Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Veterinary Immunology and Immunopathology, 29 (1991) 89–103
Elsevier Science Publishers B.V., Amsterdam

Natural killer (NK) activity of porcine blood lymphocytes against allogeneic melanoma target cells

Mathias Büttner[a], Rüdiger Wanke[b] and Bernd Obermann[a]

[a]*Institute for Medical Microbiology, Epidemiology and Infectious Diseases,* [b]*Institute of Veterinary Pathology, Veterinärstrasse 13, 8000 München 22, FRG*

(Accepted 7 August 1990)

ABSTRACT

Büttner, M., Wanke, R. and Obermann, B., 1991. Natural killer (NK) activity of porcine blood lymphocytes against allogeneic melanoma target cells. *Vet. Immunol. Immunopathol.*, 29: 89–103.

Allogeneic PM/86 melanoma cells of Munich Troll® miniature swine have been used for the demonstration of porcine peripheral blood NK cell activity. Compared with the specific lysis of xenogeneic K562-, U937- and Vero-target cells, NK cell-mediated cytotoxicity (NK-CMC) against PM/86 melanoma tumor cells was significantly lower in a 16 h chromium release assay. The target cell susceptibility to peripheral blood NK-CMC of both adult Troll® miniature swine and German Landrace sows was very similar. Cold target inhibition assays revealed the allogeneic PM/86 melanoma cells to be the most powerful inhibitors of NK-CMC.

Nylon wool non-adherent lymphocytes produced interferon (IFN)-alpha in different quantities upon contact with NK susceptible target cells. The NK effector cells could be stimulated to a higher lytic activity against all susceptible targets by a moderate dose of natural human interleukin-2 (nhuIL-2).

The role of NK-CMC in melanoma tumor rejection and/or prevention of metastases is yet unknown in swine although porcine melanoma serves as a good model for the disease in man.

ABBREVIATIONS

FCS, fetal calf serum; IFN, interferon; LeuOMe, L-leucine methyl ester; MEM, minimal essential medium; MR, maximal release; nhuIL-2, natural human interleukin-2; NK, natural killer; NK-CMC, natural killer cell-mediated cytotoxicity; PBMC, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; SPF, specific pathogen free; SR, spontaneous release, TNF, tumor necrosis factor; VSV, vesicular stomatitis virus.

0165-2427/91/$03.50

INTRODUCTION

The occurrence of NK cell activity has been investigated in various organs and in the peripheral blood of swine (Koren et al., 1978; Charley et al., 1983; Martin and Wardley, 1984; Cepica and Derbyshire, 1986). Small to medium sized lymphocytes have been identified as NK-effector cells in contrast to the large granular lymphocytes of other species (Yang et al., 1987). The NK cell population of swine is characterized as $CD2^+$ $CD8^+$ lymphocytes (Pescovitz et al., 1988; Saalmüller et al., 1988), but a specific NK cell marker is unknown for this species. Peripheral blood cells mediating SLA-unrestricted cytotoxicity were also found among the monocyte/granulocyte populations (Ohlinger and Saalmüller, 1989).

The spectrum of target cells and the reason for susceptibility in in vitro cytotoxicity assays for NK-CMC of swine are poorly understood. There is a single report on Yorkshire swine NK cell characteristics which analyses the susceptibility of xenogeneic human and murine target cells. Taking the peripheral blood lymphocytes of Yorkshire swine as effector cells, murine YAC–1 lymphoma cells were shown to be most susceptible to NK cell mediated lysis (Pinto and Fergusson, 1988).

The K562 erythromyeloma tumor cells are the most commonly used targets for the demonstration of porcine NK-CMC in chromium release assays. However, there are some discrepancies concerning spleen NK cell activity against this cell line (Takamatsu and Koinde, 1985; Yang and Schultz, 1986), which may be explained by variations in regulation of NK-CMC (Salmon et al., 1989).

Vero cells (African green monkey kidney line) have also been shown to be susceptible to NK cell mediated lysis of swine lymphocytes (Wittmann and Ohlinger, 1987), but are described as resistant to canine-CMC (Krakowka et al., 1988).

Berkelhammer et al. (1982) described leukocyte mediated cytotoxicity against allogeneic tumor cells in melanoma bearing Sinclair miniature swine but without further characterization of the effector cell population. In the Munich Troll® miniature swine there is, similar to the Sinclair miniature swine, a high incidence of heritable melanomas (Wanke and Bräuer, 1986). This situation led to the establishment of long-term in vitro culture of homogenous melanoma cells derived from a cutaneous melanoma at necropsy. Significant incidence of spontaneous regression is observed in Sinclair swine (Oxenhandler et al., 1979) as well as in Munich Troll® swine cutaneous melanoma and metastasis with manifestation of tumors in internal organs is rare. This clinical situation makes the effects of cell-mediated immunity on melanoma tumor growth and regression an object of special interest (Beattie et al., 1988; Bröcker et al., 1988).

In this report the susceptibility of Troll® miniature swine melanoma cells

to the lysis by spontaneous cytotoxic blood lymphocyte effector cells was compared with the lysis of xenogeneic target cells. Interferon production upon tumor cell contact and the influence of natural human interleukin-2 (nhuIL-2) on specific tumor cell lysis were determined.

