NATURAL KILLER CELL ACTIVITIES OF SEVERAL INBRED STRAINS OF THE GUINEA PIG AND THEIR SUSCEPTIBILITIES TO RABBIT ANTISERUM DIRECTED TO GUINEA-PIG BRAIN TISSUE

TETSURO KATAOKA, TSUNEO KAMIYAMA*, TAKAO OHNUKI,
MIKIO MIYAMOTO and TOHRU TOKUNAGA

*Department of Tuberculosis and *Department of Veterinary Science,
National Institute of Health, Kamiosaki, Shinagawa-ku, Tokyo 141*

(Received November 16, 1981. Accepted February 26, 1982)

SUMMARY: Natural killer (NK) cell activities of spleen and peripheral blood leukocytes from several inbred guinea-pig strains, established in this Institute, were assessed in an 18-hr ^{51}Cr -release assay. As target cells in the NK assay, a human myeloid cell line, K562, appeared to be the most suitable among several cultured cell lines tested. Intensities of cytolysis were different from strain to strain, but generally high in JY-2, JY-4 and JY-10 and low in JY-1. Treatment of the effector cells with rabbit anti-guinea-pig brain tissue serum plus complement reduced the NK activity, whereas treatment with rabbit anti-guinea-pig thymocyte serum, or rabbit anti-asialo GM1 serum, plus complement showed no significant effect on the NK activity.

INTRODUCTION

Natural killer (NK) cells were first demonstrated in man (Takasugi, Mickey and Terasaki, 1973) and in mice (Kiessling, Klein and Wigzell, 1975; Sendo et al., 1975), and their significant role in host resistance to tumors or to infections has been widely recognized. The NK activity was demonstrated in most animal species, but detailed studies have been concentrated to the mouse and human; only few reports have dealt with guinea-pig NK cells (Altman and Rapp, 1978; Arnaud-Battandier, Bundy and Nelson, 1978; Ermein et al., 1980). It has been known that the NK activity is different depending upon the mouse strain (Blair, 1980) and that NK cells are susceptible to rabbit antiserum to either mouse brain-associated T cell antigen (Habu et al., 1979) or a glycosphingolipid, asialo GM1 (Kasai et al., 1980). We attempted to find out whether or not these findings are true also in guinea pigs; we compared the NK activities of several inbred guinea-pig strains (JY series) established in this Institute (cited in: Chiba, Otokawa and Egashira, 1978; Kataoka and Tokunaga, 1979; Asano, Minobe and Nakagawa, 1981), and tested the guinea pig NK cells for suscep-

片岡哲朗・大拔隆雄・宮本幹夫・徳永 徹 (国立予防衛生研究所 結核部)
神山恒夫 (国立予防衛生研究所 獣疫部)

tibilities to antiserum against guinea-pig brain tissues and that against asialo GM1.

MATERIALS AND METHODS

Animals: Inbred guinea pigs of strains JY-1, JY-2, JY-4, JY-7, JY-10 and (JY-4×JY-10) F1 were obtained from the Department of Veterinary Science, National Institute of Health, Tokyo. These inbred strains were established by Drs. H. Yoda, T. Nakano, and M. Nakagawa in that Department from random-bred guinea pigs obtained in 1949 from the Rockland Farm, U.S.A. Inbred strain-2 and strain-13 guinea pigs and outbred Hartley guinea pigs were purchased from Nippon Institute for Biological Science (Ohme, Tokyo), and Funabashi Farms Co. (Funabashi, Chiba), respectively. Both male and female animals were bred conventionally and used at the age of around 10 weeks. Rabbits (Japanese white) weighing about 3 kg were purchased from Nihon Ikagakuzairyo Co. (Tokyo).

Effector cells: Spleens were removed aseptically into cold Hanks' buffered salt solution (HBSS) and teased apart on a stainless steel mesh (#200) by gentle pressing with a spatula. Packed spleen cells were suspended in sterile distilled water for 10 sec and then added was an equal volume of double-strength HBSS to lyse erythrocytes. Heparinized blood, drawn from the heart, was layered over a 0.33 volume of a Ficoll-Hypaque solution (LSM reagent; Litton Bionetics, Kensington, MD, U.S.A.) in a 5-ml glass tube and centrifuged at $400 \times g$ for 30 min at 20 C. The cells at the interface were collected and diluted with HBSS. All these cells were washed three times with HBSS.

