

Participation of CD45, NKR-P1A and ANK61 antigen in rat hepatic NK cell (pit cell) mediated target cell cytotoxicity

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Abstract

AIM Several triggering receptors have been described to be involved in natural killer (NK) cell-mediated target cytotoxicity. In these studies, NK cells derived from blood or spleen were used. Pit cells are liver-specific NK cells that possess a higher level of natural cytotoxicity and a different morphology when compared to blood NK cells. The aim of this study was to characterize the role of the NK-triggering molecules NKR-P1A, ANK61 antigen, and CD45 in pit cell-mediated killing of target cells.

METHODS ⁵¹Cr-release and DNA fragmentation were used to quantify target cell lysis and apoptosis, respectively.

RESULTS Flow cytometric analysis showed that pit cells expressed CD45, NKR-P1A, and ANK61 antigen. Treatment of pit cells with monoclonal antibody (mAb) to CD45 (ANK74) not only inhibited CC531s or YAC-1 target lysis but also apoptosis induced by pit cells. The mAbs to NKR-P1A (3.2.3) and ANK61 antigen (ANK61) had no

effect on pit cell-mediated CC531s or YAC-1 target cytolysis or apoptosis, while they did increase the Fcγ receptor positive (FcγR⁺) P815 cytolysis and apoptosis. This enhanced cytotoxicity could be inhibited by 3,4-dichloroisocoumarin, an inhibitor of granzymes.

CONCLUSION These results indicate that CD45 participates in pit cell-mediated CC531s and YAC-1 target cytolysis and apoptosis. NKR-P1A and ANK61 antigen on pit cells function as activation structures against FcγR⁺ P815 cells, which was mediated by the perforin/granzyme pathway.

INTRODUCTION

Natural killer (NK) cells can kill certain tumor cells or virus-infected cells without prior sensitization and thus play an important role in host anti-tumor defense^[1]. Cytotoxic activity of NK cells is achieved by two distinct mechanisms, i.e. necrosis (cytolysis) and apoptosis, and is believed to be regulated by triggering and inhibiting receptors on NK cells^[1-3]. Several candidate receptors involved in triggering a cytotoxic reaction, such as NKR-P1A (CD161A) and an NK-activation structure recognized by monoclonal antibody (mAb) ANK61 (ANK61 antigen), have been described on rat NK cells^[2,4,5].

NKR-P1A is a lectin-like surface molecule expressed on all rat NK cells^[4]. The ligands for NKR-P1A are oligosaccharides. Interactions of these oligosaccharides on target cells with NKR-P1A on NK cells are crucial for target cell recognition and for NK cytotoxicity^[6]. Monoclonal antibody to NKR-P1A (3.2.3) has been shown to stimulate NK cell phosphoinositide turnover, calcium mobilization, degranulation and NK cytotoxicity against certain Fcγ receptor positive (FcγR⁺) tumor target cells^[4,7]. These findings demonstrate that NKR-P1A is an activating molecule on rat NK cells that recognizes target cells and induces cytotoxicity.

Recently, a rat NK-activation structure (ANK61 antigen) recognized by mAb ANK61 has been described^[5]. Cross-linking of the ANK61 antigen and FcγR⁺ tumor targets with mAb ANK61 resulted in enhanced killing of the targets by NK cells^[5].

CD45, which is expressed by most leukocytes, including NK cells, has been shown to be involved in NK

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cell-mediated target cell cytotoxicity^[8-10]. It is reported that mAbs against CD45 inhibit NK cell-mediated target cell killing^[8,9] and CD45-negative NK cells fail to lyse tumor targets^[10]. The cytoplasmic domain of CD45 has tyrosine phosphatase activity, which is believed to play an important role in the regulation of NK activity^[8,11].

Pit cells are liver-specific NK cells^[12]. Compared to blood NK cells, rat pit cells possess a higher level of cytolytic activity against a variety of tumor target cells, including NK resistant P815 cells, which is comparable to the cytotoxicity level of lymphokine-activated killer (LAK) cells^[13]. These data indicate that pit cells have different characteristics from blood NK cells. Although much is known about the NK receptors that participate in NK cell activation and target cell killing, the surface receptors involved in pit cell-mediated target cell killing have not yet been clarified. Moreover, involvement of the above mentioned antigens in NK cell-mediated target cell apoptosis has remained elusive, since ⁵¹Cr-release assay employed in the previous studies^[4,5,10] indicates only cytolysis, rather than apoptosis. In the present work, we used mAbs against CD45, NKR-P1A, and ANK61 antigen to evaluate their roles in pit cell-mediated target cell lysis and apoptosis.

MATERIALS AND METHODS

Isolation and purification of pit cells

Male Wistar rats (Proefdierencentrum, Leuven, Belgium) weighing (250–280) g were used at an age between 8 to 12 wk. Hepatic NK cells (pit cells) were isolated according to protocols described before^[14]. The purity of the recovered pit cells was at least 90%, as evaluated by morphology on May-Giemsa-stained cytospin preparations and by flow cytometric analysis using mAb 3.2.3. The viability of the recovered cells was more than 95%, as determined by Trypan blue exclusion. The procedures used in this study were approved by the local ethical committee (license No LA1230212).