MATERIALS AND METHODS

Animals

Peripheral blood cells of 15 female Munich Troll® miniature pigs (Sambraus, 1987) at an age of 4 days to 4 months, one boar (2 years old), and German Landrace sows (approximately 100 kg at slaughter) were used. The miniature swine were raised under specific pathogen free (SPF) conditions at the Institute of Animal Pathology, University of Munich. Blood was collected from the cranial vena cava after anesthesia (Stresnil–Hypnodil, Janssen, Neuss) from Troll® pigs in which cutaneous melanoma were not detectable (neither at birth nor until the day of NK-CMC analysis). Three Troll® miniature swine, tested at an age of 4 or 16 weeks, had congenital cutaneous melanoma that showed progressive enlargement up to diameters of approximately 5 cm when the test for NK-CMC was performed (see Fig. 5). At the time the experiments were carried out, there were no signs of detrimental effects on the common health status. Blood samples of clinically healthy ($n=6$) and melanoma-bearing ($n=3$) Troll® swines were taken on the same day and Nylon non-adherent lymphocytes were analysed for NK-CMC immediately after isolation in the same test. Blood samples from German Landrace pigs were taken immediately after slaughter during exsanguination at the Munich abattoir.

Separation of effector cells

Peripheral blood mononuclear cells (PBMC) were separated from whole blood by Ficoll–Hypaque (Seromed, Berlin) gradient centrifugation (Boyum, 1976), washed twice with phosphate-buffered saline (PBS) and adjusted to 10^8 cells/ml in culture medium, RPMI 1640 (Seromed, Berlin) supplemented with 10% fetal calf serum (FCS) (Seromed, Berlin).

Removal of plastic adherent cells. One hundred millilitres of a PBMC suspension (10^8 cells in RPMI) were poured into a culture flask (175 cm^2, Falcon, Becton Dickinson, Heidelberg), precoated with FCS, and incubated for 2 h at 37°C in a humidified incubator with 5% CO_2 (Kumagai et al., 1979).

Non-adherent cells were removed by gentle agitation of the culture medium, followed by three washings with RPMI and collected in plastic tubes for centrifugation.

Separation of lymphocytes by nylon wool passage. Plastic non-adherent PBMC were adjusted to 5×10^7/ml and carefully layered on a column of nylon fibers (Travenol Lab., Morton Grove, IL) according to the method of Julius et al. (1973). After 1 h of incubation at 37°C the column was flushed with 25 ml of warm medium (37°C) to elute nylon-non-adherent cells. The eluted cells were centrifuged ($300 \times g$, 10 min, 4°C) and resuspended in culture- medium. Cell viability, determined by the dye exclusion method, was higher than 90% in every experiment.

Treatment of nylon non-adherent cells with L-leucine methyl ester (LeuOMe). To demonstrate the nature of NK-CMC, nylon non-adherent lymphocytes (2.5×10^6) were treated with 20 or 40 mM concentrations of freshly prepared LeuOMe (Sigma, Deisenhofen) in 1 ml RPMI 1640 medium at 37°C for 30 min (Shau and Golup, 1985). The cells were then washed three times by centrifugation and used as effector cells in the chromium release assays.

Target cells

K562 human erythroleukemic cells (American Type Culture Collection, ATCC, CCL 243, Andersson et al., 1979), U937, a human histiocytic lymphoma (ATCC CRL 1593, Sundström and Nillson, 1976) and Raji Burkitt lymphoma cells (ATCC CCL 86, kindly supplied by Dr. Lipp, Institute of Biochemistry, Munich) were grown in suspension in RPMI 1640 medium supplemented with 10% FCS at 37°C in a humidified incubator with 5% CO_2. Vero (African green monkey) kidney cells (ATCC CCL 81) were cultivated in minimal essential medium (MEM, Biochrom, Berlin) supplemented with 3% FCS as monolayers in Roux glass bottles.

The PM/68 Troll® miniature pig melanoma cells (European Collection of Animal Cell Cultures, ECACC No. 89013101) were derived from a congenital cutaneous melanoma from a 7-week-old male piglet with massive melanomatous alterations of the internal organs found at autopsy. Homogeneous melanoma cells were grown in Dulbecco's modified MEM (D-MEM) with 10% FCS as adherent monolayers in plastic flasks (175 cm^2, Becton Dickinson, Heidelberg) and used as target cells at the 26 culture passage. All target cell lines were checked at regular intervals from mycoplasma contamination and were found to be free. Glass or plastic adherent target cells (Vero, PM/86) were detached from the surface using a mild trypsin (0.05%)/EDTA (0.025%) salt solution, centrifuged immediately and resuspended in medium before the labelling with sodium [^{51}Cr]chromate.

Chromium release assay

A ^{51}Cr-release microcytotoxicity technique was performed as described by Yang et al. (1987). Target cells at a concentration of 2×10^6 were labelled with 100 μCi $Na_2{}^{51}CrO_4$ (Dupont NEN, Bad Homburg) for 1 h (37°C, 5%

CO_2). Labelled cells were washed three times with PBS and adjusted to 5×10^4 cells/ml with RPMI 1640 medium containing 10% FCS.