Target cells: The cultured cell lines used were: guinea-pig macrophage-like cell line K3 (Kato *et al.*, 1981) and simian virus 40-transformed cells of strain 13 (STC13) or strain JY-1 (SV-JY 1) guinea-pig kidney cells gifted by Dr. K. Kato, Department of Enteroviruses of this Institute; mouse-derived YAC-1 (Sjögren and Hellström, 1965) and RL δ 1 (Sendo *et al.*, 1975); and human-originated K562 (Lozzio and Lozzio, 1973). All cells were maintained in RPMI 1640 medium (Nissui Seiyaku, Co., Tokyo) supplemented with 10% fetal calf serum (FCS; Grand Island Biological Co., Grand Island, NY, U.S.A.), 100 units/ml penicillin and 100 μ g/ml streptomycin.

Preparation of antisera: As immunogens, thymocyte and brain homogenates of Hartley guinea pigs were suspended in HBSS and emulsified with Freund's complete adjuvant containing 0.5 mg/ml tubercle bacilli. Rabbits were immunized intradermally with the emulsion containing 2×10^8 thymocytes or 1 g of brain tissue. The animals were boosted with two iv injections with 1×10^8 thymocytes suspended in 3 ml HBSS at a 2-week interval or two sc injections with 1 g brain homogenate on days 7 and 21. Two to three weeks after the last injection, the animals were sacrificed. Sera were inactivated by heating at 56 C for 45 min.

The serum was mixed with an equal volume of packed guinea-pig erythro-

cytes, incubated at 4 C for 60 min, and then separated from the erythrocytes by centrifugation. This procedure was repeated four times. Then the serum was absorbed with an equal volume of packed EN-L₂C cells (B cell leukemia of a strain 2 guinea pig kindly gifted by Dr. E. M. Shevach, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, U.S.A.) five times in similar manner. The absorbed sera were millipored and stored at -20 C.

Rabbit anti-asialo GM1 serum was prepared and given by Dr. M. Kasai, Department of Tuberculosis of this Institute, as ammonium sulfate precipitate of whole antiserum of the rabbits injected repeatedly with purified asialo GM1 in Freund's complete adjuvant (Kasai et al., 1980).

These preparations were tested for the complement-dependent cytolytic activities against spleen cells, lymphnode cells, thymocytes, and bone marrow cells of a Hartley guinea pig and EN-L₂C cells by the ⁵¹Cr-release assay described by Shevach, Rosenstreich and Green (1973).

Treatment of effector cells with antisera: A suspension of 2×10⁶ spleen cells/ml was mixed with an equal volume of a diluted antiserum and incubated at room temperature for 45 min. The cells were washed once, resuspended in the original volume of fresh guinea-pig serum (1:2 dilution) and then incubated 37 C for 30 min. The mixture was centrifuged and the cells were washed three times with RPMI 1640 medium and resuspended in the original volume of the medium.

Microcytotoxicity assay: Five to 10-million target cells were labeled with 100 μCi/ml of sodium ⁵¹Cr (51 mCi/mg, Japan Radioisotope Association, Tokyo) for 60 min. The target (1×10⁴ unless otherwise noted) and the effector cells were mixed in a well of a round bottomed microplate (A/S Nunc, Roskilde, Denmark) to a final volume of 0.2 ml. The plate was spun at 200 ×g for 2 min and incubated in 5% CO₂ in a humidified incubator for 4 and 18 hr at 37 C. The supernatants were harvested with the Titer-teck supernatant collection systems (Flow Laboratories, Rockville, MD, U.S.A.) and counted in a gamma counter (Packard Instruments Co., Inc., Downers Grove, IL, U.S.A.). The count was made with triplicate samples. The % cytotoxicity was calculated as follows:

$$\% \text{ Cytotoxicity} = \frac{\text{Experimental count (cpm)} - \text{Minimum count (cpm)}}{\text{Maximum count (cpm)} - \text{Minimum count (cpm)}} \times 100$$

Maximum and minimum counts were made by incubation of the target cells alone in 0.1% sodium dodesyl sulfate (SDS) and in test medium, respectively.

Statistical analysis: Student's *t* assay was employed to analyze the results.