Tumor cell line

CC531s, a dimethylhydrazine-induced colon carcinoma of Wag/Rij (inbred strain of Wistar) rats^[15], P815, a murine mastocytoma, and YAC-1, a mouse T cell lymphoma, were maintained in culture medium RPMI-1640 (Gibco, Life Technologies, Gent, Belgium), supplemented with 10% fetal calf serum (Eurobiochem, Bierges, Belgium), benzylpenicillin (100 kU/L), streptomycin (100 mg/L), and glutamine (0.2 mmol/L) (Gibco, Life Technologies, Gent, Belgium).

Reagents and antibodies

The following mouse anti-rat mAbs were used: 3.2.3 (anti-NKR-P1A, IgG_{2b} or IgG₁)^[4], ANK74 (anti-CD45, IgG₁)^[8], and ANK61 (anti-rat NK activation structure, IgG₁)^[5] were developed in Department of Surgery and Pathology, Leiden University Medical Center, the

Netherlands. 3,4-dichloroisocoumarin (DCI), a granzyme inhibitor^[16] was purchased from Sigma (Bornem, Belgium).

Flow cytometry

The expression of surface antigens on the cells was measured by one or two-color flow cytometric analyses as described previously^[17]. Briefly, a quantity of 0.5×10^6 cells per sample was incubated (30 min, 4 °C) with the primary antibodies, 3.2.3, ANK74, and ANK61. Cells were then washed thrice with cold phosphate-buffered saline (PBS) containing 1% bovine serum albumin and 0.02% sodium azide. Subsequently, cells were incubated with fluorescein-conjugated anti-mouse IgG₁ and biotin-conjugated anti-mouse IgG_{2b} (Gilbertsville, PA). After incubation and washing, cells were incubated with streptavidin-phycoerythrin (Gilbertsville, PA). Then, cells were washed and fixed with 2% paraformaldehyde in PBS and analyzed (FACstar, Becton Dickinson, Mountain View, CA). Isotype-matched irrelevant antibodies were used as a control.

⁵¹Cr-release assay

Cytolysis was measured after 4 h incubation of P815 and YAC-1 cells, and 18 h for CC531s cells. ⁵¹Cr-release assay was performed using round-bottomed 96-well microplates as described previously^[18]. Briefly, 1×10^6 target cells/0.5 mL were labeled with 9.25 MBq Na ⁵¹CrO₄ (Amersham Belgium, Gent, Belgium) for 80 min at 37 °C. Before the incubation, freshly isolated pit cells were preincubated with either saturating amounts of the mAbs (final concentration for each mAb was 10 mg/L), 3.2.3, ANK61, ANK74, or irrelevant mouse IgG₁ for 15 minutes at room temperature. The mAbs were also present during the assay and the treatments with these mAbs did not affect the viability of the cells. The target cells were seeded at a concentration of 1×10^4 cells/well. Suspensions of effector cells were then added to the wells at effector to target (E:T) ratios of 10:1 in a final volume of 200 μL. All assays were done in triplicate. For the experiments using DCI, pit cells were preincubated with 50 μmol/L DCI for 30 min at 37 °C. After washing twice with medium, the cells were incubated with the target cells as mentioned above. After a incubation at 37 °C for 4 or 18 h, 100 μL of supernatant per well was aspirated and counted in a gamma counter to determine experimental release. Results were expressed as percentage of specific lysis according to the formula:

$$\% \text{ specific cytolysis} = \frac{\text{Experimental release} - \text{Spontaneous release}}{\text{Maximal release} - \text{Spontaneous release}} \times 100\%$$

Experimental release represents cpm (counts per minute) release from target cells in the presence of effector cell and mAbs. Spontaneous release was obtained from wells containing labeled target cells and medium only. Maximal release of ⁵¹Cr-labeled target cells was determined

after adding 20 μ L 10% sodium dodecyl sulfate (SDS) detergent to the labeled target cells.

Quantitative DNA fragmentation assay

DNA fragmentation in the target cells was determined as described previously^[19]. Briefly, to label the DNA of the target cells, 5×10^5 cells in 250 μ L medium were incubated with 0.37 MBq [methyl-³H] thymidine ([³H]-TdR) (Amersham, Buckinghamshire, U.K) for 3 h at 37°C. Before the coincubation, freshly isolated pit cells were preincubated with the mAbs as mentioned above. The pit cells (10^5 cells in 100 μ L) and the target cells (10^4 cells in 100 μ L) (E:T = 10:1) were placed in triplicate in 1.5 mL microcentrifuge tubes. The total volume per microcentrifuge tube was 200 μ L. For the experiments using DCI, pit cells were preincubated with 50 μ mol/L DCI for 30 min at 37°C. After washing twice with medium, the cells were coincubated with the target cells as mentioned above. After 3 h coincubation at 37°C and centrifugation at $300 \times g$ for 10 min, the incubation medium was removed from the tubes. Subsequently, the pelleted cells were lysed with 0.5 mL of cold lysis buffer (4°C) (5 mmol/L Tris, 2 mmol/L EDTA, 1% Triton X-100, pH 7.4) for 30 min at 4°C. Then, the lysates were ultracentrifuged ($10,000 \times g$ for 15 min at 4°C) to separate fragmented DNA from intact DNA. Radioactivity (cpm) in the incubation medium, in the $10,000 \times g$ supernatant and in the $10,000 \times g$ pellet was determined in a beta counter (Beckman, Fullerton, CA, USA). The percentage fragmented DNA was calculated using the following formula:

