



HOKKAIDO UNIVERSITY

Title	NONSPECIFIC CELL-MEDIATED CYTOTOXICITY OF PERIPHERAL BLOOD LYMPHOCYTES DERIVED FROM SUCKLING PIGLETS
Author(s)	ONIZUKA, Nobuyuki; MAEDE, Yoshimitsu; OHSUGI, Takeo et al.
Citation	Japanese Journal of Veterinary Research, 35(1), 41-48
Issue Date	1987-01-30
DOI	https://doi.org/10.14943/jjvr.35.1.41
Doc URL	https://hdl.handle.net/2115/3029
Type	departmental bulletin paper
File Information	KJ00002374453.pdf



NONSPECIFIC CELL-MEDIATED CYTOTOXICITY OF PERIPHERAL BLOOD LYMPHOCYTES DERIVED FROM SUCKLING PIGLETS

Nobuyuki ONIZUKA, Yoshimitsu MAEDE, Takeo OHSUGI and
Shigeo NAMIOKA

(Accepted for publication December 5, 1986)

Peripheral blood lymphocytes (PBL) from both adult pigs and suckling piglets showed natural killer (NK) activity against porcine B cell tumor cell line Mitsukaido, and this activity increased after incubation of PBL in glass Petri dishes at 37°C for 16 h. The NK activity against homogeneous Mitsukaido was higher than that against heterogenous human chronic myelogenous leukemia cell line K562.

NK activity of suckling piglets against Mitsukaido was not observed during 1 to 3 weeks of age. However, the activity rose slightly at 4 weeks old, and it remarkably at 5 weeks old. On the other hand, NK activity against K562 was not seen during 1 to 4 weeks of age, but at 5 weeks old, it was clearly recognized.

These results indicated that the NK activity of piglets at 1 to 3 weeks of age was very low compared with adult controls.

Key words: nonspecific cell-mediated cytotoxicity, piglets

INTRODUCTION

It is well known that suckling piglets are immunodeficient because of the poor differentiation of B lymphocytes into antibody-forming cells (ALLEN & PORTER, 1973; KIM, 1975; NAMIOKA et al., 1983; YABIKI et al., 1974).

Recently, SUGANUMA et al. (1986) reported that this immunodeficiency is thought to be due to an activation of suppressor T lymphocytes.

Apart from the immune system of T and B lymphocytes, there is spontaneous cytotoxicity against various targets in natural killer (NK) cells. NK activity was first observed in the mouse, and particularly high levels of NK activity were found in the "nude" mouse (HERBERMAN et al., 1975; KIESSLING et al., 1975). It has been postulated that NK cells play an important role in immune surveillance against tumors and

various infections. Though several workers reported the presence of porcine NK activity (KOREN et al. 1978; KIM et al. 1980; CEPICA & DERBYPHIRE, 1983; NORLEY & WARDLEY, 1983; MARTIN & WARDLEY, 1984; TAKAMATSU & KOIDE 1985), their results varied among the researchers.

In this investigation, NK activity of peripheral blood lymphocytes (PBL) derived from suckling piglets was examined for the purpose of comprehending to some degree immunoresponsiveness in the suckling stage. NK activity of PBL from both adult pigs and suckling piglets was tested in a 4 h ^{51}Cr -release assay against porcine B cell tumor cell line Mitsukaido and human chronic myelogenous leukemia cell line K562.

MATERIALS and METHODS

Animals

Six specific pathogen free (SPF) pigs (2.5 to 7 months old) and four piglets from a SPF sow were used. They were maintained in a minimal disease condition.

