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Detection and Characterization of Natural Killer Cells in Syrian Golden Hamsters*

DAVID M. TEALE,†‡ ROBERT C. REES,† ANTHONY CLARK§ and CHRISTOPHER W. POTTER†
Departments of †Virology and §Medical Microbiology, The University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX, U.K.

Abstract—*Natural killer (NK) cell activity was observed in the peripheral blood and spleen of 8- to 10-week-old Syrian golden hamsters, but not in the bone marrow or thymus. Low, but significant, levels of cytotoxicity were also observed in mesenteric and axillary lymph nodes and cells harvested from the peritoneum. Cytotoxicity, in a 4-hr ^{51}Cr-release assay, was found to be nylon wool non-adherent and was significantly reduced by treatment with trypsin or incubation at 37°C for 18 hr. Natural cytotoxicity was shown to be low at 1 week of age, but increased to a maximum at 8 weeks and was maintained into old age. Correlation was observed between peripheral blood cytotoxicity and the presence of large, often granular, lymphocytes following fractionation of effector cells by Percoll discontinuous gradient separation. These findings are compared with previous studies in hamsters, where the results of longer-term cytotoxicity tests differ in some respects to those of the present study.*

INTRODUCTION

NATURAL killer (NK) cells have been shown to be an important subset of lymphocytes possessing functional significance in tumour resistance and immune surveillance [1]. These cells are defined by their ability to lyse selected targets without prior deliberate sensitization to target cell antigens, and effector cells mediating natural killing have been identified in man, rats and mice [2–5]. The functional properties and characteristics of NK cells in these species have been studied extensively, although only recently has the morphology of NK cells been described as that of large granular lymphocytes (LGL) possessing an eccentric kidney-shaped nucleus and azurophilic cytoplasmic granules [2]. Enrichment of cell populations with these characteristics have been shown to correlate with increased target cell killing in the human and rat systems, and LGLs have been shown to bind selectively to NK-sensitive target cells [6, 7].

It is not known if NK cells are present in all animal species. In the hamster, Datta *et al.* [8] and Trentin and Datta [9] have reported natural cytotoxicity in long-term (16 to 18-hr) assays and shown some differences in the properties of effector cells compared with other species. Since the hamster represents an important animal model for the study of many infectious diseases [10, 11] and transplantable tumours [12–14], it is relevant to define clearly spontaneous cytotoxic reactivity in this species. We report here on the nature and properties of natural killing by hamster peripheral blood and splenic effector cells in a 4-hr ^{51}Cr-release assay. The results show that these effector cells possess similar functional and morphological properties to NK cells described for other species and may be distinct from the cytotoxic effects previously reported for the hamster [8, 9].

MATERIALS AND METHODS

Animals

Syrian golden hamsters were obtained from a closed, randomly bred colony at the University of Sheffield. The animals were weaned at age 3–4 weeks and studies performed using male hamsters aged 5–10 weeks unless otherwise stated. Graft rejection experiments (Potter and Jennings, unpublished findings) have shown these animals to be syngeneic.

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‡To whom requests for reprints should be addressed.

Target cells for natural killer cell assay

The human myeloid lymphoma line K562 was maintained as a suspension culture in medium RPMI 1640 supplemented with antibiotics and 10% newborn calf serum (RPMI-NBCS): xenogeneic target cells, including K562, have previously been used to demonstrate cytolytic reactivity [4, 15]. The human lung fibroblast cell line 2002, the Burkitt lymphoma line Raji, the mouse lymphoma line P3NS1 and the rat lymphoma G1 were also maintained in RPMI-NBCS. The African green monkey kidney cell line vero was grown in Eagle's Minimal Essential Medium and 10% fetal calf serum, whilst the baby hamster kidney (BHK) cell line was maintained in Glasgow Modified Eagle's Medium with 10% tryptose phosphate broth plus 10% newborn calf serum. Prior to cytotoxicity testing, cells were harvested from suspension culture bottles, or adherent cells were freed from the glass surface using 0.5 (w/v) trypsin (Difco 1:250) and washed ×3 in RPMI-NBCS. After washing the cells were re-suspended in 0.1 ml of RPMI-NBCS containing 100 μCi of chromium-51 (^{51}Cr) (Amersham Radiochemicals Ltd.) for 60 min as described previously [4, 15].

Preparation of effector lymphoid cells

Effector (E) cells were prepared from spleen, thymus, bone marrow and axillary and mesenteric nodes as previously described [16]. For each experiment the cells from 5–10 animals were pooled. Peripheral blood lymphocytes (PBL) were recovered from blood samples obtained by cardiac puncture, separated and partially purified on lymphocyte separation medium as described previously [17]. In addition, peritoneal cells were harvested from the peritoneum in Hank's BSS containing 10 units per ml heparin. All effector cell preparations were washed ×3 in Hank's BSS and re-suspended in RPMI-NBCS to the required concentration: cell suspensions prepared in this manner were also used for further purification by passage over nylon wool or separation on Percoll discontinuous density gradients. Human lymphocytes were prepared by centrifugation of heparinized blood on lymphocyte separation medium as previously described [18]. The cells were washed ×3, counted and re-suspended in RPMI-NBCS.