$$\% \text{ specific DNA fragmentation} = \frac{\text{cpm}_{\text{fr,exp}} - \text{cpm}_{\text{fr,spont}}}{\text{cpm}_{\text{total}} - \text{cpm}_{\text{fr,spont}}} \times 100\%$$

in which: cpm_{fr} = the radioactivity in the incubation medium plus the cpm in the $10,000 \times g$ supernatant; $\text{cpm}_{\text{total}}$ = cpm_{fr} + radioactivity in the $10,000 \times g$ pellet; exp = experimental (CC531s cells with pit cells); spont =

spontaneous (CC531s cells and medium only).

Hoechst 33342 (HO 342)/propidium iodide (PI) staining

The method of HO 342 and PI staining has been described previously^[19]. In short, target cells, at a concentration of 1×10^4 cells per well in a 96m multiwell plate, were coincubated with pit cells (E:T = 10:1). After 3 h coincubation, the cells were stained with HO 342, which stains DNA blue, and PI, which only penetrates necrotic cells through the damaged cell membrane and stains DNA red. Cells were viewed under a Leica DM IRB/E inverted fluorescence microscope (Leica, Heidelberg, Germany) with ultraviolet excitation at 340 to 380 nm. The apoptotic target cells were determined by their characteristic apoptotic morphological changes of the nucleus, i.e. condensation of chromatin and nuclear fragmentation. Target cells alone acted as spontaneous apoptosis control.

Statistical analysis

Results were given as the mean with the corresponding standard deviation. Statistical analysis was performed by Student's t test or one-way ANOVA with post-hoc multiple comparison analysis made by Duncan test, using SPSS statistical package (SPSS Inc., Chicago, IL, USA). Statistical significance between two groups was considered at the level of $P < 0.05$.

RESULTS

Expression of surface antigens on pit cells

Flow cytometric analysis was used to determine the expression of surface antigens on pit cells and target cells. In two-color flow cytometric analysis, mAb 3.2.3 was used to stain pit cells^[20]. Pit cells were shown to express CD45, which was recognized by mAb ANK74, and an NK activation structure, which was recognized by mAb ANK61 (Figure 1). CC531s, P815, and YAC-1 cells did not express these NK-associated antigens (data not shown).

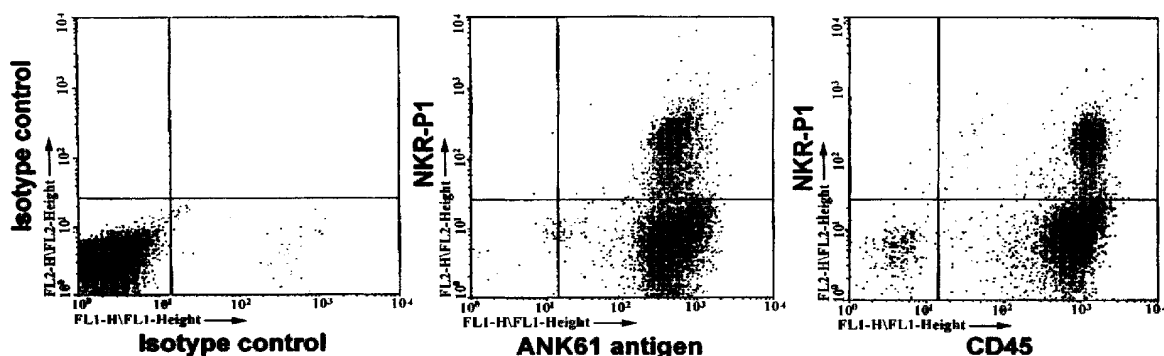


Figure 1 Expression of CD45 and ANK61 antigen on hepatic NK cells (pit cells). The cells were washed out of the liver by sinusoidal lavage. After Ficoll-Paque gradient centrifugation and nylon-wool adherence to remove erythrocytes, granulocytes, monocytes and B lymphocytes, the cells were stained with anti-NKR-P1A mAb (3.2.3) (phycoerythrin) and anti-ANK61 antigen (ANK61), anti-CD45 mAb (ANK 74) (fluorescein) and analyzed by two-color flow cytometry. CD45 and ANK61 antigen were expressed on the x-axis (fluorescein), NKR-P1A on the y-axis (phycoerythrin).

Effect of CD45, NKR-P1A, and ANK61 antigen on pit cell-mediated target cell lysis

To investigate the function of these surface antigens in pit cell-mediated cytolysis of targets, the mAbs against these antigens were used in ^{51}Cr -release assays. In the presence of anti-CD45 mAb ANK74, the lysis of CC531s and YAC-1 cells by pit cells was significantly inhibited ($P < 0.01$, $P < 0.05$) (Figure 2A, 2B). The presence of mAbs 3.2.3 and ANK61 had no effect on pit cell-mediated lysis against-CC531s and YAC-1 targets (Figure 2A, 2B). These data indicate that CD45 is involved in pit cell-mediated cytolysis against CC531s and YAC-1 targets.