Isolation of mononuclear cells from peripheral blood

Blood samples were collected from the jugular vein and mononuclear cells were isolated by Ficoll-Conray gradients (NAMIOKA et al., 1983). Briefly, blood was diluted with an equal volume of phosphate buffered saline (PBS), the diluted blood (10 ml) was layered on 3 ml of Ficoll-Conray solution (10 parts of 33.4% Conray with 24 parts of 9% Ficoll, specific gravity, 1.084) and centrifuged for 30 min at 400 x g at 20°C. The lymphocyte rich middle fraction was withdrawn and the cells were washed four times in PBS at 4°C. After that, the cells were suspended in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), antibiotics (penicillin 100 μ units/ml, streptomycin 100 μ g/ml) and HEPES (20mM/ml). This cell suspension contained about 5% cells positive for phagocytosis of yeasts.

Culture of mononuclear cells and removal of adherent cells

Mononuclear cells were adjusted to 3×10^6 /ml in RPMI 1640 medium (containing 10% FBS) and dispensed in 10 ml per glass Petri dish. They were incubated for 1 or 16 h at 37°C in a 5% CO_2 atmosphere. Nonadherent cells contained less than 1% cells which were positive for phagocytosis of yeasts.

Then, 3 ml of the cell suspensions were added to the test tubes. Nonadherent cells were collected after incubation at 37°C for 16 h under 5% CO_2 air and were also used as effector cells. Effector cells were adjusted to 10^7 /ml in RPMI 1640 medium (containing 10% FBS).

Target cell preparation

Porcine B cell tumor cell line Mitsukaido (KODAMA & OGIHARA 1986) and human myeloid leukemia cell line K562 were used as target cells for NK assay. Briefly, 1 to 10×10^5 cells were resuspended in 0.3 ml of RPMI 1640 containing 10% FBS, respectively. Then 100 μ Ci of $\text{Na}_2 \text{}^{51}\text{CrO}_4$ (specific activity 360 to 530 mCi/mg, No. MEZ-030s, New England Nuclear, USA) were added to the target cells, which were

incubated for 1 h at 37°C in a 5% CO₂ atmosphere. The labeled cells were washed twice in RPMI 1640 medium (containing 10% FBS) and adjusted to 10⁵/ml.

Assay for NK activity

A 4 h ⁵¹Cr-release assay method was used to measure cytotoxic activities for NK as described by KIM et al. (1980). Two-fold serial dilution of effector cells was incubated in the wells of a 96 well flat-bottomed microtitre plate (Corning 25860, USA) with ⁵¹Cr-labeled target cells, giving the ratios of 100:1, 50:1, 25:1 in total volumes of 200 μl of medium. The plates were incubated at 37°C for 4 h in a 5% CO₂ atmosphere. The assay was terminated by centrifuging the plates for 10 min at 200 x G and 100 μl of the supernatants were collected. Radioactivity was counted in a gamma counter (Aloka, ARC-500). The spontaneous release (SR) was determined as the counts per minute (cpm) by adding target cells to the medium alone. The maximum release (MR) was determined as the cpm in the supernatants after lysis of the targets with 1% Triton X-100.

The formula used to calculate the % specific ⁵¹Cr-release was:

$$\% \text{ specific release (lysis)} = \frac{\text{cpm Experimental} - \text{cpm SR}}{\text{cpm MR} - \text{cpm SR}} \times 100$$

SR for K562 was 8.2 ± 1.5% (mean ± SD) of MR, and SR Mitsukaido was 13.6 ± 4.0% of MR.

RESULTS and DISCUSSION

Recently, KODAMA and OGIHARA (1986) established porcine B cell tumor cell line Mitsukaido. At first, NK activity in PBL from adult pigs was observed using this cell line as a target. Table 1 shows the influences of culture vessels in which PBL are contained and the incubation times against NK activity. NK activity of PBL cultured

TABLE 1 Influences of culture vessels and times at appearance of NK activity in PBL from adult pigs¹

Vessels	Times	
	1 h	16h
Test tube	N. D. ²	4.5 ± 2.2
Glass petri dish	5.1 ± 0.7% ³	27.7 ± 19.9%

¹ NK activity of peripheral blood lymphocytes (PBL) from adult pigs at an effector/target (E:T) ratio of 100:1 was tested in a 4 h ⁵¹Cr-release assay against porcine tumor cell line Mitsukaido.