Nylon wool column fractionation

Cell suspensions containing 1.0×10^8 cells in 1.5 ml of RPMI-NBCS were incubated for 50 min at 37°C with 0.5 g of nylon wool packed into a 5.0 ml disposable syringe barrel [19]. After incubation non-adherent cells were eluted from the column with 25 ml of pre-warmed RPMI-NBCS and washed ×3 with the same medium. Adherent cells were removed from the nylon wool by gently teasing in RPMI-NBCS medium: these cells were washed ×3 in RPMI-NBCS medium prior to use in cytotoxicity tests.

Treatment with trypsin and 18-hr incubation at 37°C

Nylon wool column (NWC)-eluted peripheral blood lymphocytes were washed ×2 in Hank's BSS and re-suspended in pre-warmed (37°C) PBS or 0.5% (w/v) trypsin for 10 min. After incubation the cells were washed ×2 in RPMI-NBCS and re-suspended to the required concentration for testing in a 4-hr ^{51}Cr-release assay. Other NWC-eluted PBLs were washed ×3 in RPMI-NBCS and stored in 25-ml plastic tissue culture flasks overnight (18 hr) at 37 or 4°C. After incubation the cells were washed ×2 in RPMI-NBCS and re-suspended to the required concentration before testing in a 4-hr release assay.

Evaluation of effector cell morphology

Effector cells (2×10^5) in an 0.2-ml volume were centrifuged for 10 min at 900 revs/min on to microscopic slides using a cytospin centrifuge (Sandon Elliott). The preparations were air dried, fixed for 10 min in ethanol and stained for 25 min with 10% Giemsa (Fisher Scientific Company, Fairborn, N.J.) diluted in PBS. The preparations were examined by using the oil immersion objective and >200 cells identified for each preparation.

Non-specific esterase (ANBE) staining

Air-dried cell preparations, prepared as described for Giemsa staining, were fixed at room temperature for 30 sec, incubated with hexazotized pararosaniline and α-naphthyl butyrate for 45 min at 37°C, washed and counterstained with 1.5% methyl green: this method has been described previously in detail [20].

Discontinuous density gradient separation

Effector cells were separated by Percoll discontinuous density gradient fractionation as previously described [4, 21]. A stock Percoll solution of 310–315 mosm/kg H_2O was prepared and adjusted to isotonic strength with ×10 concentrated phosphate-buffered saline, pH 7.4 (PBS), and used to prepare 7-step density gradients; the varying concentrations of Percoll were prepared by diluting the stock solution with RPMI-NBCS. The following Percoll concentrations were used to prepare step gradients: 70% (1.5 ml), 65% (1.5 ml), 60% (1.5 ml), 55% (1.5 ml), 50% (2.5 ml), 45% (2.5 ml) and 40% (2.0 ml) Percoll in 15-ml conical centrifuge tubes. Up to 5×10^7 NWC-eluted cells in a 1.5-ml volume were

layered on top of the gradient, which was centrifuged at 550 *g* for 30 min. The cells at each interface were harvested, washed ×3 in RPMI-NBCS and their viability and per cent recovery was assessed by trypan blue exclusion. The cells were identified morphologically by Giemsa and non-specific esterase (ANBE) staining and their cytolytic activity measured in a 4-hr ^{51}Cr-release assay.

Cytotoxicity assay

The details of this test have been described previously [4, 15]. Briefly, 1.0×10^4 ^{51}Cr-labelled target (T) cells in an 0.1-ml volume of RPMI-NBCS were incubated with an equal volume of effector (E) cells in round-bottomed wells of microtitre plates (Limbro Scientific Company, Mamden, U.S.A.): the E:T ratio ranged between 100:1 and 3:1. After incubation for 4 hr at 37°C, a 0.1-ml volume of supernatant fluid was removed from each well and assessed for isotope content. In addition, the remaining supernatant and cells were assessed for radioactivity; the amount of ^{51}Cr present was measured in a γ-spectrophotometer. The isotope release in the presence of effector cells was compared with that of target cells incubated in medium alone (control). All tests were carried out in triplicate and the per cent Cr-release and cytotoxicity calculated by the formulae:

$$\% \text{ release} = \frac{(\frac{1}{2}\,\text{SN}) \times 2}{(\frac{1}{2}\,\text{SN}) + (\frac{1}{2}\,\text{SN} + \text{cells})} \times 100,$$

where SN = supernatant, and

$$\% \text{ cytotoxicity} = \frac{\begin{array}{c}\text{counts/min of test group} \\ \text{- counts/min in medium} \\ \text{control}\end{array}}{\begin{array}{c}\text{total counts/min in } 10^4 \\ \text{targets - counts/min of} \\ \text{counter background}\end{array}} \times 100.$$

Some results are expressed as lytic units (LU)/10^6 cells, with an LU being the number of effector cells required to cause 20% cytotoxicity.

