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Natural Killer (NK) Activity and Interferon (IFN) Production by a Fraction of Spleen and Blood Lymphocytes in Swine

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ABSTRACT

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After carbonyl iron treatment and gradient isolation, spleen and blood pig lymphocytes exhibited NK activity and produced IFN after viral induction. Removal of plastic-adherent cells, including the majority of B cells, did not change these activities. The plastic-non-adherent cells were further separated into two subsets of roughly similar size by panning using a monoclonal, anti-T, and anti-null cell antibodies (81+cells). NK activity and IFN production were found in the 81− cell fraction. A significantly higher proportion of null lymphocytes from blood and of splenic Fc-gamma receptor-bearing lymphocytes was also found among the 81− cell fraction as compared to the 81+ fraction, without any change among other subsets. Similar proportions of helper (PT4+), cytotoxic (PT8+) and total T cells (MSA4+) were found among lymphocytes bound to target K562 cells and among the whole lymphocyte population. In contrast, lymphocytes that bound K562 cells demonstrated a striking increase in the proportion of Fc-gamma receptor-positive cells of high affinity. These results show that NK cells and IFN-producing cells are mainly included in the same blood and spleen fraction, and suggest that among 81− cells only those expressing an Fc-gamma receptor of high affinity are active.

INTRODUCTION

NK activity may well be an essential part of early defense mechanism against infections (Welsh, 1978). This possibility was recently supported in swine by the demonstration of a delayed onset of clinical disorders caused by a coronavirus in piglets which received adoptively transferred blood lymphocytes ex-

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hibiting in vitro NK activity (Cepica and Derbyshire, 1984). However, NK activity against the tumor cell line K562 was not detected in either whole cell or in plastic-adherent depleted spleen cells of germ-free or specific-pathogen-free pigs (Kim et al., 1980). In contrast, Takamatsu and Koide (1985) described NK activity in conventional pig spleen from either whole cells isolated on a Ficoll/Triosil gradient or from populations depleted of plastic-adherent cells, with a higher activity among spleen than among blood cells. More recently, Yang and Schultz (1986) did not find NK activity in conventional pig spleens.

These discrepancies may be related to variations in the regulatory processes of NK activity. Thus, in pigs as in other species (review by Welsh, 1984) exogenous alpha-IFN increases NK activity (Chung et al., 1982; Charley et al., 1983; Lesnick and Derbyshire, 1988) and, in humans, a positive feedback exists in one (Timonen et al., 1980; Djeu et al., 1982) or two lymphocyte subsets (Minato et al., 1980). In mice, all types of spleen cells are capable of secreting IFN after influenza induction (Ito et al., 1978). We showed previously that a monoclonal antibody 81B reacted with a specific surface antigen expressed on a fraction of T and null lymphocytes (Kelley et al., 1984, 1988), the distribution of which was similar to that of a suppressor PT8+ cell (Pescovitz et al., 1984). In this report, a specific panning technique (Mage et al., 1977) was used to show that 81− lymphocytes from both blood and spleen include the lymphocyte population exhibiting NK activity and viral-induced IFN production. Furthermore, this subset is enriched into lymphocytes bearing a receptor of high avidity for IgG.

MATERIALS AND METHODS

Animals

Five Large White×Landrace crossbred pigs, 6 to 24 months of age, were used. Pigs were raised conventionally at Thiverval-Grignon. Blood was collected from the cranial vena cava after anesthesia (Stresnil-Hypnodil, Lathevet). Additionally, spleens were collected from four, 6-months-old pigs immediately after exsanguination at the abattoir.

Antisera and plate preparation

A rabbit affinity-purified anti-mouse IgG, prepared in our laboratory, was used that reacted only with the IgG class. Antibody-coated Petri dishes were prepared by adding 5 ml of cold saline to each Petri dish for tissue culture (Corning Cat. No. 25020) for 1 h followed by cold phosphate-buffered saline (PBS) containing 100 μg of rabbit affinity-purified anti-mouse IgG plus 100

μg of purified normal rabbit gamma globulins for an additional 1 h at room temperature. This incubation was followed by washes with cold PBS, and plates, containing 5 ml of saline, were stored at 4°C. A murine monoclonal antibody of IgG2 isotype, 81B, was prepared against porcine thymocytes, and was used as a supernatant from producing clones (Kelley et al., 1984).