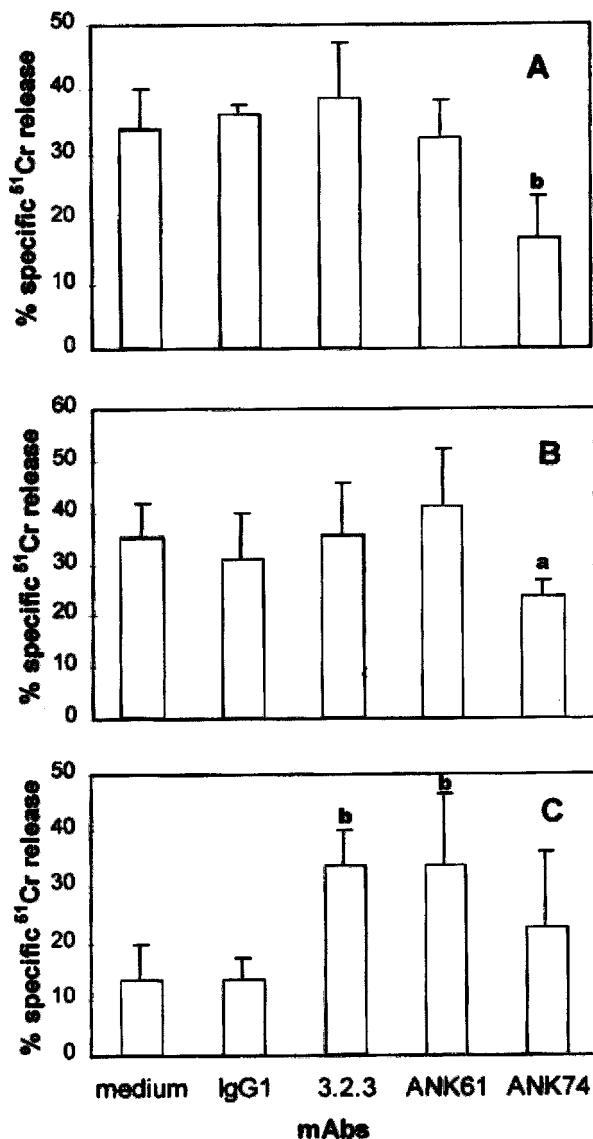


Figure 2 Effect of the mAbs on pit cell-mediated CC531s (A), YAC-1 (B) and P815 (C) target cell cytolysis. ^{51}Cr -labeled target cells were incubated at an E:T ratio of 10:1 with freshly isolated pit cells in the absence or presence of the mAbs. (A), Cytolysis of CC531s cells was measured in an 18h ^{51}Cr -release assay. (B), Cytolysis of YAC-1 cells was measured in a 4h ^{51}Cr -release assay. (C), Cytolysis of P815 cells was measured in a 4h ^{51}Cr -release assay. Values are mean \pm SD from four different experiments. ^a $P < 0.05$, ^b $P < 0.01$ vs the control.

It is reported that mAbs 3.2.3 or ANK61 can induce cytolysis in $\text{Fc}\gamma\text{R}^+$ target cells by NK cells by redirected antibody-dependent cellular cytotoxicity (rADCC)^[4,5]. In order to investigate whether these mAbs play a similar role in pit cells, cytolytic activity of pit cells was measured by a 4-h ^{51}Cr -release assay using the $\text{Fc}\gamma\text{R}^+$ P815 cell line^[21], a murine mast ocytoma, as target cells. As shown in Figure 2C, the mAbs 3.2.3 and ANK61 significantly increased the cytolysis of P815 cells by pit cells ($P < 0.01$). Anti-CD45 mAb ANK74 did not significantly increase the cytolysis (Figure 2C).

Effect of CD45, NKR-P1A, and ANK61 antigen on pit cell-mediated target apoptosis

When CC531s^[19], P815 (Vermijlen D, unpublished data) or YAC-1 targets (Figure 3) were coincubated with pit cells, these target cells showed typical morphological characteristics of apoptosis like nuclear fragmentation. To address the effect of the surface antigens in pit cell-mediated apoptosis of these targets, a quantitative DNA fragmentation assay was used. The results showed that the anti-CD45 mAb ANK74 significantly inhibited pit cell-mediated DNA fragmentation of CC531s and YAC-1 cells ($P < 0.05$, $P < 0.01$) (Figure 4A, 4B). The presence of mAbs 3.2.3, ANK61 had no effect on DNA fragmentation of CC531s and YAC-1 cells by pit cells (Figure 4A, 4B), but increased the DNA fragmentation of P815 cells induced by pit cells ($P < 0.01$) (Figure 4C). The anti-CD45 mAb ANK74 did not significantly affect the DNA fragmentation of P815 cells by pit cells (Figure 4C).