RESULTS

Organ distribution of cytotoxicity

Effector cells were obtained from various organs removed from adult hamsters and tested for cytotoxicity against K562 target cells in 4-hr ^{51}Cr-release assays. The results are shown in Table 1. Peripheral blood lymphocytes gave 54.9 and 31.7% cytotoxicity in two separate 4-hr assays at an E:T ratio of 100:1; similar levels of cytotoxicity were observed for spleen lymphocytes. However, lymphocytes from mesenteric or axillary lymph nodes, thymus, peritoneal or bone-marrow cells showed insignificant levels of cytotoxicity or weak killing at high E:T ratios (Table 1). Thymocytes and bone-marrow cells in particular failed to cause significant killing of K562 targets.

To study further the cytotoxicity of PBL and spleen-cell suspensions these cells were passed over a nylon wool column and the adherent and non-adherent cell populations examined for cytotoxicity against K562 in 4-hr ^{51}Cr-release tests. The results are shown in Table 2. Nylon wool-adherent effector cells from PBL showed reduced levels of cytotoxicity compared with unfractionated cells, whilst NWC-eluted cells showed comparatively high cytolytic activity. NWC-retained splenic effectors also showed reduced levels of cytotoxicity when compared to unfractionated cells. NWC-eluted cells, however, although showing higher cytolytic activity than retained cells, also showed lower levels of cytotoxicity as compared to unfractionated spleen cells.

Natural cytotoxicity of PBL and spleen cells from hamsters of different ages

PBL and spleen cells from hamsters aged 1 week–1½ yr were passed over nylon wool and the non-adherent cells examined for cytotoxicity against K562 cells in a 4-hr ^{51}Cr-release assay. The results are shown in Fig. 1. Cytotoxicity was demonstrable for both spleen and PBL cells at all ages tested, except hamsters aged 1 week (spleen

Table 1. Organ distribution of natural cytotoxicity in hamsters

Source of effector cells	Effector: target-cell ratio									
	Experiment 1				Experiment 2					
	100:1	50:1	25:1	12:1	100:1	50:1	25:1	12:1	6:1	3:1
Peripheral blood	54.9	39.0	31.5	21.9***	31.7	36.6	36.9	25.6	14.8***	1.2*
Spleen	43.4	30.5	23.6	15.0***	51.8	47.6	42.2	38.3	28.5***	8.7***
Mesenteric lymph node	0.7^{NS}	1.5*	0.3^{NS}	0.0^{NS}	5.4*	3.6*	0.0^{NS}	2.0^{NS}	NT	NT
Axillary lymph node	1.4*	1.2*	0.0	0.1^{NS}	5.7**	1.1^{NS}	0.0	1.4^{NS}	NT	NT
Thymus	0.4	0.0	0.1	0.0	0.0	1.1^{NS}	0.0	2.4^{NS}	NT	NT
Peritoneal cells	2.8*	1.4*	1.0^{NS}	0.0^{NS}	4.2**	3.8**	2.6^{NS}	0.9^{NS}	NT	NT
Bone marrow	0.0	1.0^{NS}	0.0	0.0	0.0	0.0	0.0	0.0	NT	NT

Cytotoxicity assessed by Student's *t* test; *$P = <0.05$; **$P = <0.01$; ***$P = <0.001$; NS = not significant; NT = not tested. All experiments were performed using unfractionated effector cells in 4-hr ^{51}Cr-release assays.

Table 2. Cytotoxicity of hamster peripheral blood and spleen cells against the target line K562 following nylon wool column fractionation