NK cell activity to different target cells was determined by mixing nylon non-adherent lymphocytes with ^{51}Cr-labelled target cells in the wells of round bottom microtiter plates (Greiner, Nürtingen) in a total volume of 0.2 ml. Four different effector to target cell ratios (E:T) 50, 25, 12.5 and 6:1 were assayed in triplicate in a 16 h incubation test.

The percentage cytotoxicity was calculated as follows:

$$\text{Percent cytotoxicity} = \frac{\text{mean c.p.m. experimental} - \text{mean c.p.m. SR}}{\text{mean c.p.m. MR} - \text{mean c.p.m. SR}} \times 100$$

where SR (spontaneous release) is defined as the radioactivity released from target cells incubated in medium alone and MR (maximal release) as counts per min (c.p.m.) in the supernatants of target cells lysed with TritonX100. The c.p.m. of SR did not exceed 25% of the c.p.m. values of MR from any target cells used. Supernatant fluid (0.1 ml) from each well was collected and radioactivity was determined in a gamma counter (Beckman Instruments, Munich). The percentage of specific lysis was calculated after data transfer (PC Ready Plus, Beckman Instruments, Munich) in a personal computer.

Cold target inhibition assays

For cold target competition experiments unlabelled (cold) target cells were added to lymphocyte effector cells at E:T ratios of 10:1 and 80:1 and incubated as triplets in microplates (0.1 ml/well) for 1 h at 37°C in a humidified incubator (5% CO_2). Thereafter [^{51}Cr] radiolabelled (hot) target cells (0.1 ml) were added to achieve a constant E:T ratio of 50:1. Results are presented as the specific lysis at an E:T (hot targets) ratio of 50:1 in the presence of the two different amounts of cold target cells and compared with the lytic activity without adding cold target cells.

Stimulation of effector lymphocytes with interleukin-2

Natural human interleukin-2 (nhuIL-2, Lymphocult-T-HP, Biotest, Frankfurt) dissolved in RPMI 1640 was added in the microtoxicity assay to effector/target mixtures at a concentration of 100 units U/ml. The percentage of specific target cell lysis from EL-2 treated and non-treated effector cells at an E:T ratio of 50:1 and 6:1 were compared.

Interferon assays

Effector (2.5×10^6/ml nylon non-adherent lymphocytes) cells were incubated for 16 h (37°C, 5% CO_2) together with non-radiolabelled target cells at an E:T of 50:1 in a total volume of 2 ml RPMI 1640 supplemented with 10% FCS. At the end of incubation, cells were centrifuged for 10 min at $300\times g$. The supernatants were collected and filtered through 0.22 μm filters (Sarto-

rius, Göttingen). In some experiments the clarified supernatants were finally acidified with 1 N HCl to pH 2.5. The antiviral activity found was characterized as IFN activity according to current criteria (Lockart, 1973) and by neutralization with a specific antiserum containing 450 000 neutralizing units (NU) anti-human IFN-alpha and 3000 NU anti-human IFN-beta/ml (kindly provided by Prof. Cantell, Helsinki).

IFN titers were determined on bovine MDBK cells (ATCC CCL 22) challenged with vesicular stomatitis virus (VSV), strain Indiana as described (Rubinstein et al., 1981). Twofold serial dilutions of supernatants (50 μl) were made by dilution of the samples in MEM in 96-well microtiter plates (Nunclon, Nunc, Wiesbaden).

On each plate, four wells were filled with 50 μl of medium to serve as virus- or cell-controls. Each well was then seeded with 5×10^4 MDBK cells in 50 μl MEM supplemented with 2% FCS. After 6 h of incubation at 37°C the monolayers were challenged with 50–80 plaque-forming units per well of VSV. By the time the virus control cell sheet showed complete destruction, the highest protective dilutions were scored.

Since there is no porcine standard IFN available all titers were expressed in human units by including a laboratory standard IFN in each independent assay. This recombinant standard IFN-alpha (Boehringer, Vienna) had a mean titer of 1200 international units (IU)/ml when assayed in MDBK cells against the international reference alpha$_2$-IFN Gxa01-901-535 (National Institute of Health, U.S.A.)

The 50% endpoints were determined by visual examination after staining the monolayers with crystal violet (Merck, Darmstadt).

Statistical analysis

Results were expressed as means ± standard deviation (s.d.), and statistical significance was analysed by Student's *t*-test (unpaired or paired as appropriate). *P* values less than 0.05 were considered significant.

RESULTS

Susceptibility of target cells to NK-CMC

Allogeneic PM/86 melanoma cells showed reduced susceptibility to lysis by peripheral blood lymphocytes of both, German Landrace and Munich Troll® miniature swine, compared with the NK activity against the xenogeneic target cells U937, K562 and Vero. In Fig. 1 the susceptibility of the target cells to NK-CMC of peripheral blood lymphocytes from German Landrace and Munich Troll® miniature swine is expressed in percent of specific lysis at E:T ratios of 50:1 and 6:1.