Inhibition of anti-NKR-P1A and ANK61 antigen mAb-enhanced P815 cell cytotoxicity by DCI

To address whether mAbs 3.2.3 and ANK61 enhanced $\text{Fc}\gamma\text{R}^+$ P815 cell killing by pit cells via the perforin/granzyme pathway, we used DCI, an inhibitor of granzymes in intact cells, to investigate the role of granzymes in the process. When pit cells were preincubated with 50 $\mu\text{mol/L}$ DCI, the mAb-enhanced P815 cytolysis and apoptosis by pit cells were completely inhibited ($P < 0.05$, $P < 0.01$) (Figure 5A, 5B). These results suggest that mAbs 3.2.3 or ANK61 induce cytotoxicity in $\text{Fc}\gamma\text{R}^+$ P815 cells by pit cells via the perforin/granzyme pathway.

DISCUSSION

Pit cells are liver-specific NK cells and, together with Kupffer cells, constitute the natural cellular defense against invading cancer cells in the liver^[12]. It has been shown that pit cells are four to eight times more cytotoxic against YAC-1 and CC531s cells than blood NK cells and are able to kill NK-resistant, LAK-sensitive P815 cells^[13]. However, the surface receptors involved in pit cell-mediated cytotoxicity against tumor targets have remained elusive. Present data show that the mAbs against the NK-triggering molecules NKR-P1A and ANK61 antigen increased not only cytolysis but also apoptosis of $\text{Fc}\gamma\text{R}^+$ P815 cells, but had no effect on $\text{Fc}\gamma\text{R}$ YAC-1 and

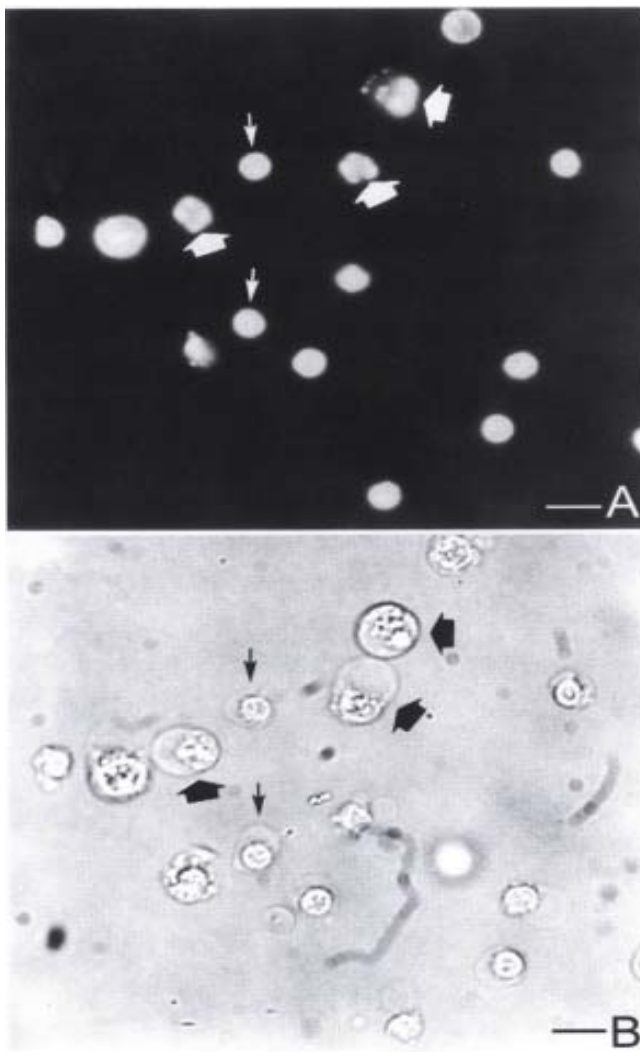


Figure 3 Fluorescence and light micrographs of YAC-1 cells coincubated with pit cells at an E:T ratio of 10:1 for 3 h. (A) Fluorescence micrograph showing the apoptotic YAC-1 cells with fragmented nuclei (thick arrows) and pit cells (thin arrows). Light micrograph shows the same field as (A). Bar = 5 μ m.

CC531s cytotoxicity induced by pit cells. These results indicate that NKR-P1A and ANK61 antigen on pit cells function as triggering molecules against $Fc\gamma R^+$ target cells, but not against $Fc\gamma R^-$ targets. It has been demonstrated that mAbs 3.2.3 and ANK61 enhance cytolytic activity of NK and LAK cells only against $Fc\gamma R^+$ tumor target cells by rADCC^[4,5,7]. Other evidence to support this observation was that, in our experiments, cross-linking of NKR-P1A on pit cells with mAb 3.2.3 did not induce granule exocytosis from pit cells, as observed in May-Giemsa-stained cytospin preparations (data not shown). This is in contradiction with a report describing that mAb 3.2.3 can induce degranulation and release of granzymes from mNK and IL-2 activated NK cells^[22]. Furthermore, after incubation of pit cells with mAb 3.2.3, the medium and the pit cells were separated by centrifugation. The medium