Source of Effectors	Experiment No.	Unfractionated E:T ratio 100	50	25	12	6	3	Retained E:T ratio 100	50	25	12	6	3	Eluted E:T ratio 100	50	25	12	6	3
PBL	1	30.2	26.4	22.0	18.0	9.8	5.2	15.4	12.0	8.2	5.3	0.0	1.2[NS]	38.2	36.0	33.1	23.0	15.0	2.6
	2	49.1	45.3	36.1	28.4	NT	NT	22.4	19.1	14.0	9.6	NT	NT	55.8	49.2	45.7	33.2	NT	NT
	3	32.1	27.1	22.2	19.3	NT	NT	19.3	17.0	13.5	11.7	NT	NT	42.9	36.9	31.5	25.0	NT	NT
	4	NT	NT	20.4	12.9	9.0	5.9	NT	NT	2.3	1.7[NS]	0.0	0.0	54.0	51.0	34.0	22.7	10.4	6.2
	5	NT	NT	NT	NT	NT	NT	22.4	19.1	14.0	9.6	NT	NT	55.8	49.2	45.7	33.2	NT	NT
	6	NT	NT	NT	NT	NT	NT	NT	6.2	3.8	1.8	NT	NT	33.0	23.2	19.0	NT	NT	NT
	7	NT	NT	NT	NT	NT	NT	NT	NT	0.5	0.1	0.0	0.0	NT	NT	20.5	18.7	14.0	NT
Spleen	8	51.8	47.6	42.2	38.3	28.5	8.7	18.9	13.6	7.4	0.0	0.0	0.0	48.6	35.9	31.9	12.1	5.9	0.0
	9	32.3	28.7	23.6	16.2	NT	NT	15.5	14.2	10.9	8.5	NT	NT	25.5	23.2	23.1	18.0	NT	NT
	10	NT	NT	NT	NT	NT	NT	21.0	14.6	9.7	NT	NT	NT	39.3	35.1	28.0	NT	NT	NT
	11	NT	NT	NT	NT	NT	NT	NT	14.6	9.3	5.6	5.3	NT	NT	54.7	42.7	24.4	13.9	NT

Unless otherwise stated, all cytotoxicity values were statistically significant ($P = <0.05$–0.001) by Student's t test; NS = not significant; NT = not tested. All results were obtained in 4-hr ^{51}Cr-release assays.

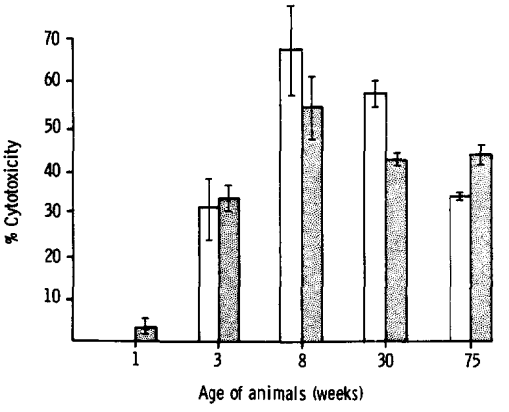

Fig. 1. Age distribution of NK activity in nylon wool column-eluted peripheral blood (white bars) and nylon wool column-eluted spleen lymphocytes (dark bars). Eight hamsters were used per group at each age bracket.

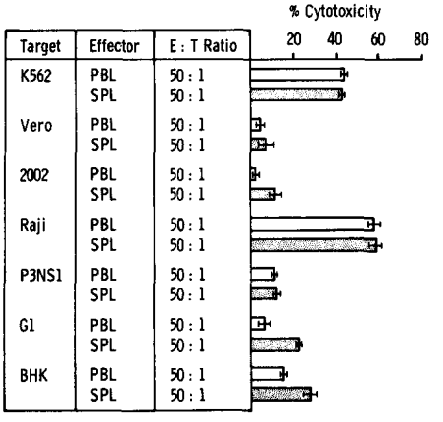

Fig. 2. Target cell susceptibility, in a 4-hr chromium-release assay, to peripheral blood (white bars) and spleen (dark bars) nylon wool column-eluted lymphocytes. K562 = human leukaemia; vero = African green monkey kidney; 2002 = human lung fibroblast; Raji = Burkitt's lymphoma; P3NS1 = mouse lymphoma; G1 = rat lymphoma; BHK = baby hamster kidney. Other experiments showed a similar trend in target-cell susceptibility.

cells only). The highest level of target-cell killing was seen at age 8 weeks and the results suggest that natural cytotoxicity is highest in young adults, declining slightly with age (Fig. 1). However, there is no evidence from these studies that, except for weanling hamsters, there was an age restriction on natural cytotoxicity.

Target-cell susceptibility

Previous studies have shown that NK cells can lyse a variety of target cells and that the spectrum for cytolysis may vary for NK cells from different species. Hamster natural cytotoxicity was assayed against a variety of target cells using nylon wool-non-adherent PBLs and spleen effector cells from adult hamsters. The results are shown in Fig. 2. Hamster PBLs and spleen cells were cytotoxic for

K562, although by comparison vero and 2002 cell targets were weakly susceptible to hamster PBL killing and slightly more susceptible to effector cells derived from hamster spleen. In contrast, the Raji cells showed approximately 60% cytotoxicity with PBL and spleen effector cells at an E:T ratio of 50:1 (Fig. 2). P3NS1, G1 and BHK targets were shown to be susceptible to both hamster PBL and spleen-cell cytotoxicity ($P<0.01$), although the level of activity was less than that observed towards K562 and RAJI targets. Repeat experiments showed a similar trend in target-cell susceptibility using both PBL and spleen effector cells.