The kinetics of NK activity by Troll® miniature swine peripheral blood lymphocytes are similar for K562 and PM/86 target cells, but the values of

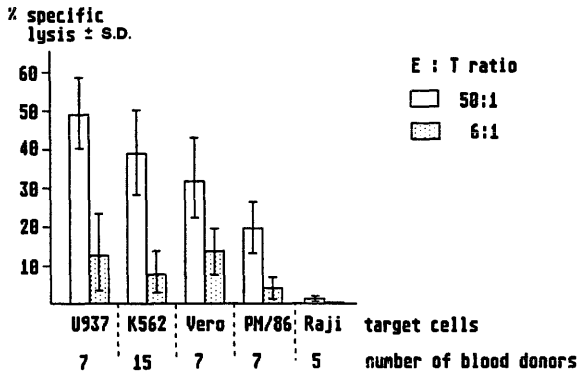

Fig. 1. NK activity of peripheral blood lymphocytes (German Landrace and Troll® miniature swine) against one allogeneic and four xenogeneic target cells. Data from Troll® miniature swine and German Landrace swine (equal number of blood donors) are not shown separately as there is no significant ($P > 0.05$) difference in target cell killing. Mean spontaneous ^{51}Cr-release (SR) was 21.4 ± 2.6% of maximal possible release (MR) for K562, 22.8 ± 1.9% for U937, 23.7 ± 3.1% for PM/86 and 15.3 ± 8.3% for Vero target cells, respectively.

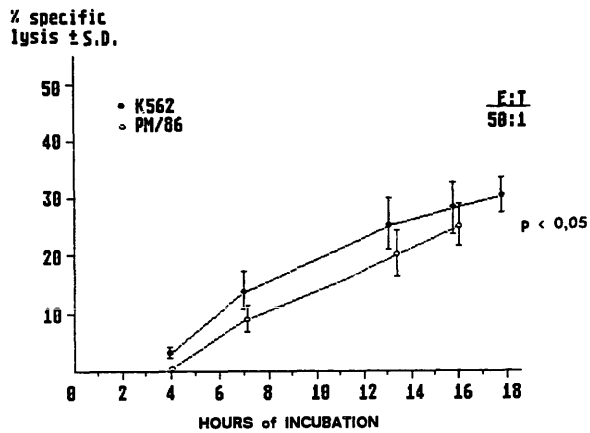

Fig. 2. The kinetics of NK-CMC by Troll® Munich miniature swine peripheral blood lymphocytes, $n = 4$ pigs, 4 months old.

specific lysis of allogeneic melanoma cells are significantly ($P < 0.05$) lower (Fig. 2). As already observed with K562 target cells (Yang et al., 1987; Pinto and Ferguson, 1988), porcine blood lymphocytes do not mediate remarkable lysis before 6 h of incubation. This phenomenon is also seen when allogeneic melanoma cells are used as targets. The plateau of cytotoxicity is not shown for PM/86 target cells because of an increase of spontaneous ^{51}Cr release to values above 25% of maximal release after incubation for more than 16 h.

The U937 tumor cell line could be lysed most efficiently by blood lymphocyte effector cells from the two different races of swine, whereas the Burkitt lymphoma line Raji was almost completely resistant to NK lysis in a 16 h chromium release assay (Fig. 1).

The treatment of nylon non-adherent lymphocytes with 40 or 20 mM of the lysosomotropic agent, L-leucine methyl ester, completely abolished NK cell activity against all susceptible target cells tested (data not shown).

Nylon non-adherent lymphocytes of Troll® miniature swine were able to lyse K562 and melanoma target cells when blood samples of animals older than 2 weeks are taken as the source of the effector cells. Almost no NK cell activity could be seen with lymphocytes from animals younger than 1 week of age (data not shown). A significantly ($P<0.05$) lower cytotoxicity against PM/86 melanoma cells compared with K562 lysis was detected at all E:T ratios tested, when the lymphocytes of adult animals were taken as effector cells, regardless of the race in the blood donor (Fig. 3). In Troll® miniature swine the grade of melanoma target cell lysis was not significantly different between clinically healthy animals and three animals bearing cutaneous melanoma (Figs. 4 and 5).

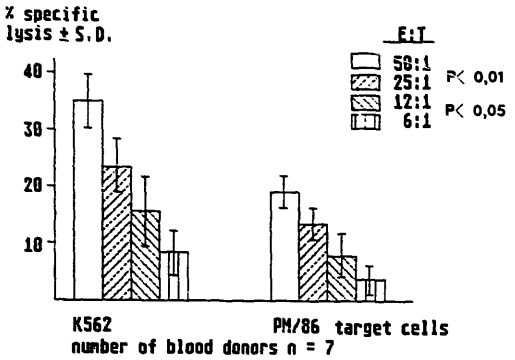

Fig. 3. NK cell activity of German Landrace swine peripheral blood lymphocytes against allogeneic PM/86 melanoma cells and xenogeneic K562 tumor cells (mean values of specific lysis ± s.d. from three independent experiments, seven different blood donors in each experiment, 16 h chromium release assay).