RESULTS

First, we searched optimal conditions for assaying the guinea-pig NK activity using spleen leukocytes from Hartley guinea pigs as effector cells and cells of lines K562, RL δ 1 and YAC-1 as target cells. The target cells were known to

TABLE I

Natural cytotoxicity of Hartley guinea-pig spleen cells against different target cells

Exp. No.	Incubation period (hr)	Target cell	Cytotoxicity (%)		
			E/T=25	50	100
1	4	K562	0.8	2.4	7.6
		YAC-1	1.7	1.1	3.5
		RL δ 1	2.4	4.2	5.9
	18	K562	10.3	13.7	22.9
		YAC-1	-2.4	-0.4	6.1
		RL δ 1	-2.5	-0.4	6.8
2	18	K562	7.8	12.7	21.1
		K3	7.9	6.7	10.6
		STC13	NT*	NT	-6.0
		SV-JY1	NT	NT	1.2

* NT: Not tested.

be susceptible to human and mouse NK cells. The effector and ^{51}Cr -labeled target cells were incubated for 4 or 18 hr at effector to target (E/T) ratios of 25, 50, and 100 (Exp. 1 of Table I). It was indicated that significant lysis occurs only in the system using K562 as the target and an incubation period of 18 hr. RL δ 1 and YAC-1 cells showed much lower sensitivity to guinea-pig NK cells.

Next, we surveyed guinea-pig-originated target cells for availability for guinea-pig NK assay. Cultured cell lines of K3, STC13 and SV-JY 1 were tested for their susceptibilities to NK cytotoxicity with spleen leukocytes from Hartley, strain 2, strain 13 and strain JY-1 guinea pigs. None of these target cells was lysed significantly by 18-hr incubation with the effectors even at an E/T ratio of 100 (Exp. 2 of Table I). Susceptibilities of the guinea-pig target cells were lower than that of K562 cells to guinea-pig NK cells. Therefore, we used K562 in 18 hr ^{51}Cr -release assay for further experiments.

The spleen and peripheral blood leukocytes obtained from several inbred guinea-pig strains of JY series were tested for the NK activities (Table II). It seems that there is strain difference in the NK activity, though the intensities of cytotoxicity varied among the individuals of the same strain. The strains with high NK activities were JY-2, JY-4 and JY-10, and that with a low activity was JY-1. Strain 2 guinea pigs seemed to have an intermediate activity. The cytotoxicities were comparable between spleen and peripheral blood effector cells in all strains except JY-4.

Splenic effector cells from Hartley strain guinea pigs were treated with each antiserum and complement before the NK assay. The pretreatment of the effector cells with rabbit anti-guinea-pig thymocytes serum and complement did not change the NK activity (Table III). On the contrary, the pretreatment

Fig. 1. Specificities of rabbit anti-guinea-pig thymocytes, anti-guinea-pig brain tissue, and anti-asialo GM1 sera.

A 100- μ l portion of each of 1×10^6 /ml ^{51}Cr -labeled guinea-pig thymocytes (○), lymph-node cells (△), spleen cells (●), bone marrow cells (▲) and EN-L₂C leukemia cells (□), was mixed with 100 μ l of an indicated dilution of antiserum and fresh guinea-pig serum (1:2 dilution) in a well of 96-well round bottomed microplates. The mixtures were incubated in a CO₂ incubator at 37 C. After 45 min, the plates were centrifuged at 500 $\times$ g for 10 min and a 100- μ l aliquot was transferred from each well into a plastic tube, which was counted in an autogramma spectrophotometer (Packard Instruments Inc.). The percentage cytotoxicity was calculated by the following equation:

$$\frac{\text{cpm in wells with antiserum} - \text{cpm in wells with medium}}{\text{cpm in wells with SDS} - \text{cpm in wells with medium}} \times 100$$

Each point expresses the mathematical mean of triplicate wells.

with rabbit anti-guinea-pig brain tissue serum plus complement reduced the NK activity remarkably. The pretreatment with anti-asialo GM1 plus complement did not significantly reduce the NK activity.

DISCUSSION

In the present study, we first tested various target cell lines, including human, murine and guinea-pig tumor cells, for their susceptibilities to the splenic effector cells. Among them, only human myeloid cell K562 showed a relatively high and constant susceptibility to the NK cells, as reported by Altman and Rapp (1978). Other two groups of workers employed other human-derived tumor cell lines as the target (Arnaud-Battandier et al., 1978; Ermein et al., 1980). In all of these reports, an incubation period for longer than 18 hr