Effect of trypsin on cytolytic activity

Nylon wool column-eluted hamster PBLs were suspended in either PBS or 0.5% (w/v) trypsin and incubated at 37°C for 10 min, washed ×3 in RPMI-NBCS and tested for cytotoxicity towards K562 target cells. The results are shown in Fig. 3. Lymphocytes pretreated with trypsin demonstrated significantly reduced cytolytic activity. Examination of the morphology of trypsin-treated effector cells did not indicate that the enzyme treatment was toxic with respect to cell numbers or phenotypic characteristics.

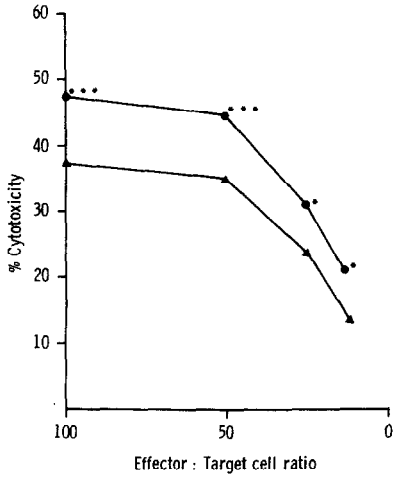

*Fig. 3. Per cent cytotoxicity, in a 4-hr release assay, of nylon wool column-eluted peripheral blood lymphocytes after 10 min incubation at 37°C in PBS (●) or trypsin (▲). Cytotoxicity was assessed by Student's t test at each E:T ratio for treated and untreated effectors. ***P = <0.001; *P = <0.05. The results were shown to be reproducible in several repeat experiments.*

Effect of 18-hr incubation at 4 or 37°C on cytolytic activity

Nylon wool column-eluted PBLs were stored overnight in RPMI-NBCS at 4 or 37°C and the following day tested for cytolytic activity towards K562 targets. As shown in Fig. 4, PBLs incubated at 37°C were significantly less cytotoxic ($P<0.001$) than effector cells incubated at 4°C. No significant difference in cell numbers or individual cell-type morphology was observed between the two cell populations.

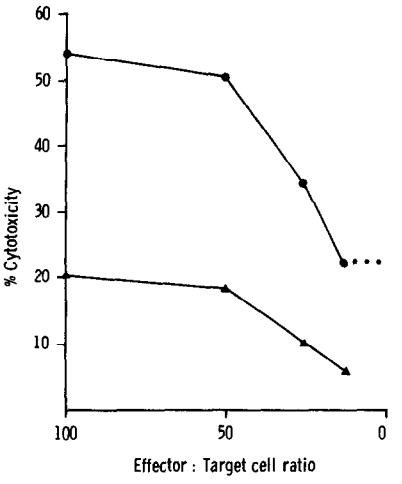

*Fig. 4. Per cent cytotoxicity in a 4-hr release assay of nylon wool column-eluted peripheral blood lymphocytes after 18 hr incubation at 4 (●) or 37°C (▲). Cytotoxicity was assessed by Student's t test at each E:T ratio between the two curves. ***P = <0.001 at each point. The results were shown to be reproducible in three repeat experiments.*

Percoll discontinuous density gradient fractionation

Nylon wool-non-adherent hamster PBLs were layered onto a 7-step Percoll gradient and centrifuged at 550 *g* for 30 min. The cells were collected from the gradient interfaces and tested for cytolytic activity against K562 cells in a 4-hr ^{51}Cr-release assay. In addition, each cell population was counted and identified by Giemsa and non-specific esterase staining. The results are shown in Fig. 5. Lymphocytes were found in the highest concentrations in fractions 4, 5 and 6 respectively and were predominantly large lymphocytes, with a small percentage (5–10%) possessing cytoplasmic granules. In addition, these fractions were shown to possess high cytolytic activity for K562 target cells. Significantly lower levels of cytolytic activity against K562 targets were found for cells recovered from fractions 1 and 3, which were composed mainly of polymorphonuclear cells and unidentifiable cells, probably a mixture of neutrophils and lymphocytes which had been distorted during the separation procedure. Low levels of small lymphocytes were recovered from fractions 5 and 6, but their numbers failed to correlate with any increase in cytotoxicity.