² Not done

³ Data represent the mean (±SD) %

for 16 h was five times higher than that cultured for 1 h, while PBL cultured in test tubes for 16 h showed lower NK activity than those cultured in glass Petri dishes. This result agrees with the findings of KOREN et al. (1978) and TAKAMATSU & KOIDE (1985). According to the results mentioned above, PBL cultured in glass Petri dishes at 37°C for 16 h were used as the effector cells throughout the study to measure NK activity of PBL.

Figure 1 shows the NK activity at different effector/ target (E : T) cell ratios against Mitsukaido and K562. Cytotoxic activity of the PBL both against Mitsukaido and K562 was augmented with the increase in the number of effector cells. Higher cytotoxic activity of the adult PBL was shown against Mitsukaido than heterogeneous K562 in the same adult pigs No. 5.

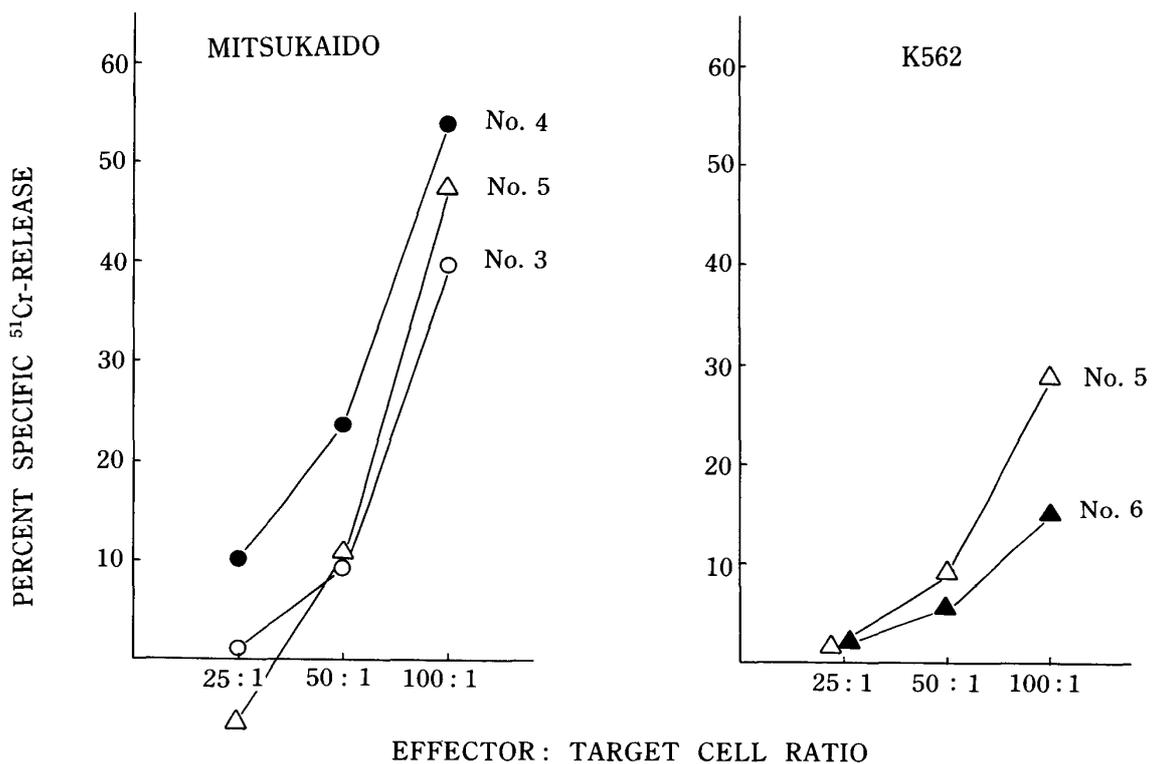


FIGURE 1 Effect of effector/target (E : T) ratio on target cells lysis. Porcine B cell tumor cell line Mitsukaido and human chronic myelogenous leukemia cell line K562 were used as target cells for NK assay. Effector cells were peripheral blood lymphocytes (PBL) from adult pigs (Nos. 3, 4, 5 and 6) and incubated for 16 h at 37°C before NK assay.