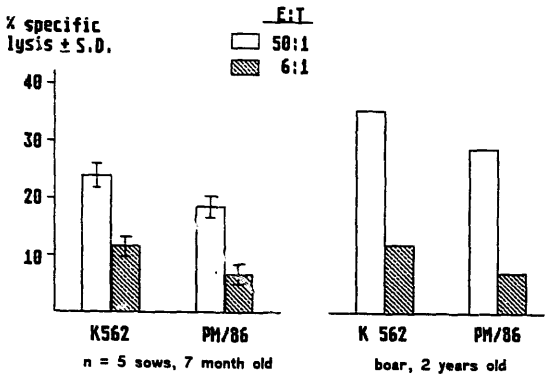

Fig. 4. NK cell activity of Munich Troll® miniature swine peripheral blood lymphocytes against allogeneic melanoma PM/86, and xenogeneic K562 target cells (16 h chromium release assay).

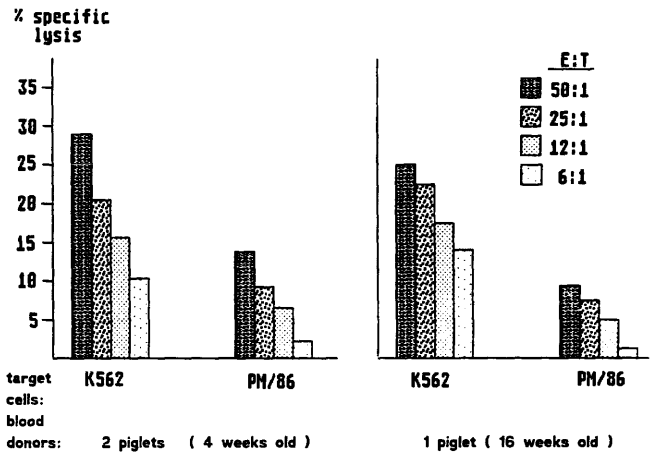

Fig. 5. Peripheral blood NK cell activity of Troll® miniature swine bearing cutaneous melanomas against allogeneic PM/86 melanoma cells and xenogeneic K562 target cells.

TABLE 1

Inhibition of K562 and U937 target cell lysis (E:T = 50:1) by competitive interaction of effector cells with allogeneic or xenogeneic tumor cells

Effector cells	Cold competitors	Ratio: effector/ cold targets	Percent inhibition of K562 target cell lysis	Percent inhibition of U937 target cell lysis
Nylon-NA lymphocytes	U937	10:1	63 (±6)	
$n=7$ blood donors		80:1	11 (±4)	
Troll® minipigs	K562	10:1		50 (±12)
		80:1		5 (±2)
	PM/86	10:1	90 (±5)	61 (±15)
		80:1	37 (±11)	14 (±4)
	Raji	10:1	41 (±18)	18 (±6)
		80:1	16 (±8)	4 (±3)

Cold target competition

As expected, the NK-CMC resistant Raji cells are the weakest competitors in chromium release tests with radiolabelled U937, K562 and PM/86 target cells. The allogeneic PM/86 melanoma cells are strong inhibitors of NK-CMC when K562 or U937 radiolabelled target cells were used, but the hindrance of U937 specific tumor cell lysis is about 30% less than that of K562 cells.

The use of K562 and U937 cold target cells as competitors in cytotoxicity experiments with (^{51}Cr) labelled cells of the different tumor line resulted in a slightly stronger inhibition of K562 lysis by U937 cells than vice versa (Table 1).

Stimulation of NK-CMC with interleukin-2

A moderate dose (100 U/2.5×10^6 lymphocytes) of nhuIL-2, added to effector/target cell mixtures during the NK-CMC test time (16 h) enhanced

TABLE 2

Enhancement of NK-CMC against K562, U937 and PM/86 melanoma target cells by the addition of 100 U/ml of natural human IL-2 in a 16 h chromium release assay

Effector: target	Medium RPMI +10% FCS	Medium 10% FCS +100 U/ml IL-2	Percent increase of NK-CMC
K562			
50:1	64.9 (±15.2)	84.6 (±20.2)	30.2
6:1	15.0 (±4.1)	20.8 (±6.5)	38.2
U937			
50:1	73.1 (±14.8)	88.9 (±8.5)	21.5
6:1	23.8 (±5.4)	44.0 (±15.5)	80.3
PM/86			
50:1	31.4 (±9.5)	39.0 (±9.0)	23.8
6:1	21.1 (±8.7)	31.8 (±3.8)	50.7

Percent specific lysis (±s.d.); $n=7$ blood donors (German Landrace swine).

TABLE 3

Content of IFN-alpha in cell-free supernatants of effector/tumor cell mixtures after 16 h of cocultivation

Blood donors	Effector/target cell supernatant IU/ml interferon-alpha (E:T=50:1)			
	K562	U937	PM/86	Raji
German Landrace swine ($n=14$)	1553 (±606)[1]	340 (±140)	<20	<20
Munich Troll® miniature swine ($n=6$)	640 (±350)	213 (±120)	<20	<20

[1] ±s.d.

the specific lysis of xenogeneic K562- and U937 tumor cells significantly ($P<0.05$). This effect was more pronounced at the low E:T ratio of 6:1 (Table 2). The addition of nhuIL-2 to assays of lymphocyte cytotoxicity against PM/86 melanoma target cells also resulted in higher rates of specific lysis. The NK cell stimulation with nhuIL-2 in the allogeneic system too, was more clearly visible at the low E:T ratio of 6:1 (Table 2). The relative percentage of NK-CMC enhancement is comparable with that obtained in the assays with xenogeneic tumor cells as the targets.