The results are representative of those obtained in other similar experiments, where killing was associated with cells recovered from interface fractions between 50 and 65% Percoll and where

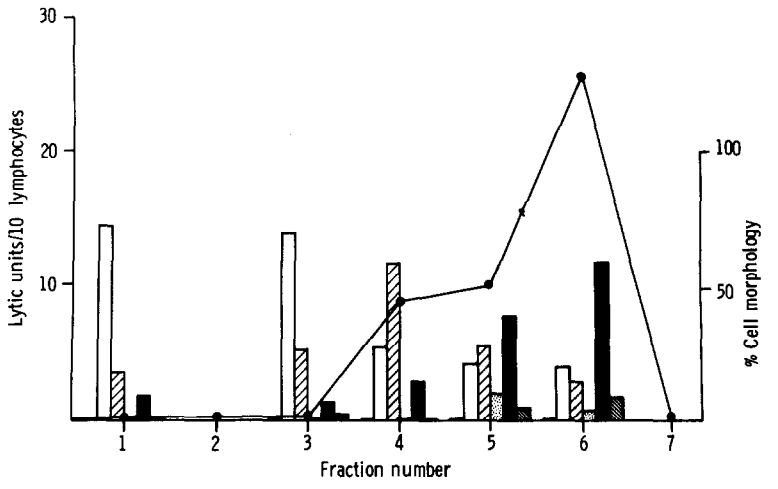

Fig. 5. Lytic units obtained from the interfaces of a 7-step Percoll discontinuous density gradient plotted with the percentage of cell types found at each interface. One lytic unit = No. of lymphocytes/10^6 lymphocytes that are capable of giving 20% cytotoxicity. □ = Possibly neutrophils, distorted lymphocytes and dead cells; ▨ = neutrophils; ▩ = small lymphocytes; ■ = large lymphocytes; ■ = large granular lymphocytes. Monocytes were shown to be less than 1.0% in all fractions. These results were found to be reproducible in three repeat experiments. The percentages of cell recovery at each interface are as follows: fraction 1, 2.0%; fraction 2, 0.0%; fraction 3, 4.0%; fraction 4, 11.0%; fraction 5, 23.0%; fraction 6, 52.0%; fraction 7, 0.0%.

the highest number of large, and sometimes granular, lymphocytes fractionated. This is in contrast to rats, mice and humans, where cells mediating natural killing separated in lower-density Percoll fractions [2–5].

In experiments where human and hamster NWC-eluted PBLs were fractionated on 7-step Percoll gradients simultaneously, natural killer activity towards K562 targets was associated with fractions 2, 3 and 4 for the human and fractions 4, 5 and 6 for the hamster. The distribution of large, often granular lymphocytes was also associated with fractions possessing high cytotoxicity (Table 3).

It therefore appears that the cell type responsible for cytotoxicity in a 4-hr ^{51}Cr-release assay in hamsters, although morphologically very similar to those described in other systems, is more dense. Staining for non-specific esterase showed that less than 1% of NW-non-adherent cells were esterase-positive and Percoll fractions enriched for cytotoxic activity were devoid of such cells.

DISCUSSION

A previous report has demonstrated the existence of spontaneous cell-mediated cytotoxicity in hamsters using 16- to 18-hr cytotoxicity assays [8, 9]. The results from such studies, however, differ from those reported here in a 4-hr assay and may be due to the presence of effector cell subsets which mediate cytolytic activity in longer-term assays and which may show a difference in organ distribution and target-cell susceptibility. Employing a 4-hr cytotoxocity test, we have evaluated natural killing against the NK-sensitive target line K562 and determined some

Table 3. Comparison of cytotoxicity of hamster and human PBL following separation on discontinuous 7-step Percoll gradients

Fraction No.	Hamster % cell recovery	Hamster LU/10^6 lymph	Hamster % LGL	Human % cell recovery	Human LU/10^6 lymph	Human % LGL
1	5.0	<1.0	0.0	0.1	<1.0	0.0
2	0	0	0	0	0	0
3	3.0	<1.0	2.1	5.2	8.3	32.0
4	17.7	<1.0	3.2	16.3	10.0	38.0
5	27.0	3.2	4.1	34.0	1.1	7.0
6	20.7	5.0	4.4		1.0	0.0
7	7.0	<1.0	1.0	6.0	<1.0	0.0
Pellet	3.4	<1.0	0	1.8	<1.0	0.0

LU = lytic units expressed as the number of lymphocytes per 1 × 10^6 lymphocytes giving 20% cytotoxicity against K562 target cells.

differences in the properties of the effector cells to those reported previously [8, 9]. The results shown here indicate that peripheral blood and spleen cells in particular are a rich source of spontaneous cytolytic activity. Spleen and peripheral blood effector cells were consistently cytotoxic towards the NK-sensitive target K562 as well as to BHK and Raji target cell lines, while other target cells (vero, 2002, P3NS1 and G1 cells) were relatively insensitive to both spleen and PBL effectors. Datta *et al.* [8] have reported that spleen, bone-marrow and mesenteric lymph-node cells possess high cytotoxic activity in a 16- to 18-hr assay against the Simian adenovirus 7-induced lymphoma cell line, and that thymocytes were inactive under these assay conditions. However, the organ distribution of effector cells in a 4-hr assay against K562 targets shows spleen cells and PBL to be rich in cytolytic activity, while bone marrow and thymus were inactive; weak, but sometimes significant, activity was demonstrated in cells removed from the peritoneum, mesenteric and axillary lymph-node cells. In particular, the bone-marrow cell reactivity demonstrated in 16- to 18-hr assays would suggest the presence of an effector cell population distinct from cells mediating cytolytic activity in 4 hr [8, 9].