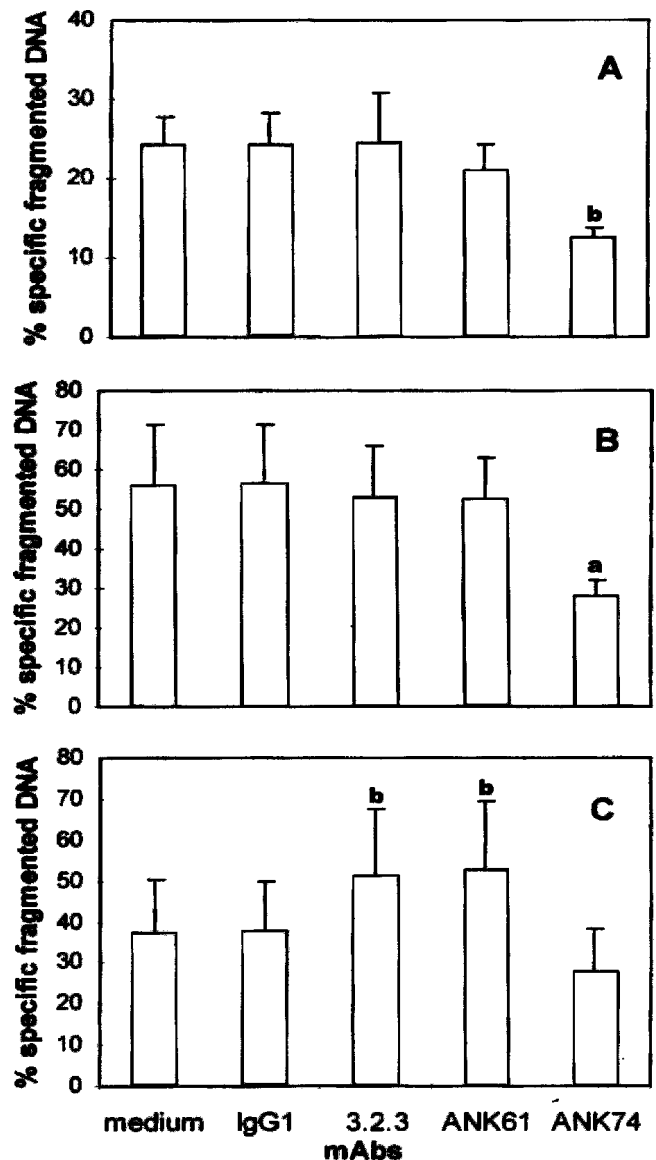


Figure 4 Effect of the mAbs on pit cell-induced CC531s (A), YAC-1 (B) and P8 15 (C) target cell apoptosis. [3H]-TdR labeled target cells were incubated at an E:T ratio of 10:1 with freshly isolated pit cells for 3 h in the absence or presence of the mAbs. Values are mean \pm SD from four different experiments. ^a $P < 0.05$, ^b $P < 0.01$ vs the control.

did not kill CC531s and YAC-1 cells, whereas the pit cells' cytotoxicity against these targets was comparable to freshly isolated pit cells (data not shown). Moreover, it has also been shown that NKR-P1A negative NK clones are able to lyse the same type of target cells as NKR-P1A positive NK clones^[23] and expression of NKR-P1A is not an absolute requirement for NK activity^[24]. These data suggest that NKR-P1A is not the sole activation molecule on NK cells. Actually, a diversity of activation molecules on NK cells has been described^[2,3,5,25]. NK cells might use these different activation molecules to lyse different target cells.

It is believed that mAbs 3.2.3 or ANK61-enhanced cytotoxicity in $Fc\gamma R^+$ target cells by NK cells is a rADCC reaction^[4,5]. The present data show further that these