TABLE II

NK activities of several inbred strains of guinea pig

Exp. No.	Strain	Animal No.	Cytotoxicity (%)					
			Spleen cells			Peripheral blood leukocytes		
			E/T=25	50	100	E/T=25	50	100
1.	JY 1	1	1.8	1.4	3.1	2.9	4.7	5.1
		2	5.7	4.4	NT	5.5	5.0	NT
		3	5.3	6.4	NT	6.3	8.0	8.8
		4	-0.8	-0.2	NT	6.0	7.1	8.1
	JY 2	1	12.5	20.1	31.7	37.6	38.2	NT
		2	19.5	34.6	52.2	12.7	20.2	27.9
		3	18.7	33.6	50.0	23.4	27.4	NT
	JY 4	1	20.4	31.6	25.5	1.4	0.8	6.4
		2	16.5	18.4	26.6	4.6	6.2	2.7
		3	13.5	25.6	42.6	NT	NT	NT
	JY 7	1	3.4	1.6	-1.3	-4.7	-5.2	-7.7
		2	12.3	19.0	30.6	7.1	17.0	17.4
		3	-0.8	-1.3	1.1	0.8	6.6	0.3
	JY 10	1	7.5	14.5	26.0	7.1	17.7	17.6
		2	8.7	9.9	19.6	11.9	8.0	12.9
		3	4.7	10.0	NT	7.5	7.5	NT
		4	11.8	20.4	NT	15.1	18.1	NT
	(JY 4×JY 10)	1	15.4	19.4	33.9	3.0	8.1	13.9
		2	15.2	11.0	30.5	6.0	10.1	12.4
	Strain 2	1	12.9	16.0	18.7	10.1	7.0	NT
		2	10.3	13.0	16.7	9.0	11.1	15.8
		3	3.1	7.0	7.5	16.5	18.7	28.0
2.	JY 1	Spleen cells						
		E/T=20 40 80 160 320						
		5	3.2	2.7	7.9	4.0	2.8	
		6	3.8	3.6	4.7	5.1	3.5	
		7	3.4	2.9	5.0	4.3	3.0	

K562 cells were used as the target cells.

NT: Not tested.

was required to detect significant NK activity. The cytotoxic mechanism of guinea-pig NK cells may be different from those of human and mouse NK cells, because 4-hr incubation is enough to detect significantly high NK activities of those species.

Possible contribution of macrophages to the cytotoxicity detected in the system can be excluded by the following facts: when ^{51}Cr -labeled K562 cells were incubated with mineral oil-induced peritoneal adherent cells at E/T 20

TABLE III

Effects of treatment of effector cells with anti-thymocytes, anti-brain or anti-asialo GM1 plus complement on guinea-pig NK activity

Experiment No.	Treatment	Serum dilution	Cytotoxicity (%)	Reduction in NK activity (%)	t-assay*
1	C' alone		28.3		
	Anti-thymocytes	1:20	26.8	5.2	NS
	+C'	1:40	25.7	9.2	NS
	Anti-brain	1:20	14.6	48.3	<0.01
	+C'	1:40	22.2	21.6	<0.01
2	C' alone		34.9		
	Anti-brain	1:20	26.8	23.2	<0.05
	+C'	1:40	27.9	20.1	NS
	Anti-asialo GM1	1:20	38.1	-9.2	NS
	+C'	1:40	41.0	-17.4	NS

Spleen cells of a Hartley guinea pig were treated with or without an antiserum plus complement, then mixed with ^{51}Cr -labeled K 562 cells at an E/T ratio of 100.

Cytotoxicity obtained by untreated spleen cells were 29.1% (exp. 1) and 36.7% (exp. 1).

* P-value, NS: Not significant.

or 40 for 18 hr, ^{51}Cr -release into the culture supernatant was not greater than that with K562 cells alone. This was consistent with the reports of Altman and Rapp (1978) and Arnaud-Battandier et al. (1978).

Some fluctuations in individuals of the same strain were observed, especially in JY-7. Since the animals used in this study were bred under conventional conditions, the influence of environmental factor(s) on the NK activity must be considered. The animals were kept carefully in the same breeding room and were sacrificed at almost the same age after birth, however, a tendency different cytolytic activities among the strains, especially a low activity in strain JY-1, was always observed in repeated experiments. Altman and Rapp (1978) also noticed that the strain 2 guinea pig had NK activity significantly higher than strain 13. In the mouse (Blair, 1980) and rat (Shellam and Hogg, 1977) systems, the presence of such a strain with a high or low NK activity has also been indicated.

The treatment with anti-brain serum plus complement considerably reduced the NK activity, whereas that with anti-thymocyte and complement did not, coinciding with the results with the mouse NK system (Habu et al., 1979). However, it must be considered that anti-thymocyte and anti-brain-tissue sera used in our experiments are not specific enough, differing from anti-Thy 1 and anti-brain-associated θ antigen in the mouse system. The treatment with rabbit anti-asialo GM1 serum plus complement did not affect the guinea-pig NK activity, while this antiserum eliminated the mouse NK activity (Kasai et al., 1980). It was found by enzyme-linked immunosorbent assay that the anti-brain

serum used here contained large amounts of both anti-asialo GM1 and anti-GM1 antibodies and moderate amounts of both anti-guinea-pig brain lipid and anti-Forsman antigen antibodies (T. Momoi, personal communication). Therefore, it seems that the guinea-pig NK cells are not sensitive to anti-asialo GM1 serum, indicating that glycolipids on the NK-cell surface are different between guinea pigs and mice.