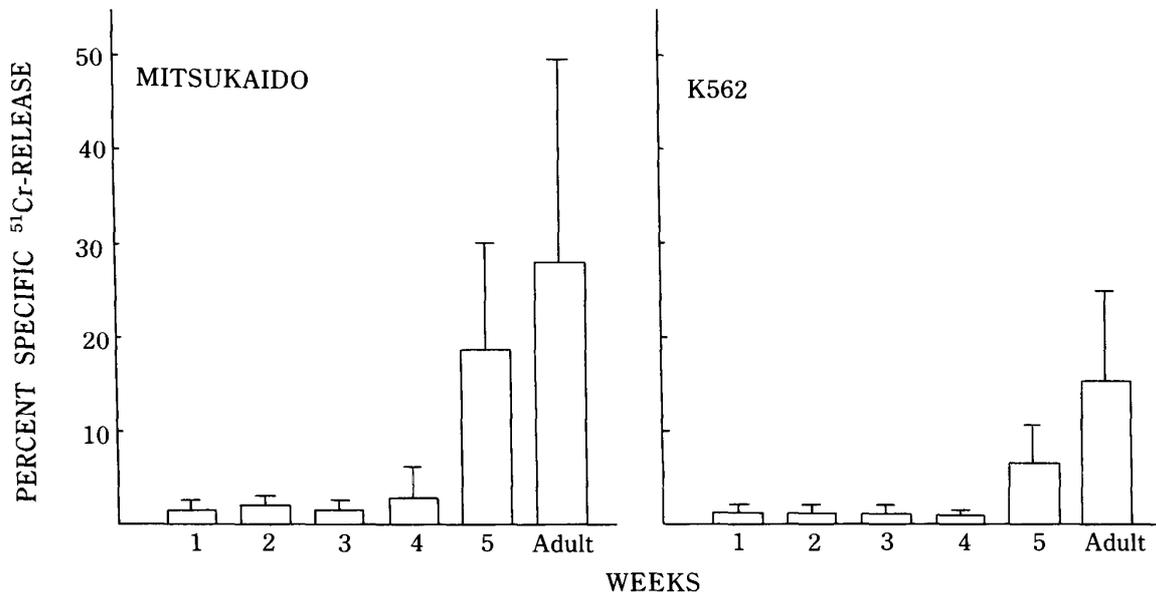


FIGURE 2 Development of NK activity in PBL from suckling piglets. Homogeneous Mitsukaido and heterogenous K562 were used as target cells for NK assay. PBL from suckling piglets incubated for 16h at 37°C before NK assay. Data represent the mean (\pm SD) of the NK activity at an E : T ratio of 100 : 1.

Figure 2 shows NK activity of PBL from piglets at 1, 2, 3, 4 and 5 weeks of age against Mitsukaido and K562. Cytotoxic activity against Mitsukaido was not observed during 1 to 3 weeks of age. However, the activity was observed slightly at 4 weeks old, and it increased remarkably, reaching 65.9 per cent of the adult NK activity, at 5 weeks old. On the other hand, cytotoxic activity against K562 was not seen during 1 to 4 weeks of age, but at 5 weeks old, it was clearly recognized. Cytotoxic activity of suckling piglets against homogeneous Mitsukaido was higher at 4 to 5 weeks of age than that with heterogeneous K562.

