCUSSION**

PI-9 mRNA is generally determined by means of Northern blot analysis, which has demonstrated the presence of PI-9 mRNA in NK cells, CTLs, B cells, as well as little or no expression of PI-9 mRNA in the megakaryocytic cell line MEG-01, the myeloid cell line HL-60 and the T cell line Jurkat (Sun et al. 1996). However, Northern blot analysis provides no information about the precise quantities of PI-9 mRNA. In contrast, our real-time quantitative PCR assay identified such quantities of PI-9 mRNA in a CD8\(^+\) T lymphocyte clone, the NK cell line YT-N10, the myeloid cell line HL-60, a B cell clone transformed with the Epstein-Barr virus (EBV), the new B-ALL cell line U-Tree02, the B cell line Raji and the megakaryocytic cell line CMK. The strongest expression of PI-9 mRNA was observed in YT-N10 cells, while no PI-9 mRNA expression was noted in Jurkat cells. These findings show that real-time quantitative PCR is suitable for detailed identification of the regulatory mechanism of PI-9 mRNA.

First, changes in PI-9 mRNA expression after activation of the CD8\(^+\) T lymphocyte clone and YT-N10 cells were investigated. PMA is believed to be a chemical agent which is capable of activating various hematopoietic cells via intracellular c-kinase (Nishizuka 1984). We investigated the effect of PMA on mRNA expressions of PI-9 and GrB and were able to demonstrate for...
the first time that PMA enhances PI-9 mRNA expression in the CD8+ T lymphocyte clone and YT-N10 cells prior to GrB mRNA expression. Similar results were obtained for PI-9 mRNA expression after combined IL-2- and IL-12-induced activation. These results suggest that earlier synthesis of PI-9 seems to be essential for the prevention of autolysis of CTLs and NK cells.

The in vitro flow cytometric assay using the PI-9 polyclonal antibody and the GrB monoclonal antibody GrB-7 showed hardly any changes in PI-9 and GrB antigens, although more sensitive methods may be able to detect minor changes. A recent study using Western blot analysis showed that the amount of PI-9 antigen in a CTL clone increased 6 days after the addition of a combination of concanavalin A and PMA (Hirst et al. 2003). Longer incubation of a CTL clone and a NK cell line with various kinds of agonists may therefore result in a further increase in the quantities of PI-9 and GrB antigens. However, since the in vivo activation of CTLs and NK cells is believed to occur soon after stimulation, we postulate that the longer incubation of a CTL clone and a NK cell line with agonists does not reflect the in vivo physiological phenomena. Further, PMA was also found to enhance PI-9 mRNA expression in the EBV-transformed B cell clone and CMK cells, which indicates that c-kinase in hematopoietic cells may play a crucial role in the regulation of PI-9 mRNA.

Buzza et al. (2001) reported that PI-9 mRNA expression in endothelial and methothelial cells was upregulated by PMA. They postulated that inflammatory mediators such as TNF-α, IL-1β and IFN-γ upregulated PI-9 mRNA. IL-2 and IL-12 are known to induce perforin and GrB gene expression in CTLs and NK cells (Damle and Doyle 1987; Gately et al. 1992; DeBlaker-Hohe et al. 1995) and to function as physiological activators for these cells in vivo. Examination of the effect of IL-2 combined with IL-12 showed that these cytokines had a similar differential effect on the expression of PI-9 and GrB mRNAs in the CD8+ T lymphocyte clone and YT-N10 cells.

To the best of our knowledge, no reports have dealt with the precise role of c-kinase in B cells. If this role can be clarified, the significance of the regulation of PI-9 mRNA via c-kinase activation in B cells can be determined. IL-6 is known to enhance alloantigen expression by B cells (McKenzie 1988) and increase IgM and IgG production by B cells (Bertolini and Benson 1990). On the other hand, our study showed that IL-6 had no effect on PI-9 mRNA in the EBV-transformed B cell clone. These findings show that IL-6 induced activation of B cells may not be related to the regulation of PI-9 mRNA. PMA has been found to induce mature megakaryocytic cells which produce platelets from CMK cells (Nagano et al. 1992). Normal platelets are believed not to possess any PI-9 antigen, so that the increase of PI-9 mRNA in CMK cells observed in response to PMA may not reflect a physiological event.

Lastly, on the assumption that patients with GVHD after allogeneic HSCT may have activated CTLs and NK cells, we observed the changes in the PI-9 mRNA of patients who had undergone allogeneic HSCT. Jaksch et al. (2003) reported they had detected increased levels of GrB mRNA in patients with acute GVHD, although this increase did not correlate with clinical severity. In our study, an increase in GrB mRNA was observed in only one of the four patients without GVHD after allogeneic HSCT. On the other hand, such an increase was observed in three of the four patients with GVHD. The clinical features of all these cases were previously reported in detail (Takahashi et al. 2003). Our assumption that an increase in GrB mRNA in patients after allogeneic HSCT may reflect the intensity of CTL activation leads to the hypothesis that PI-9 mRNA may be enhanced in patients who have undergone allogeneic HSCT. Surprisingly, PI-9 mRNA was found to have decreased in all eight patients after allogeneic HSCT, which seems to indicate that a reduction in PI-9 contributes to an increase in tissue damage induced by GrB after allogeneic HSCT. These results suggest that the expressions of PI-9 and GrB mRNAs may be controlled through different regulatory pathways.

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References