In the mouse, NK cell activity has been shown to be age-restricted, being maximum at 8 weeks of age and declining to insignificant levels by 12–14 weeks of age [1]. However, studies in rats [22–24] and in humans [25, 26] have shown that NK cell activity is maintained into old age. Our studies in hamsters indicate that natural cytotoxicity against xenogeneic K562 target cells is present at 3, but not at 1, weeks of age, is maximum at 8 weeks and remains high up to 1½ yr of age. Results of longer-term cytotoxicity tests [9] demonstrate the presence of natural cytotoxicity in 4-day-old hamsters, with activity increasing progressively with age, but in the present study cytolytic activity appeared to decline with age. In addition, natural cytotoxicity in 4-hr assays was sensitive to trypsin treatment, while effectors mediating cytotoxicity in 16- to 18-hr assays were resistant. These studies further indicate the possible existence of at least 2 distinct populations of cells mediating natural cytotoxicity, and in view of the importance of certain hamster tumours as models for the study of neoplastic disease [9, 12, 14, 27–29], a clear definition of natural effector mechanisms is therefore essential.

Studies in the rat, mouse and human have shown natural killer cell activity to be associated with lymphocytes of a distinct morphological type [2–5] which have been described as large lymphocytes with an eccentric nucleus and azurophilic granules in their cytoplasm. These effector cells fractionate on Percoll discontinuous gradients in a distinct and reproducible manner, and interface fractions rich in large granular lymphocytes (LGL) have been shown to possess maximum NK cell activity. In these studies LGLs were found to bind selectively to NK-sensitive target cells, while other cell types (LGL-depleted cell populations) showed reduced cytotoxic activity and binding capacity [6, 7]. It was therefore particularly relevant to determine the morphology of natural cytotoxic effector cells in the hamster since it has been inferred that LGL morphology may be a universal marker for NK cells. In keeping with the observations reported for other rodents and for man, hamster effector cells fractionated on Percoll discontinuous gradients showed good correlation between cytolytic activity and the presence of large, sometimes granular, lymphocytes. However, this cell population was shown to separate between 50 and 65% Percoll, showing them to be more dense than those found in rats, mice and humans. There was no evidence that small lymphocytes were responsible for cytotoxicity, and although polymorphs were present in cytotoxic fractions, it is unlikely that these cells are responsible for mediating cytotoxicity since they also constituted a dominant cell population in fractions devoid of cytotoxic activity; also, other studies have shown them to be devoid of NK cell activity [15]. Initial studies on the extent to which different effector cell populations bind to NK-sensitive targets indicate that both large lymphocytes and polymorphonuclear (PMN) leukocytes form conjugates with K562 target cells (Teale *et al.*, unpublished results), and it is likely that natural cytotoxicity is mediated by the large lymphocyte population.

REFERENCES

1. HERBERMAN RB, HOLDEN HT. Natural cell-mediated immunity. *Adv Cancer Res* 1978, **27**, 305–377.
2. TIMMONEN T, SAKSELA E, RANKI A, HAYRY P. Fractionation, morphological and functional characterization of effector cells responsible for human NK activity against cell line targets. *Cell Immunol* 1979, **48**, 133–148.
3. TIMMONEN T, ORTALDO J, HERBERMAN RB. Characteristics of human large granular lymphocytes and relationships to NK and K cells. *J Exp Med* 1981, **153**, 569–582.