HUH et al. (1981) reported that the colostrum-deprived piglets obtained by aseptic hysterectomy developed NK activity at 3-4 weeks of age, whereas the activity was seen at 2-3 weeks of age in colostrum-fed piglets which were naturally-farrowed. According to the results of CEPICA & DERBYSHIRE (1984), who applied the 16 h ⁵¹Cr-release assay, there was a lack of spontaneous cell mediated cytotoxicity of piglets against transmissible gastroenteritis virus infected cells during the first week of life, but significant activities developed during the second week and usually increased further by the sixth week.

In our study, NK activity was not observed in the piglets at 1–3 weeks of age, while the NK activity was clearly recognized at 5 weeks old. This result differs somewhat from that of other studies as was mentioned above: the difference may be due to considerable variations in the method of culture of PBL, the type of target cells chosen and the incubation time for the assay of cytotoxicity. In particular, the ^{51}Cr -release assay time for detecting the NK activity varied according to the workers, which indicates the need for a standardized detection method.

However, it was interesting that the appearance of clear NK activity of piglets at five weeks coincided with the period of the disappearance of the suppressor activity of T lymphocytes (SUGANUMA et al. 1986). Therefore it is reasonable to consider that there might be a relationship between productivity of interleukin 2 (IL2) and NK activity. In humans, it is well known that NK activity of cord blood and newborn peripheral blood is still low compared with that of adult. Recently, it was reported that the NK activity of newborn peripheral blood could be enhanced by interferon (IFN) and IL-2 (SATO et al., 1979; ANTONELLI et al., 1981; KOHL et al., 1981; UENO et al., 1985; SEKI et al., 1985).

As was described previously, in pigs, the potency of the suppressor activity of newborn T lymphocytes may be inversely proportional to the production of IL-2 from helper T lymphocytes; therefore it is reasonable to consider that the NK activity of newborn peripheral blood up to 4 weeks of age is low.

In this connection, comparative studies are required to measure the production of IFN and IL-2 between suckling and adult pigs.

ACKNOWLEDGEMENTS

We are grateful to Dr. M. KODAMA (National Institute of Animal Health) for providing the Mitsukaido cell line, and to Dr. I. AZUMA (Research Section of Chemistry, Institute of Immunological Science, Hokkaido University) for his kind offer of the K562 cell line.

REFERENCES

- 1) ALLEN, W. D. & PORTER, P. (1973): The relative distribution of IgM and IgA cells in intestinal mucosa and lymphoid tissues of the young unweaned pig and their significance in ontogenesis of secretory immunity. *Immunology*, **24**, 493–501
- 2) ANTONELLI, P., STEWART H, W. & DUPONT, B. (1981): Distribution of natural killer cell activity in peripheral blood, cord blood, thymus, lymph nodes and spleen and the effect of *in vitro* treatment with interferon preparation. *Clin. Immunol. Immunopathol.*, **19**, 161–169
- 3) CEPICA, A. & DERBYSHIRE, J. B. (1983): Antibody-dependent cell-mediated cytotoxicity and spontaneous cell-mediated cytotoxicity against cells infected with porcine transmissible gastroenteritis virus. *Can. J. Comp. Med.*, **47**, 298–303
- 4) CEPICA, A. & DERBYSHIRE, J. B. (1984): Antibody-dependent and spontaneous cell-