Other monoclonal antibodies were PT4 and PT8 of helper and suppressor phenotypes, respectively (Pescovitz et al., 1984) and MSA4 directed against the sheep red blood cell receptor of T cells (Hammerberg and Schurig, 1986). These reagents were also used as hybridoma tissue culture supernatants.

Cell separation procedures

Lymphocyte purification. Spleen cells were liberated in Earle medium (MEM) with 10% fetal bovine serum (FBS), filtered through gauze, centrifuged and adjusted at 1.2×10^6 cells/ml. Carbonyl iron (FeC) treatment consisted of 25 ml of cell suspension mixed with 4 ml of gum arabic and FeC (Salmon, 1979) for 1 h at 37°C and non-phagocytic cells were then recovered on a Ficoll/Triosil gradient. Blood lymphocytes were isolated on a Ficoll/Triosil gradient from 150 ml of glass bead defibrinated blood either before or after a FeC step in order to remove phagocytic cells (Salmon, 1979). Fifty percent of the original cells were recovered for both spleen and blood.

Removal of plastic-adherent cells. Five ml of a lymphocyte suspension (10^7 cells in MEM with 10% FBS) were poured onto Petri dishes and incubated for 1 h at room temperature. Non-adherent cells were removed by gentle agitation followed by ten washings with MEM; plastic-adherent lymphocytes were then removed by gentle flushing with 10% FBS-199 medium from a 20-ml syringe.

Separation of plastic-non-adherent lymphocytes into 81+ and 81− fractions. Plastic-non-adherent lymphocytes were pelleted by centrifugation and then resuspended with 81B monoclonal antibody at a ratio of 100 μl of tissue culture supernatant for 2×10^8 cells for 45 min at 4°C. After three washes, 5×10^7 cells were poured into each antimouse Ig-coated dish for 30 min at 4°C. At the end of incubation, non-adherent (negative cells) and adherent cells (positive cells) were harvested as above. Efficiency of the panning technique was tested by incubation of each fraction with a fluorescent swine anti-mouse immunoglobulin (Nordic). Ninety to 98% of lymphocytes in the 81+ cell fraction were labelled and only 3% in the 81− cell fraction. In a control experiment, reincubation of each cell fraction with 81B antibody did not modify the proportion of labelled cells.

Morphological identification of cells

Mononuclear phagocytes, granulocytes and cytoplasmic granules in lymphocytes were identified on the basis of their morphology and staining properties with May Grunwald Giemsa, and alpha-naphthyl acetate esterase on cytocentrifuged cell suspensions (Salmon, 1983; Salmon et al., 1987).

Natural killer assay

Chromium labelling of target cells was performed according to Kim et al. (1980). In brief, K562 human leukemia cells were grown in suspension, aliquoted and frozen. For each experiment, an aliquot was thawed; 2×10^6 cells were resuspended and incubated in 0.2 ml RPMI medium containing 40 μCi $Na_2{}^{51}CrO_4$ (Amersham, U.K.) for 1 h at 37°C. The cells were then washed extensively with RPMI supplemented with 10% FBS and kept 30 min at room temperature before the final wash. Labelled K562 cells were adjusted to a final concentration of 10^5 cells/ml in RPMI with 10% FBS.

The NK assay was performed as previously described (Charley et al., 1983). Porcine cells were preincubated overnight at 37°C and then assayed at three different effector to target cell ratios (E:T), 25, 50 and 100:1, each in triplicate, in a 4-h incubation test.

The percentage cytotoxicity was calculated as follows:

$$\%\ \text{cytotoxicity} = \frac{\text{mean cpm experimental} - \text{mean cpm SR}}{\text{mean cpm MR} - \text{mean cpm SR}} \times 100$$

where SR (spontaneous release) is defined as the radioactivity released from target cells incubated in medium alone (RPMI with 10% FBS) and MR (maximal release) as cpm in the supernatants of target cells lysed with Triton X100.