ACKNOWLEDGEMENTS

The authors thank Drs. M. Nakagawa and T. Asano, Department of Veterinary Science of this Institute, for supplying inbred guinea pigs and Miss M. Takegawa and Mrs. N. Katsu for their technical assistance. This work was partly supported by Research Grants from the Ministry of Health and Welfare, and Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

- ALTMAN, A. AND RAPP, H. J. (1978): Natural cell-mediated cytotoxicity in guinea pigs: Properties and specificity of natural killer cells. *J. Immunol.*, **121**, 2244-2252.
- ARNAUD-BATTANDIER, F., BUNDY, B. M. AND NELSON, D. L. (1978): Natural killer cells in guinea pig spleen bear Fc receptors. *Europe. J. Immunol.*, **8**, 400-406.
- ASANO, T., MINOBE, Y. AND NAKAGAWA, M. (1981): Polymorphism of serum protein in guinea pigs of inbred strains. *Exptl. Animal*, **30**, 41-46.
- BLAIR, P. B. (1980): Immunogenetics of natural immunity. p. 401-415. *In* R. B. HERBERMAN [ed.], *Natural Cell-Mediated Immunity Against Tumors*. Academic Press, New York.
- CHIBA, J., OTOKAWA, M. AND EGASHIRA, Y. (1978): Serological studies on the major histocompatibility complex of new inbred strains of guinea pig. *Microbiol. Immunol.*, **22**, 545-555.
- ERMEIN, O., COOMBS, R. R. A., ASHBY, J. AND PLUMB, D. (1980): Natural cytotoxicity in the guinea pig: The natural killer (NK) cell activity of the Kurloff cell. *Immunology*, **41**, 367-378.
- HABU, S., HAYAKAWA, K., OKUMURA, K. AND TADA, T. (1979): Surface markers on natural killer cells of the mouse. *Europe. J. Immunol.*, **9**, 938-942.
- KASAI, M., IWAMORI, M., NAGAI, Y., OKUMURA, K. AND TADA, T. (1980): A glycolipid on the surface of mouse natural killer cells. *Europe. J. Immunol.*, **10**, 175-180.
- KATAOKA, T. AND TOKUNAGA, T. (1979): Restriction by the major histocompatibility complex of antigen-induced T lymphocyte proliferation in new inbred guinea pig strains. *Microbiol. Immunol.*, **23**, 197-205.
- KATO, K., KATAOKA, T., OHNUKI, T. AND TOKUNAGA, T. (1981): Establishment of macrophage-like cell lines of the guinea pig. *Microbiol. Immunol.*, **23**, 197-205.
- KIESSLING, R., KLEIN, E. AND WIGZELL, H. (1975): "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. *Europe. J. Immunol.*, **5**, 112-117.
- LOZZIO, C. B. AND LOZZIO, B. B. (1973): Cytotoxicity of a factor isolated from human spleen. *J. Nat. Cancer Inst.*, **50**, 535-538.
- SENDO, F., AOKI, T., BOYSE, E. A. AND BUOFO, C. K. (1975): Natural occurrence of lymphocytes showing cytotoxic activity to BALB/c radiation-induced leukemia RL σ 1 cells. *J. Nat. Cancer Inst.*, **55**, 603-609.
- SHELLAM, G. R. AND HOGG, N. (1977): Gross virus-induced lymphoma in the rat. IV. Cytotoxic cells in normal rats. *Int. J. Cancer*, **19**, 212-224.
- SHEVACH, E. M., ROSENSTREICH, D. L. AND GREEN, I. (1973): The distribution of histocompatibility antigens on T and B cells in the guinea pig. *Transplantation*, **16**, 126-133.
- SJÖGREN, H. O. AND HELLSTRÖM, I. (1965): Induction of polyoma specific transplantation antigenicity in Moloney leukemia cells. *Exptl. Cell Res.*, **40**, 208-212.
- TAKASUGI, M., MICKEY, M. R. AND TERASAKI, P. I. (1973): Reactivity of lymphocytes from normal persons or cultured tumor cells. *Cancer Res.*, **33**, 2898-2902.