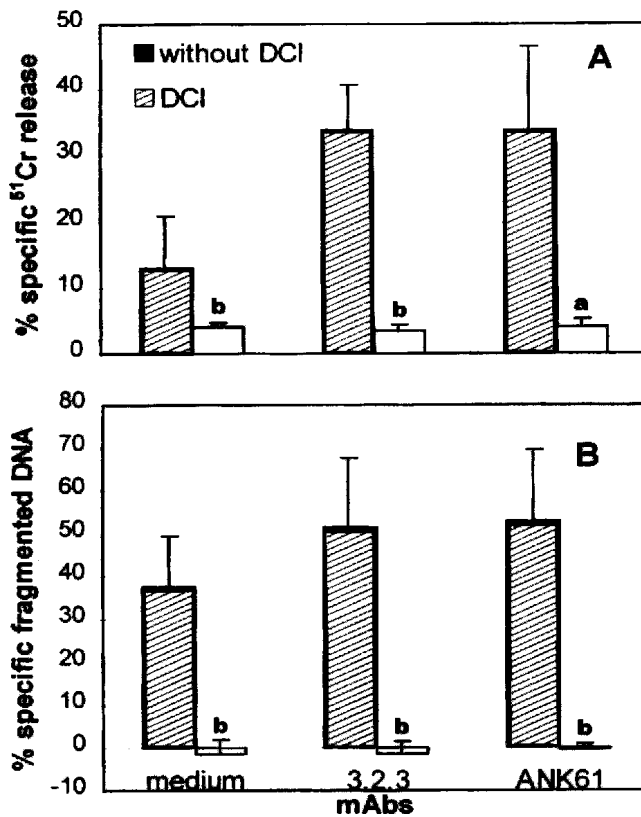


Figure 5 Effect of DCI on mAbs 3.2.3 and ANK61-enhanced Fc γ R⁺ P815 cell cytotoxicity (A) and apoptosis (B) by pit cells. Pit cells were preincubated with 50 μ mol/L DCI for 30 min at 37°C. After washing twice, the cells were coincubated with P815 cells as mentioned in materials and methods. Values are mean \pm SD from three different experiments. ^a P < 0.05, ^b P < 0.01 vs the corresponding control.

mAb-enhanced cytotoxicity and apoptosis of P815 cells by pit cells are mediated by the perforin/granzyme pathway because this enhanced cytotoxicity can be completely inhibited by the granzyme inhibitor DCI.

Although the function of CD45 is well known in NK cells^[8-11], its role in pit cells has not yet been clarified. We showed here that CD45 was involved in the pit cell induced cytotoxicity and apoptosis of CC531s and YAC-1 cells but not of P815 cells. The reason that CD45 on pit cells had different effects on pit cell-mediated cytotoxicity against different targets is not clear. It has been reported that CD45 is not essential for Fc γ R-mediated function in NK cells, since NK cells in CD45^{-/-} mice have normal cytotoxic activities, comparable to normal mice (CD45^{+/+})^[26]. Another possible explanation is that CD45 plays a regulatory role in signal transduction through surface receptors on NK cells, such as NKR-P1A, LFA-1, and killer cell inhibitory receptors (KIR) in NK cell-mediated target killing^[8,10,11]. A diversity of receptors is found to be involved in NK cell activation^[2,3]. It could be that CD45 plays a different regulating role in different NK activation receptors. On the other hand, the regulatory role of CD45 in the inhibitory signal pathway via KIR is dependent on the expression level of MHC class I on target

cells^[8]. The anti-CD45 mAb inhibits only the lysis of target cells expressing low level of MHC class I, such as CC531s^[8] and YAC-1^[27], whereas the lysis of target cells expressing high level of MHC class I, such as P815^[27], is not inhibited^[8].

In summary, CD45 on pit cells participated in pit cell-mediated CC531s and YAC-1 but not P815 cytotoxicity and apoptosis. NKR-P1A and ANK61 antigen on pit cells were involved in pit cell-mediated Fc γ R⁺ P815 cell cytotoxicity and apoptosis, but not in Fc γ R⁻ CC531s and YAC-1 cell cytotoxicities. The mAbs 3.2.3 and ANK61-induced cytotoxicities of Fc γ R⁺ P815 cells by pit cells were mediated by the perforin/granzyme pathway. These findings provide evidence that the same molecules on pit cells and blood or spleen-derived NK cells regulate target cell lysis and apoptosis. The difference in cytotoxic capacity between these cell types therefore may be on a quantitative rather than a qualitative level.

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REFERENCES

- 1 Brittenen D, Heys SD, Ross J, Eremin O. Natural killer cells and cancer. *Cancer*, 1996;77:1226-1243
- 2 Lanier LL. NK cell receptors. *Annu Rev Immunol*, 1998;16:359-393
- 3 Timonon T, Helander TS. Natural killer cell-target cell interactions. *Curr Opin Cell Biol*, 1997;9:667-673
- 4 Chambers WH, Vujanovic NL, DeLeo AB, Olszowy MW, Herberman RB, Hiserodt JC. Monoclonal antibody to a triggering structure expressed on rat natural killer cells and adherent lymphokine activated killer cells. *J Exp Med*, 1989;169:1373-1389
- 5 Giezeman Smits KM, Gorter A, Nagelkerke JF, van Vlierberghe RLP, van Eendenburg J, Eggermont AMM, Fleuren GJ, Kuppen PJK. Characterization of three new membrane structures on rat NK cells which are involved in activation of the lytic machinery. *Immunobiology*, 1997;197:429-443
- 6 Bezouska K, Yuen CT, O'Brien J, Childs RA, Chai W, Lawson AM, Drbal K, Fiserova A, Pospisil M, Feizi T. Oligosaccharide ligands for NKR-P1 protein activate NK cells and cytotoxicity. *Nature*, 1994;372:150-157
- 7 Ryan JC, Niemi Ec, Goldfien RD, Hiserodt JC, Seaman WE. NKR-P1, an activating molecule on rat natural killer cells, stimulates phosphoinositide turnover and a rise in intracellular calcium. *J Immunol*, 1991;147:3244-3250
- 8 Giezeman Smits KM, Gorter A, van Vlierberghe RLP, van Eendenburg J DH, Eggermont AMM, Fleuren GJ, Kuppen PJK. The regulatory role of CD45 on rat NK cells in target cell lysis. *J Immunol*, 1999;163:71-76
- 9 Starling GC, Hart DN. CD45 molecule cross linking inhibits natural killer cell mediated lysis independently of lytic triggering. *Immunology*, 1990;71:190-195
- 10 Bell GM, Dethloff GM, Imboden JB. CD45 negative mutants of a rat natural killer cell line fail to lyse target cells. *J Immunol*, 1993;151:3646-3653
- 11 Poggi A, Pardi R, Pella N, Morelli L, Sivori S, Vitale M, Revello V, Moretta A, Moretta L. CD45 mediated regulation of LFA 1 function in human natural killer cells. Anti CD45 monoclonal antibodies inhibit the calcium mobilization induced via LFA 1 molecules. *Eur J Immunol*, 1993;23:2454-2463
- 12 Wisse E, Luo D, Vermijlen D, Kanellopoulou C, Zanger RD, Braet F. On the function of pit cells, the liver specific natural killer cells. *Seminars in*