Interferon induction and assay

Each cell preparation was incubated overnight at 4°C, then adjusted at 10^7 cells/ml in RPMI medium and primed for 3 h at 37°C by human alpha interferon (100 I.U./ml from Institut Pasteur Production, Garches, France). The cell suspension was then infected with 200 hemagglutinating units of human influenza virus (A/Philippines, H3-N2) and the incubation proceeded for 18 h at 37°C. Thereafter, the total cell suspension was frozen and thawed once in order to lyse the cells, clarified by low speed centrifugation and finally acidified to pH 2.5 using 1 *N* HCl. On three occasions two different viruses of porcine origin were tested; transmissible gastroenteritis virus, Purdue 115-strain (coronavirus) and Aujesky's disease (herpes virus), both used at a multiplicity of infection of one plaque-forming unit per cell.

IFN titers were determined on bovine MDBK cells challenged with vesicular

stomatitis virus, as previously described (La Bonnardiere and Laude, 1981), except that 2-fold serial dilutions instead of 3-fold dilutions were used for better accuracy. No IFN international reference is available for either bovine or porcine species, so all titers were expressed in human IFN units by including a laboratory standard IFN in each independent assay. This standard IFN had a mean titer of 1000–1200 I.U./ml when assayed in MDBK cells against the human international reference alpha-IFN B/69-19 (NIH, U.S.A.).

Surface markers

Surface receptors. T, B and L lymphocytes, characterized by the presence of receptor for sheep red blood cells, receptor for C3b and receptor for IgG (FcR), were each assayed by rosette formation with sheep red blood cells (SRBC) in the presence of dextran (SRBC rosette), C3b-coated zymosan (zymosan rosette) and IgG-sensitized bovine red blood cells (EA rosette), respectively (Salmon, 1979). Cells without receptors, null cells, were calculated as [100% – (T+B+L)].

Adding dextran in the EA test (EAD rosette) allows the detection of T lymphocytes with an Fc-gamma receptor of low avidity (Binns and Licence, 1981). Thus, by calculation (i.e. EAD% – EA%), the proportion of T cells with Fc-gamma receptor may be evaluated. Rosette-forming cells (RFC) were determined on 200 lymphocytes in the presence of acridine orange in order to exclude the counting of any non-lymphocytic cells.

Surface antigens. Cells (5×10^6) were suspended in 100 μl of monoclonal antibody (see below) and then incubated on ice for 60 min. After three washes, the pellet was further incubated in 100 μl of appropriately diluted FITC-conjugated swine anti-mouse antibody (Nordic) for 1 h, then the cells were washed thrice. After the last wash, the pellet was resuspended in 20 μl of PBS–glycerol (50%) and then deposited on a microscope slide with a coverslip.

Lymphocytes with surface immunoglobulins (SIg) were revealed using fluorescent (FITC) rabbit $F(ab')_2$ anti-pig IgG (heavy and light chain, Cappel).

Morphology and phenotype of K562-lymphocyte conjugates

K562 cells and blood lymphocyte suspensions were each adjusted to 5×10^6 cells/ml and mixed *v/v* on a vortex. The mixture was then centrifuged at 60*g* for 10 min at room temperature. After gentle resuspension, an aliquot was cytocentrifuged (Salmon, 1983) and stained with May Grunwald Giemsa. Enumeration of lymphocytes bearing the Fc-gamma receptor among the K562–lymphocyte conjugates was made by mixing lymphocytes, EA indicator cells and FITC-labelled K562 cells (Salmon, 1986 and Fig. 1).