- mediated cytotoxicity against transmissible gastroenteritis virus infected cells by lymphocytes from sows, fetuses and neonatal piglets. *Ibid.*, **48**, 258–261
- 5) HERBERMAN, R. B., NUNN, M. E. & LAVRIN, D. H. (1975): Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors I. Distribution of reactivity and specificity. *Int. J. Cancer*, **16**, 216–229
 - 6) HUH, N. D., KIM, Y. B., KOREN, H. S. & AMOS, D. B. (1981): Natural killing and antibody-dependent cellular cytotoxicity in specific-pathogen-free miniature swine and germ-free piglets. II. Ontogenic development of NK and ADCC. *Ibid.* **28**, 175–178
 - 7) KIESSLING, R., KLEIN, E. & WIGZELL, H. (1975): "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. *Eur. J. Immunol.*, **5**, 112–117
 - 8) KIM, Y. B. (1975): Developmental immunity in the piglet. In: Immunodeficiency in man and animals, 549–557 Sinauer Associates, Inc., Massachusetts
 - 9) KIM, Y. B., HUH, N. D., KOREN, H. S. & AMOS, D. B. (1980): Natural killing (NK) and antibody-dependent cellular cytotoxicity (ADCC) in specific-pathogen-free (SPF) miniature swine and germfree piglets. I. Comparison of NK and ADCC. *J. Immunol.*, **125**, 755–762
 - 10) KODAMA, M. & OGIHARA, K. (1986): Establishment of B lymphoblast cell line (named Mitsukaido) derived from porcine leukemia pig. (in preparation)
 - 11) KOHL, S., FRAZIER, J. J., GREENBERG, S. B., PICKERING, L. K. & LOO, Lian-Sim (1981): Interferon induction of natural killer cytotoxicity in human neonates. *J. Pediatr.*, **98**, 379–384
 - 12) KOREN, H. S., AMOS, D. B. & KIM, Y. B. (1978): Natural killing and antibody-dependent cellular cytotoxicity are independent immune functions in the Minnesota miniature swine. *Proc. Natl. Acad. Sci., USA*, **75**, 5127–5131
 - 13) MARTIN, S. & WARDLEY, R. C. (1984): Natural cytotoxicity detected in swine using Aujeszky's disease virus infected targets. *Res. Vet. Sci.*, **37**, 211–218
 - 14) NAMIOKA, S., OHSUGI, T., FUJIMOTO, T. & MAEDE, Y. (1983): Lymphocyte transformation of peripheral blood lymphocytes and plaque forming cells of the spleen and the mesenteric lymph node in suckling piglets with and without immunopotentiators. *Jpn. J. Vet. Res.*, **31**, 53–64
 - 15) NORLEY, S. G. & WARDLEY, R. C. (1983): Investigation of porcine natural killer cell activity with reference to African swine-fever virus infection. *Immunology.*, **49**, 593–597
 - 16) SATO, T., FUSE, A. & KUWATA, T. (1979): Enhancement by interferon of natural cytotoxic activities of lymphocytes from human cord and peripheral blood of aged persons. *Cell. Immunol.*, **45**, 458–463
 - 17) SEKI, H., UENO, Y., TAGA, K., MATSUDA, A., MIYAWAKI, T. & TANIGUCHI, N. (1985): Mode of *in vitro* augmentation of natural killer cell activity by recombinant human interleukin 2: A comparative study of Leu-11⁺ and Leu-11⁻ cell populations in cord blood and adult peripheral blood. *J. Immunol.*, **135**, 2351–2356
 - 18) SUGANUMA, A., ISHIZUKA, A., SAKIYAMA, Y., MAEDE, Y. & NAMIOKA, S. (1986): B lymphocyte differentiation and suppressor activity by T lymphocytes derived from

- neonatal and suckling piglets. *Res. Vet. Sci.*, **40**, 400–405
- 19) TAKAMATSU, H. & KOIDE, F. (1985): Augmentation of porcine spontaneous cell-mediated cytotoxicity *in vitro*. *Jpn. J. Vet. Sci.*, **47**, 749–759
 - 20) UENO, Y., MIYAWAKI, T., SEKI, H., MATSUDA, A., TAGA, K., SATO, H. & TANIGUCHI, N. (1985): Differential effects of recombinant human interferon- γ and interleukin 2 on natural killer cell activity of peripheral blood in early human development. *J. Immunol.*, **135**, 180–184
 - 21) YABIKI, T., KASHIWAZAKI, M. & NAMIOKA, S. (1974): Quantitative analysis of three classes of immunoglobulins in serum of newborn pigs and milk of sows. *Am. J. Vet. Res.*, **35**, 1483–1489