Release of interferon upon contact of lymphocytes with target cells

Many tumor cell lines, including melanoma, are able to induce interferon on contact with human lymphocytes (Trinchieri et al., 1977). After incuba-

tion of porcine nylon non-adherent lymphocytes with K562 myeloma cells high levels of VSV antiviral activity were found in cell-free supernatants (Table 3). Because of its stability at pH 2.0 and its neutralization with an anti-IFN-alpha serum (1 NU of serum neutralized 4 IU porcine IFN) the antiviral activity in the supernatants is characterized as IFN-alpha (Lockart, 1973).

The contact of lymphocytes from Landrace swine with PM/86 melanoma cells did not lead to detectable IFN activity in the supernatants of lymphocyte/target mixtures. IFN titers, higher than 20 IU/ml could not be measured (Table 3). Incubation of Troll® miniature swine blood lymphocytes together with PM/86 melanoma cells did not initiate the release of IFN-alpha titers greater than 20 IU/ml. Neither the cocultivation with Raji lymphoma cells nor the incubation in medium alone stimulated the release of IFN, detectable in the VSV antiviral assay with MDBK cells. For the two inductive tumor cell lines K562 and U937 the kinetics of IFN production were similar (data not shown). IFN detection in supernatants was possible about 4 h after the tumor cells were added to porcine lymphocytes. The IFN titers increased steadily until reaching a plateau between 16 and 24 h of incubation. Thereafter the titers did not change even when incubation was continued up to 72 h.

DISCUSSION

NK cell activity mediated by porcine blood lymphocytes exhibits characteristics distinct from effector cell activity in man and rodents. Not only the morphology and tissue distribution of NK cells but also the kinetics of tumor cell lysis in vitro are typical for this species (Yang and Schultz, 1986; Yang et al., 1987).

The aim of this study was to compare the susceptibility of an allogeneic and four xenogeneic tumor cell lines to NK cell lysis mediated by peripheral blood lymphocytes of Troll® miniature and German Landrace swine. A possible role of self-regulatory cytokines such as IL-2 and IFN had to be watched in this system since a stimulatory effect of exogenous IFN-alpha and IL-2 is well known (Cepica and Derbyshire, 1986; Bhagyam et al., 1988; Lesnick and Derbyshire, 1988). In the human system different melanoma cell lines have been shown to be very heterogeneous concerning their capacity to induce IFN when cocultivated with lymphocytes (Trinchieri et al., 1977).

The generation of IFN-alpha after contact of porcine blood lymphocytes with the melanoma cells failed or yielded non-detectable low titers, whereas highly susceptible xenogeneic target cells regularly induced high titers of IFN. The induction of high IFN titers (> 160 IU/ml) on target/effector cell contact at an E:T of 50:1 seems to be a signal concerning the susceptibility of tumor cells for porcine NK-CMC, however, IFN induction by tumor cells might not be a reliable qualitative parameter since the NK cell mediated lysis

of U937 was more effective than K562 tumor cell lysis even the IFN titers after K562 cell contact were always the highest.

The presence of other cytotoxic cytokines such as perforin, tumor necrosis factor (TNF) or natural killer cell cytotoxic factor (NKCF) may play the crucial role in target cell destruction by swine lymphocytes as described for human NK-CMC (Carpen and Saksela, 1988). Synergistic effects with IFN are likely, because, for example human alpha, beta and gamma IFNs, are modulators of IL-2 production (Rosztoczy et al., 1986). Indirect enhancement of tumor cell lysis via NK cell activation by IL-2 and/or IFN is also well established in the porcine system (Cepica and Derbyshire, 1986; Charley and Fradelizi, 1987; Lesnick and Derbyshire, 1988).

In our experiments we found, that in a 16 h ^{51}Cr release assay a moderate dose of natural human IL-2 is sufficient for the stimulation of NK blood lymphocytes of older (> 1 week) Troll® miniature and German Landrace swine to kill susceptible target cells more efficiently. The addition of natural human IL-2 at a dose of 100 U/ml during the test time resulted in about the same enhancement of NK-CMC regardless of whether xenogeneic or allogeneic target cells were used. These findings indicate that NK-CMC under the influence of IL-2 might compensate for the diminished susceptibility of PM/86 melanoma cells.

As the cold target competition assays revealed, allogeneic PM/86 melanoma cells strongly interact with NK effector cells, but are lysed to a lower degree than the xenogeneic tumor cells. This observation is not restricted to Troll® miniature swine blood donors with melanoma inheritance; it is equally seen with blood lymphocytes from German Landrace swine. It might be caused by an inefficient killing mechanism, possibly because of a lack in directly cytotoxic or stimulatory cytokines as already suggested by Pinto and Fergusson (1988).

Structures mediating the recognition of tumor target cells by NK cells are yet unknown but their function is thought to be distinct from that triggering the cytolytic process (Targan and Newman, 1983; Chervenak and Wolcott, 1988). This has previously been shown in studies on the mechanisms of defective NK cell activity in patients with acquired immunodeficiency syndrome (AIDS) (Bonavida et al., 1986; Sirianni et al., 1988). Low NK cell activity has also been described by Hersey et al. (1979) in familial melanoma patients and their relatives. The assessment of peripheral blood NK cell activity in tumor patients as a diagnostic or prognostic parameter suffers from many imponderabilities (Pross, 1986).