Alternatively, to identify the lymphocyte subset in contact with K562 and

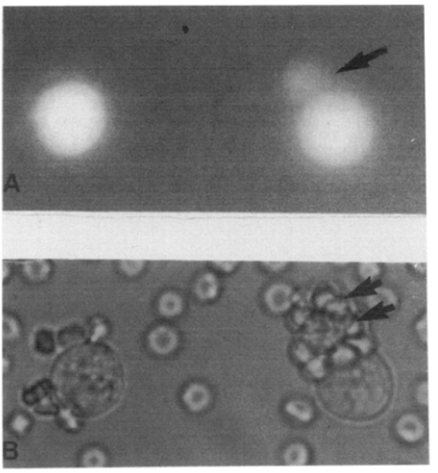

Fig. 1. Presence of Fc-gamma receptor on blood lymphocyte bound to K562 cells (magnification × 1000). For the purpose of demonstration both blood lymphocytes and target K562 cells were labelled by FITC; thereafter they were mixed with IgG-sensitized bovine red blood cells and processed for EA rosette formation (see Materials and Methods, wet preparation).
A. Cell suspension under short-wave blue illumination showing to the left a K562 cell alone; to the right the binding of lymphocyte (arrowed) to K562 cell.
B. The same microscopic field under bright illumination, showing the presence of EA indicator cells (arrowed) around the same lymphocyte as in A, indicating the presence of Fc-gamma receptors on lymphocyte bound to K562 cells.

bearing the Fc-gamma receptor, blood lymphocytes were first labelled by TRITC (50 μl of 10^8 lymphocytes in Earle–lactalbumin medium containing 4% FCS and 50 μl TRITC (2 μl/ml) for 15 min at 37°C). Cells were washed three times in MEM, and then incubated with monoclonal antibody followed by an FITC swine anti-mouse IgG (TEBU). Thereafter, lymphocytes, EA indicator cells and K562 cells were mixed by vortex and centrifuged at 60*g* for 5 min at room temperature. After gentle resuspension, an aliquot of the mixture was examined under an oil-immersion objective of large aperture (×40, d=1.05) using a fluorescence Leitz microscope equipped for epifluorescence with a 200-W HBO lamp and specific filters for FITC and RITC.

RESULTS

Lymphocyte recovery after separation

After FeC treatment and gradient separation, we recovered 50% of the original blood and spleen cell preparation. At this stage, the proportion of non-lymphoid cells was less than 2% in suspensions from both sources. Plastic-adherent cells represented 10% of spleen and blood suspensions; after panning

into 81+ and 81− cells, the proportion of recovered 81+ and 81− cells among harvested cells conformed to the proportion of positive and negative 81 cells (as revealed by immunofluorescence) in the original cell suspension. In the spleen, 42% were 81+ cells (versus 48% expected) while 41% were 81− cells (versus 47% expected). In the blood, 37% were 81+ cells (versus 45% expected) and 42% were 81− cells (versus 48% expected). Thus, the cell loss was low during the different steps of the procedure. In both spleen and blood, most of the 81+ cells (91%) were small lymphocytes, in contrast to 81− cells which contained essentially medium-sized lymphocytes. Granular lymphocytes amounted to 0.5% in both 81+ and 81− cells in the blood, and 1.7% and 0.3%, respectively, in the spleen.

NK activity and IFN production after removal of phagocytic and plastic-adherent cells

Removal of phagocytic cells (Table 1) from spleen did not significantly change NK and IFN activities. In the case of blood, this procedure led to increased NK activity, as shown by others (Lotzova and Herberman, 1986), without a change in the level of IFN production.

Spleen plastic-adherent cells were enriched for C3b- and SIg-positive cells without a change in any other subsets (Table 2A); these plastic-adherent cells exhibited a very low NK activity and IFN production (Table 1). In addition, plastic-non-adherent cells depleted significantly of C3b-positive cells did not exhibit any change in NK and IFN activities.