4. REYNOLDS CW, TIMMONEN T, HERBERMAN RB. NK activity in the rat. I. Isolation and characterization of the effector cells. *J Immunol* 1981, **127**, 282–287.
5. LUINI W, BORASCHI D, ALBERTI S, ALEOTTI A, TAGLIALIVE A. Morphological characterization of a cell population responsible for natural killer activity. *Immunology* 1981, **43**, 663–668.
6. LANDAZURI M, LOPEZ-BOTET M, TIMONEN T, ORTALDO JR, HERBERMAN RB. Human large lymphocytes: spontaneous and interferon-boosted NK activity against adherent and nonadherent tumor cell lines. *J Immunol* 1981, **127**, 1380–1383.
7. TIMMONEN T, ORLANDO JR, HERBERMAN RB. Characteristics of human large granular lymphocytes and relationship to natural killer and K cells. *J Exp Med* 1981, **153**, 569–582
8. DATTA SK, GALLAGHER MT, TRENTIN JJ. Natural cell-mediated cytotoxicity in hamsters. *Int J Cancer* 1978 **23**, 728–734.
9. TRENTIN JJ, DATTA SK. Natural killer (NK) cells in hamsters and the modulation in tumorigenisis. *Adv Exp Med Biol* 1981, **134**, 153–164.
10. JENNINGS R, POTTER CW. The hamster as a model for the study of immunity to influenza virus infection. *Fed Proc* 1978, **37**, 2072–2073.
11. JENNINGS R, DENTON MD, POTTER CW. The hamster as an experimental animal for the study of influenza. I. The role of antibody in protection. *Med Microbiol Immunol* 1976, **162**, 217–226.
12. SHORTLAND J, MOUSAWY KM, REES RC, POTTER CW. Tumourgenicity of Herpes virus hominis type-2 transformed cells (line 333-8-9) in adult hamsters. *J Pathol* 1979, **129**, 169–178.
13. MOUSAWY KM, REES RC, POTTER CW. Immunogenic properties of hamster tumours of Herpes virus hominis aetiology. *Cancer Immunol Immunother* 1980, **8**, 119–126.
14. WALKER JR, REES RC, TEALE DM, POTTER CW. Properties of a Herpes virus-transformed hamster cell line—I. Growth and culture characteristics of sublines of high and low metastatic potential. *Eur J Cancer Clin Oncol* 1982, **18**, 1017–1026.
15. HASSAN ZM, REES RC, POTTER CW. *Corynebacterium parvum* stimulation of adherent and non-adherent cytotoxicity in mice. *Br J Cancer* 1981, **44**, 532–538.
16. ORTIZ DE LANDAZURI M, HERBERMAN RB. Immune responses to Gross-virus induced lymphoma. III. Characteristics of the cellular immune response. *J Natl Cancer Inst* 1972, **49**, 147–154.
17. OEHLER JR, HERBERMAN RB, CAMPBELL DA, DJEU JY. Inhibition of rat mixed lymphocyte cultures by suppressor macrophages. *Cell Immunol* 1977, **29**, 238–250.
18. BOYUM A. Isolation of mononuclear cells and granolocytes from human blood. *Scand J Clin Lab Invest* 1968, **21** (Suppl. 97), 77–81.
19. JULIUS MH, SIMPSON E, HERZENBERG LA. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur J Immunol* 1973, **3**, 645–649.
20. KOSHI IR, POPLACK DE, BLAESE RM. A nonspecific esterase stain for the identification of monocytes and macrophages. In: BLOOM BR, DAVID JR, eds. In Vitro *Methods in Cell Mediated and Tumour Immunity*. New York, Academic Press, 1976, 359–362.
21. TIMMONEN T, SAKSELA E. Isolation of human natural killer cells by density gradient centrifugation. *J Immunol Methods* 1980, **36**, 285–291.
22. NUNN ME, DJEU JY, GLASER M, LAVARIN DM, HERBERMAN RB. Natural cytotoxic reactivity of rat lymphocytes against syngeneic Gross-virus induced lymphoma. *J Natl Cancer Inst* 1976, **56**, 393–398.
23. BROOKS CG, FLANNERY GR. Quantitative studies of natural immunity to solid tumours in rats. Persistence of natural immunity throughout reproductive life and absence of suppressor cells in infant rats. *Immunology* 1980, **39**, 187–194.
24. ZOLLER M, MATSKU S. Characterization of natural cytotoxicity *in vitro* in a spontaneous rat tumour model. *J Immunol* 1980, **124**, 1683–1690.
25. JONDAL M, PROSS H. Surface markers on human B- and T-lymphocytes. VI. Cytotoxicity against cell lines as fractional marker for lymphocyte subpopulations. *Int J Cancer* 1975, **15**, 596–605.
26. TAKASUGI M, MICKEY MR, TERASAKI PI. Reactivity of lymphocytes from normal persons on cultured tumour cells. *Cancer Res* 1973, **33**, 2898–2902.
27. COGGIN JM, AMBROSE KR, ANDERSON NG. Fetal antigens capable of inducing transplantation immunity against SV40 hamster tumour cells. *J Immunol* 1970, **105**, 524–526.
28. POTTER CW, SHORTLAND JR, MOUSAWY KM, REES RC. Comparative studies of Herpes virus hominis type 1 and 2 transformed hamster cells. *Br J Exp Pathol* 1982, **63**, 205–298.

29. MOUSAWY KM, REES RC, POTTER CW, SHORTLAND JR. Immune response of hamsters to transplanted herpes virus-induced tumours. In: LATNANSKY K, ed. *Biology of the Cancer Cell. Proceedings of the Vth Meeting of the European Association of Cancer Research.* Vienna, Kugler Medical Publications, 1980, 127–132.