There was no significantly higher NK cell activity observed, mediated by lymphocytes of Troll® miniature swine, neither bearing melanoma congenitally, nor having developed melanoma during the time after birth, when compared with the blood lymphocyte cytotoxic activity of three animals affected by cutaneous melanoma (Figs. 4 and 5). This is contrary to recent findings

by Richerson and Misfeldt (1989) in Sinclair swine, where in vitro NK-CMC is suggested to correlate with stages of tumor progression or regression.

The observation of "normal NK-CMC" mediated by blood lymphocytes of melanoma-bearing Troll® miniature swine older than 4 weeks of age has to be proven by determination of NK-CMC from a higher number of animals where no tumor regression occurred. Nevertheless NK-CMC against the allogeneic melanoma cells again is also significantly ($P<0.01$) reduced in the melanoma diseased animals if lymphocyte mediated lysis of K562 tumor cells is taken as the reference system for the detection of porcine NK cell activity. The role of blood NK cell activity for melanoma regression in the Munich Troll® miniature swine might be of lesser importance compared with the defense mechanisms at the site of tumor growth. Experiments for the characterization and determination of functional properties of tumor infiltrating leukocytes may provide more information about the role of NK cells.

ACKNOWLEDGEMENTS

The authors thank Fr. Andrea Aigner for excellent technical assistance. We also wish to thank Fr. Dr. Kahlau at the Landesamt für das Gesundheitswesen, Südbayern, for regular screening of the tumor cells on mycoplasma contamination.

REFERENCES

Anderson, L., Nilsson, K. and Gohmberg, C., 1979. K562-a human erythroleukemic cell line. Int. J. Cancer, 23: 143–147.

Beattie, C.W., Tissot, R. and Amoss, M., 1988. Experimental models in human melanoma research: a logistic perspective. Sem. Oncol., 15: 500–511.

Berkelhammer, J., Ensing, B.M. and Hook, R.R., 1982. Growth and spontaneous regression of swine melanoma: relationship of in vitro leukocyte reactivity. J. Nat. Cancer Inst., 68: 461–468.

Bhagyam, R.C., Jarrett-Zaczek, D. and Ferguson, F.G., 1988. Activation of swine peripheral blood lymphocytes with human recombinant interleukin-2. Immunology, 64: 607–613.

Bonavida, B., Katz, J. and Gottlieb, M., 1986. Mechanism of defective NK cell activity in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. I. Defective trigger on NK cells for NKCF production by target cells and partial restoration by IL-2. J. Immunology, 137: 1157–1163.

Boyum, A., 1976. Isolation of lymphocytes, granulocytes and macrophages. Scand. J. Immunol., 5, Suppl. 5: 9–15.

Bröcker, E.B., Zwaldo, G., Holzmann, B., Macher, E. and Sorg, C., 1988. Inflammatory cell infiltrates in human melanoma at different stages of tumor progression. Int. J. Cancer, 41: 562–567.

Carpen, O. and Saksela, E., 1988. Directed exocytosis in the NK-cell-mediated cytotoxicity. Nat. Immun. Cell Growth Regulation, 7: 1–12.

Cepica, A. and Derbyshire, J.B., 1986. The tissue distribution of spontaneous cell-mediated cytotoxicity effector lymphocytes in swine. J. Comp. Pathol., 96: 353–355.

Cepica, A. and Derbyshire, J.B., 1986. The role of interferon in spontaneous cell-mediated cytotoxicity in pigs. Vet. Microbiol., 11: 69–77.

Charley, B. and Fradelizi, D., 1987. Differential effects of human interleukin 2 on natural killing (NK) activity of newborn piglets and adult pigs lymphocytes. Ann. Rech. Vet., 18: 227–232.

Charley, B., Petit, E., Laude, H. and La Bonnardiere, C., 1983. Myxovirus and Coronavirus induced in vitro stimulation of spontaneous cell-mediated cytotoxicity by porcine leukocytes. Ann. Virol., 134E: 119–126.

Chervenak, R. and Wolcott, R.M., 1988. Target cell expression of MHC antigens is not (always) a turn-off signal to natural killer cells. J. Immunol., 140: 3712–3716.

Hersey, P., Edwards, A., Honeyman, M. and McCarthy, W.H., 1979. Low natural killer cell activity in familial melanoma patients and their relatives. Br. J. Cancer, 40: 113–122.

Julius, M.H., Simpson, E. and Herzenberg, L.A., 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. Eur. J. Immunol., 3: 645–647.

Koren, H.S., Amos, D.B. and Kim, Y.B., 1978. Natural killing and antibody-dependent cellular cytotoxicity are independent immune functions in the Minnesota miniature swine. Proc. Natl. Acad. Sci. U.S.A., 75: 5127–5132.

Krakowka, S., Cummings, J.M. and Ringler, S.S., 1988. The effects of human interferon-alpha upon in vitro canine immune responses. Vet. Immunol. Immunopathol., 19: 185–196.