In Table 2B, it can be seen that removal of plastic-adherent cells from blood led to a significant decrease of C3b+ and SIg+ cells, from 3% and 11% to 1%

TABLE 1

NK[1] activity and IFN[2] production of separated fractions from spleen and blood after depletion of phagocytic cells by FeC treatment and adherence onto plastic

Cell fraction	Spleen[3]		Blood[4]	
	NK	IFN	NK	IFN
Origin	15 ±7.3	19900 ± 10300	22	10000
FeC treatment	7.3 ±4.4	17800 ± 4500	55	10000
Plastic-non-adherent	16	28000	55	5000
Plastic-adherent	0.01	300	ND[5]	ND

[1]Expressed as % cytotoxicity against K562 cells at effector to target ratio 100:1.
[2]Expressed as IFN units/ml for 10^7 cells.
[3]Mean ± s.e.m. of three spleens.
[4]One representative experiment.
[5]Not done.

TABLE 2

Distribution of lymphocyte subsets defined by rosette formation (RFC) and surface immunoglobulins among the cell preparations of spleen (A) and blood (B)

Cell source	Lymphocyte subset (%)[1]					
	T	FcR+	FcDR+	C3bR+	SIg+	Null
	SRBC RFC	EA RFC	EAD RFC	ZC RFC		
A, Spleen						
FeC treatment ($n=3$)	45± 5	21±3	38±1	10±1[a]	29± 1	24± 7
Plastic-adherent ($n=2$)	33± 2	30±6.5	40±0.5	23±8	58±10[b]	12±17
Plastic-non-adherent ($n=3$)	54±13	23±6	34±5	4±0.4[a]	26± 4[b]	19±18
B, Blood						
Fec treatment ($n=4$)	59± 3	1±0	11±4	3±0.5[c]	11±0.6[d]	33± 4
Plastic-adherent ($n=6$)	13± 4	8±4	41±4	35±2	96±2	44± 5
Plastic-non-adherent ($n=4$)	59± 4	2±1	12±3	1±0.3[c]	2±0.2[d]	37± 5

[1]Mean percentage ± s.e.m.; for percentages with the same superscript in a column, $P<0.05$ by t-test.

and 2%, respectively. Moreover, total activities found in the cells before plastic adherence were totally recovered in plastic-non-adherent cells. Thus, we concluded that C3b+ and SIg+ cells in the spleen and blood did not exhibit NK and IFN activities. In addition, results in Table 3 show that after the plastic adherence step, NK activity and IFN production were similar in spleen and blood: 11.3% and 13.4% for NK activity and 26 500 and 18 800 I.U./ml for IFN.

NK activity and IFN production of 81+ and 81− cells

Incubation with the 81B antibody did not result in any modification of NK activity (data not shown). In addition, there was no correlation between the level of NK activity and IFN production, as tested in each organ.

Removal of 81+ cells from plastic-non-adherent cells did not impair the original NK activity (Table 3). NK activity of 81+ cells in spleen and blood was lower ($P<0.05$) than that of plastic-non-adherent cells. Only in spleen was the NK activity of 81+ cells significantly lower than that of 81− cells ($P<0.05$). It can be seen in Fig. 2A and B that the dose–response curve of 81−

TABLE 3

NK[1] activity and IFN[2] production in 81− and 81+ cell fractions from spleen ($n=4$) and blood ($n=5$)

Cell fraction	Spleen		Blood	
	NK	IFN	NK	IFN
Plastic-non-adherent	11.3 ± 3.9^{a}	26500 ± 1011	13.4 ± 3.4	18800 ± 5600^{b}
81−	15.1 ± 5^{b}	33800 ± 950	10.1 ± 4	26000 ± 5000^{b}
81+	$3.7 \pm 3.4^{a,b}$	860 ± 200	4.2 ± 2.8^{a}	1000 ± 300

[1]NK activity expressed as % cytotoxicity against K562 cell at E:T ratio of 100:1. For percentages with the same superscript in a column, $P<0.05$ by t-test.
[2]IFN production after influenza virus induction in I.U./ml for 10^7 cells. For numbers with the same superscript in a column, $P<0.05$ by t-test.