- liver Disease*, 1997;17:265-286
- 13 Vanderkerken K, Bouwens L, Wisse E. Characterization of a phenotypically and functionally distinct subset of large granular lymphocytes (pit cells) in rat liver sinusoids. *Hepatology*, 1990;12:70-75
 - 14 Kanellopoulou C, Seynaeve C, Crabbé E, Baekeland M, Vermijlen D, Vermoesen A, Braet F, Zanger RD, Wisse E. Isolation of pure pit cells with a magnetic cell sorter and effect of contaminating cells on their cytolytic capability against CC531. In: Wisse E, Knook DL and Balabaud C, editors. Cells of hepatic sinusoid. The Kupffer cell foundation. Leiden, The Netherlands. 1997;6:471-473
 - 15 Marquet RL, Westbroek DL, Jeekel J. Interferon treatment of a transplantable rat colon adenocarcinoma: importance of tumor site. *Int J Cancer*, 1984;33:689-692
 - 16 Otake S, Kam CM, Narasimhan L, Poe M, Blake JT, Krahenbuhl O, Tschopp J, Powers JC. Human and murine cytotoxic T lymphocyte serine proteases: subsite mapping with peptide thioester substrates and inhibition of enzyme activity and cytolysis by isocoumarins. *Biochemistry*, 1991;30:2217-2227
 - 17 Luo D, Vanderkerken K, Bouwens L, Kuppen PJK, Baekeland M, Seynaeve C, Wisse E. The role of adhesion molecules in the recruitment of hepatic natural killer cells (pit cells) in rat liver. *Hepatology*, 1996;24:1475-1480
 - 18 Bouwens L, Wisse E. Hepatic pit cells have natural cytotoxic (NC) activity against solid tumor derived target cells. In: Wisse E, Knook DL, Decker K, editors. Cells of the Hepatic Sinusoid. The Kupffer Cell Foundation, Rijswijk, The Netherlands, 1989;2:215-220
 - 19 Vermijlen D, Luo D, Robaye B, Seynaeve C, Baekeland M, Wisse E. Pit cells (hepatic natural killer cells) of the rat induce apoptosis in colon carcinoma cells by the perforin/granzyme pathway. *Hepatology*, 1999;29:51-56
 - 20 Luo D, Vanderkerken K, Bouwens L, Kuppen PJK, Crabbé E, Wisse E. The number and distribution of hepatic natural killer cells (pit cells) in normal rat liver: an immunohistochemical study. *Hepatology*, 1995;21:1690-1694
 - 21 van de Griend RJ, Bolhuis RLH, Stoter G, Roozmond RC. Regulation of cytolytic activity in CD3 and CD3 + killer cell clones by monoclonal antibodies (anti CD16, anti CD2, anti CD3) depends on subclass specificity of target cell IgG FcR. *J Immunol*, 1987;138:3137-3144
 - 22 Velitti F, Palmieri G, D'Ambrosio D, Piccoli M, Frati L, Santoni A. Differential expression of granzyme A and granzyme B proteases and their secretion by fresh rat natural killer cells (NK) and lymphokine activated killer cells with NK phenotype (LAK-NK). *Eur J Immunol*, 1992;22:1049-1053
 - 23 Brissette Storkus C, Appasamy PM, Hayes LA, Kaufman CL, Ildstad ST, Chambers WH. Characterization and comparison of the lytic function of NKR P1⁺ and NKR P1 rat natural killer cell clones established from NKR P1 bright/TCR $\alpha\beta$ -cell lines. *Nat Immun*, 1995;14:98-113
 - 24 Pinard D, Olsson NO, Chambers WH, Martin F. High expression of NKR P1 is not an absolute requirement for natural killer activity in BDIX rats. *Cancer Immunol Immunother*, 1996;42:15-23
 - 25 Bauer S, Groh V, Wu J, Steile A, Phillips JH, Lanier LL, Spies T. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science*, 1999;285:727-729
 - 26 Yamada H, Kishihara K, Kong YY, Nomoto K. Enhanced generation of NK cells with intact cytotoxic function in CD45 exon 6 deficient mice. *J Immunol*, 1996;157:1523-1528
 - 27 Haridas V, Saxena RK. Role of major histocompatibility complex class I antigens in modulating the performance of murine tumour cells in cold target competition assays. *Immunology*, 1995;84:86-90

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