Kumagai, K., Itho, K., Hinuma, S. and Tada, M., 1979. Pretreatment of plastic petri dishes with fetal calf serum. A simple method for macrophage isolation. J. Immunol. Methods, 29: 17–21.

Lesnick, C.E. and Derbyshire, J.B., 1988. Activation of natural killer cells in newborn piglets by interferon induction. Vet. Immunol. Immunopathol., 18: 109–117.

Lockart, R.Z., 1973. Criteria for acceptance of a viral inhibitor as an interferon and a general description of the biological properties of known interferons. In: N.B. Finter (Editor), Interferon and Interferon Inducers. Elsevier, Amsterdam, p. 11.

Martin, S. and Wardley, R.C., 1984. Natural cytotoxicity detected in swine using Aujeszky's disease virus infected targets. Res. Vet. Sci., 37: 211–218.

Ohlinger, V. and Saalmüller, A., 1989. Sorted porcine leukocyte populations expressing the 74.22.15- and 8/1-antigen mediate SLA-unrestricted spontaneous cellular cytotoxicity. Immunobiology, Suppl. 4: 61.

Oxenhandler, R.W., Adelstein, E.H., Haigh, J.P., Hook, R.R. and Clark, W.H., 1979. Malignant melanoma in the Sinclair miniature swine. Am. J. Pathol., 96: 707–720.

Pescovitz, M.D., Lowman, M.A. and Sachs, D.H., 1988. Expression of T-cell associated antigens by porcine natural killer cells. Immunology, 65: 267–271.

Pinto, A. and Fergusson, F., 1988. Characteristics of Yorkshire swine Natural Killer cells. Vet. Immunol. Immunopathol., 20: 15–29.

Pross, H.F., 1986. Natural killer cell activity in human malignant disease. The prognostic value of repetitive natural killer testing. In: E. Lotzova and R.B. Herberman (Editors), Natural Immunity, Cancer and Biological Response Modification. Karger, Basel, pp. 196–205.

Richerson, J.T. and Misfeldt, M.L., 1989. Host environment as a modulating factor of swine natural killer cell activity. Vet. Immunol. Immunopathol., 23: 309–319.

Rosztoczy, I., Siroki, O. and Beladi, I., 1986. Effects of interferons-α, -β, and -γ on human interleukin-2 production. J. Interferon Res., 6: 581–589.

Rubinstein, S., Familletti, P.C. and Festka, S., 1981. Convenient assay for interferons. J. Virol., 37: 755–758.

Saalmüller, A., Reddehase, M.J., Buehring, H.-J., Jonjic, S. and Koszinowski, U.H., 1988. Simultaneous expression of CD4 and CD8 antigens by a substantial proportion of resting porcine T lymphocytes. Eur. J. Immunol., 17: 1297–1301.

Salmon, H., Charley, B., Labonnardiere, C., Olivier, M., Kelley, K. and Paraf, A., 1989. Natural Killer (NK) activity and interferon (IFN) production by a fraction of spleen and blood lymphocytes in swine. Vet. Immunol. Immunopathol., 23: 113–128.

Sambraus, H.H., 1987. Atlas der Nutztierrassen. Ulmer Verlag, Stuttgart, 2nd edn., 248 pp.

Shau, H. and Golup, S.H., 1985. Depletion of NK cells with the lysosomotropic agent L-Leucine methyl ester and the in vitro generation of NK activity from NK precursor cells. J. Immunol., 134: 1136–1141.

Sirianni, M.C., Soddus, S., Malorni, W., Arancia, G. and Aiuti, F., 1988. Mechanism of defective Natural Killer cell activity in patients with AIDS is associated with defective distribution of tubulin. J. Immunol., 140: 2565–2568.

Sundström, C. and Nilsson, K., 1976. Establishment and characterization of a human histiocytic lymphoma cell line (U-937). Int. J. Cancer, 17: 565–577.

Takamatzu, H. and Koinde, F., 1985. Augmentation of porcine spontaneous cell-mediated cytotoxicity in vitro. Jpn. J. Vet. Sci., 47: 749–759.

Targan, S.R. and Newman, W., 1983. Definition of a "trigger" stage in the NK cytolytic reaction sequence by a monoclonal antibody to the glycoprotein T-200. J. Immunol., 131: 1149–1153.

Trinchieri, G., Santoli, D. and Knowles, B.B., 1977. Tumor cell lines induce interferon in human lymphocytes. Nature, 17: 611–613.

Wanke, R. and Bräuer, H., 1986. Spontane Melanome in einer Sonderlinie des Münchener Miniatureschweines Troll. Ber. Muench. Tieraertzl. Wochenschr., 99: 69.

Wittmann, G. and Ohlinger, V., 1987. Aujeszky's disease vaccination and infection of pigs with maternal immunity: effects on cell- and antibody-mediated immunity. Arch. Virol., 92: 87–101.

Yang, W.C. and Schultz, R.D., 1986. Ontogeny of Natural Killer cell activity and antibody dependent cell mediated cytotoxicity in pigs. Dev. Comp. Immunol., 10: 405–418.

Yang, W.C., Schultz, R.D. and Spano, J.S., 1987. Isolation and characterization of porcine Natural Killer (NK) cells. Vet. Immunol. Immunopathol., 14: 345–356.