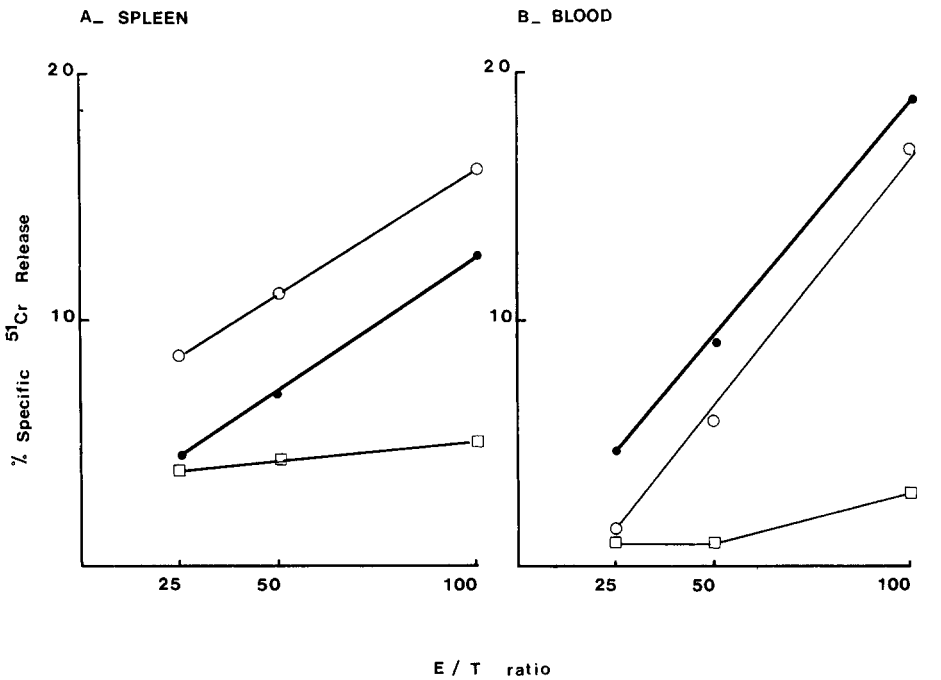

Fig. 2. NK activity of plastic-non-adherent unseparated cells (●—●) and separated 81− (○—○) and 81+ (□—□) cell fractions from spleen (A) and blood (B): effect of effector to target ratio on target cell lysis. Results were fitted to linear regression. In the case of spleen, results are the mean of four experiments; for blood, results are representative of four experiments (mean of triplicate).

TABLE 4

Interferon production by 81− and 81+ lymphocyte fractions from blood: comparison of three different inducer viruses

Cell fraction	Influenza virus	Aujesky virus	Gastro-enteritis virus
Plastic-non-adherent	20000[1]	5000	1000
81−	30000	4000	1200
81+	<100	<100	<100

[1]Results are expressed in International Units for 10^7 cells/ml.

cells parallels that of plastic-non-adherent cells: the same lytic activity required twice the E:T ratio for plastic-non-adherent cells as compared to the E:T ratio for 81− cells in the spleen. In contrast, the 81+ cell fraction in spleen did not exhibit any lytic activity. In the blood (Fig. 2B), the same lytic activity was achieved with the same E:T ratio for both 81− and plastic-non-adherent cells: 81+ cells exhibited a questionable activity.

When 81+ and 81− cells were mixed in equal proportions, the resulting NK activity was reduced two-fold relative to the original activity, as expected for the dilution effect (data not show). Thus, we concluded that the NK activity was supported only by 81− cells in the spleen, preferentially 81− cells in the blood, and that each subset 81+ and 81− behaved independently.

In spleen and blood, the 81− cell fraction supported the original IFN production (Table 3), regardless of the types of inducer viruses (Table 4). However, IFN production by 81− spleen cells was slightly enhanced as compared to the original population, 26 000 and 18 800 I.U./ml ($P<0.05$), respectively. In contrast, 81+ cells exhibited a very low level of IFN production, corresponding on a per cell basis, as a dilution of 81− cells, to a factor of 16.

Distribution of surface receptors among 81+ and 81− cells

Results in Table 5 show that each subset, as estimated by surface receptors, was present in the plastic-non-adherent 81+ and 81− cell fractions, whether in spleen or blood. Comparison of the RFC distribution between the 81− cell fraction and the original population did not reveal any significant difference in the spleen (Table 5A); in contrast, blood lymphocytes showed (Table 5B) a lower proportion of T cells in the 81− cell fraction by comparison with the original population. There was also a concomittant increase in the proportion of null cells.

Comparing 81+ and 81− cell fractions in the spleen, there was a higher proportion of FcR+ in the 81− cell fraction than in the 81+ cell fraction, either in the absence (33% compared to 12%; $P<0.05$) or in the presence of dextran (42% compared to 23%; $P<0.05$). No significant difference was de-

TABLE 5

Distribution of lymphocyte subsets in 81+ and 81− fraction from spleen (A) and blood (B) as assessed by rosette formation (RFC)

Cell source	Lymphocyte subset (%)[1]				
	T	FcR+	FcDR	C3b+	Null
	SRBC RFC	EA RFC	EAD RFC	ZC RFC	
A, Spleen					
Plastic-non-adherent	54±13	23±6	34±5	4 ±1	19±18
81+	62± 7	12±5[a]	23±2	6 ±2	20± 7
81−	48±14	33±8[a]	42±4	5 ±1	14±19
B, Blood					
Plastic-non-adherent	60± 5[b]	2±1	13±6	1 ±0.6	36± 6[c]
81+	74±11[b]	2±1	10±1	0.5±0.3	24±12
81−	38± 9	4±2	13±8	1 ±0.5	56±11[c]

[1]Mean percentage ±s.e.m. of four spleen and three blood preparations. Percentages with the same superscript in a column are significantly different ($P<0.05$ by t-test).

tected within the other subsets, and particularly in that subset defined by low avidity Fc-gamma receptor (EAD−EA).

In the blood, a significantly higher proportion of T cells was found among the 81+ than 81− cells, 74% as compared to 38% ($P<0.01$). Conversely, there was a lower proportion of null cells in 81+ (24%) than in 81− cells (56%) ($P<0.02$). No significant change was seen in other subsets although there was a trend towards a higher proportion of EA-RFC in the 81− cell fraction (2%) than in the 81+ cell fraction (4%).

Morphology and phenotype of K562-lymphocyte conjugates

Cytocentrifuged preparations of the conjugates showed the presence of granular lymphocytes among doublets, including a lymphocyte and a K562 cell (data not shown). The proportion of cells labelled by various monoclonal antibodies (MSA4, 81B, PT4, PT8) was similar in the whole lymphocyte population and in the K562–lymphocyte conjugates (Table 6), thus suggesting that MSA4, 81, PT8, or PT4 were specific markers of K562-conjugated lymphocytes. In contrast, a ten-fold increase (from 2% to 23%), in the proportion of EA-RFC was observed among conjugates as compared to the whole population, suggesting that FcR was a specific marker of NK cells. Conversely, although EA-RFC were enriched in conjugates, we also found the same proportion of MSA4, 81, PT8 and PT4 cells among the EA-RFC whether or not bound to

TABLE 6

Comparative distribution of surface antigens (as assessed by immunofluorescence with monoclonal antibody) and Fc-gamma receptor (as assessed by EA rosette) among unbound and K562-bound lymphocytes

Lymphocyte marker	% positive cells among	
	Unbound lymphocyte	K562-bound lymphocyte
MSA4	79	70
81[1]	66	56
MSA4, 81[2]	87	85
PT4	25	24
EA	2	23

[1]Similar proportions with PT8 antibody.
[2]Mixture of MSA4 and 81B antibody.

K562. This finding suggests that neither MSA4 nor 81 were specific markers of Fc-gamma positive lymphocytes.

DISCUSSION

Using similar procedures for cell preparations, comparable NK activity and IFN production were found in pig spleen and in blood lymphocytes. Differences in spleen cell preparation procedure might explain why others have not detected NK activity in swine spleen cells (Kim et al., 1980; Yang and Schultz, 1986). Although removal of phagocytic cells from blood increased NK activity, such a treatment was inefficient with spleen cells, due to a very low proportion of phagocytic cells in the original spleen cell preparation. In both organs, NK and IFN activities were caused by lymphocytes. Moreover, the plastic adhesion step showed that neither SIg+, nor C3B+, B-cells (i.e., that subset of SIg+ cells bearing stable membrane immunoglobulin, Salmon, 1979) were active, a result in accordance with the phenotype of human NK cells (Tedder et al., 1983). The lymphocyte subset responsible for NK and IFN activity must therefore be included in the T, L or null lymphocytes, such subsets being defined by the presence of surface receptors leading to rosette formation; T cells include SRBC rosette-forming cells (some of which expressed an FcR of low avidity) and null lymphocytes (Binns, 1982). However, determination of the precise lineage of these subsets is largely dependent on the sensitivity of the technique used. Although our previous results indicated that EA-RFC were independent of T cells as judged by SRBC rosetting (Salmon, 1979), results of this study indicate that a large fraction of EA-RFC exhibit the MSA4 antigen. In addition, the MSA4 antigen has been detected on 50% of B cells, although at a lower density (Lunney and Pescovitz, 1989).

Using a panning technique with the 81B antibody, cell recovery increased to 93 and 95%, thus allowing a valuable comparison of the activity of separated fractions. It could be argued that low activity of either NK or IFN in 81+ cells was due to the physical detachment of these cells from plastic; however, 81-positive lymphocytes were 100% viable and responded normally to PHA stimulation and IL-2 induction (data not shown). Alternatively, the observation can be explained on the basis of either a minute contamination of 81+ cells by 81− cells or of 81+ cells being at a different stage of differentiation (Reynolds and Ortaldo, 1987). Since IFN has been reported to act as an internal positive feedback mechanism in the activation of NK cells (Timonen et al., 1980), it could be argued that 81+ cells do not lyse K562 cells due to the absence of IFN-producing cells; however, this hypothesis is not tenable as mixing 81+ and 81− cells did not result in an enhanced activity of 81− cells.

Absence of IFN activity from the 81+ cell fraction cannot be explained by the absence of any subset, such as human null cells (Peter et al., 1980), non-SRBC rosetting T cells bearing Fc-gamma receptor (Trinchieri et al., 1978) or large granular lymphocytes (Timonen et al., 1981; Djeu et al., 1982). In mice, IFN-producing cells are included among T and B cells after influenza virus induction (Ito et al., 1978). Since the IFN titre was enhanced in the 81− cell fraction of spleen, with a concurrent increase in the proportion of FcR+ cells, we favour the view that these cells are responsible for IFN production in blood and spleen.

Blood cell phenotype involved in NK activity was studied at the single-cell level by a double fluorescent labelling and rosette formation. Only a drastic enrichment of plastic-non-adherent FcR+ cells was found, suggesting that the Fc-gamma receptor is a specific marker of NK cells. This conclusion is in accordance with our results of enhanced NK activity of spleen 81− cells, which were enriched two-fold as compared with the original cell suspension. Since we showed earlier (Salmon, 1979) the independence of FcR+ cells from the SRBC rosetting cells, these results agree with the general view that pig NK cells are non-SRBC rosetting T cells (Koren et al., 1978; Norley and Wardley, 1983; Yang et al., 1987). In contrast to observations made on NK activity against TGE virus-infected cells (Cepica and Derbyshire, 1986), we did not find any increase in the proportion of cells with Fc-gamma receptor of low avidity among 81− cells. The presence of granular lymphocytes among conjugates is at variance with the results from Yang et al. (1987) and agrees with the general view that granular lymphocytes in humans, mice and rats are effector cells for NK activity (Savary and Lotzova, 1986). However, granular lymphocytes were also found among non-active 81+ cells of spleen, which suggests that there are functional subsets of granular lymphocytes.

Although previous studies have suggested that producing cells alpha-IFN and NK cells are largely overlapping if not identical populations, Abb et al. (1983) showed that human blood lymphocytes depleted of Leu 7+, NK cells, which were presumably FcR+, were still fully able to synthesize alpha-IFN

after induction by influenza virus. Our results indicate that the FcR+ subset mediates NK and IFN activities.

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