Clin. exp. Immunol. (1978) 34, 78–86.

Organ distribution of natural cytotoxicity in the rat

M. R. POTTER & M. MOORE *Immunology Division, Paterson Laboratories, Christie Hospital & Holt Radium Institute, Manchester*

(*Received* 30 *March* 1978)

SUMMARY

The natural (spontaneous) cytotoxicity (NC) of cell populations from different lymphoid organs of the rat were examined using a human myeloid cell line (K562) and a rat fibrosarcoma cell line (Mc40) as target cells. Rat blood and spleen lymphoid cell populations gave high cytotoxicity against K562, while lymph node cells and bone-marrow cells gave low levels of cytotoxicity and thymus cells virtually no activity. Addition of thymus or lymph node cells to spleen effector cells did not suppress the high cytotoxicity of spleen cells. A similar organ distribution of reactivity was observed against Mc40 cells, but the levels of cytotoxicity were much lower than for K562.

A strain difference was monitored in the levels of natural cytotoxicity and cell populations from inbred Wistar rats consistently gave higher activity on a cell-to-cell basis than the corresponding population from PVG/c rats.

Natural cytotoxicity was not removed when spleen cell populations were depleted of cells adhering to nylon-fibre columns or plastic surfaces, or depleted of cells ingesting carbonyl iron. In agreement with other studies using human and animal lymphoid cells, the natural killer cell in this system was found to be non-adherent and non-phagocytic and its distribution did not correspond to the established organ distribution of T or B lymphocytes.

INTRODUCTION

Lymphoid cell populations from humans and experimental animals express spontaneous (natural) cytotoxicity (NC) against a variety of tissue-culture cell lines. This effect has been demonstrated using lymphoid cells and target cells in syngeneic, allogeneic and xenogeneic combinations (Herberman *et al.*, 1973; Takasugi, Mickey & Terasaki, 1973; Greenberg & Playfair, 1974; Rosenberg *et al.*, 1974; Herberman, Nunn & Lavrin, 1975a; Jondal & Pross, 1975; Pross & Jondal, 1975; Kiessling, Klein & Wigzell, 1975a; Zarling, Nowinski & Bach, 1975; Nunn *et al.*, 1976). NC has been shown to be the property of a population of lymphocytes which lack the characteristics of T or B lymphocytes (Pross & Jondal, 1975; Herberman *et al.*, 1975b; Kiessling *et al.*, 1975b; Kiuchi & Takasugi, 1976), and may be used as a functional marker for this population. The human myeloid cell line K562 is particularly susceptible to natural cytotoxicity and measurement of ^{51}Cr release from these cells provides a sensitive and reproducible assay for this activity (Jondal & Pross, 1975; West *et al.*, 1977). The majority of studies on NC have been conducted using effector cells from human peripheral blood and rat or mouse spleen. The activity of cells from different lymphoid tissues has not been well-defined. Using Moloney virus-induced mouse leukaemia (Kiessling *et al.*, 1975b) and Gross virus-induced rat lymphoma (Nunn *et al.*, 1976; Shellam & Hogg, 1977) target cells, syngeneic spleen cells were found to be more cytotoxic than other lymphoid cells. In the case of Rauscher virus-induced-tumour target cells, high activity has been reported for spleen, lymph node and blood lymphocytes (Herberman *et al.*, 1975a). We have examined the natural

Correspondence: Dr M. R. Potter, Immunology Division, Paterson Laboratories, Christie Hospital & Holt Radium Institute, Manchester, M20 9BX.

0099-9104/78/0100-0078$02.00

cytotoxic activity of cell populations from different lymphoid organs of the rat using the K562 and Mc40 cell lines as target cells and have also examined some characteristics of the active population.

MATERIALS AND METHODS

Animals. Young adult rats of two inbred strains, Nottingham Wistar (W/Not) and PVG/c, maintained in these laboratories were used.

Lymphoid cell preparations. Heparinized blood samples were obtained by cardiac puncture and lymphocyte-enriched cell populations prepared by centrifugation on Ficoll–Triosil, as previously described for human blood (Potter & Moore, 1975). Spleen, thymus, peripheral lymph-node (cervical, axillary and apical) and mesenteric lymph-node cell suspensions were prepared by teasing chopped tissue through a stainless steel mesh into RPMI 1640 medium (RPMI). Bone-marrow cells were obtained by removing the femur and washing through with phosphate-buffered saline. In all experiments pooled lymphoid cells from at least four animals were used. Unless otherwise stated all lymphoid cell suspensions were sedimented by centrifugation, treated with distilled water for 5 seconds to lyse contaminating erythrocytes and washed three times with RPMI. In some experiments the effect of using 0·75% NH_4Cl in Tris buffer pH 7·2 (Tris-NH_4Cl) for erythrocyte lysis was examined.

The techniques used for depleting adherent cells by nylon-fibre-column filtration and adherence to plastic surfaces, and depleting phagocytic cells by incubation with carbonyl iron, have been described previously for human cells (Potter & Moore, 1975; 1977). Nylon-fibre-column filtration of rat-spleen cells reduced the number of surface immunoglobulin (Ig) bearing cells from 33% to 6% (mean values) as determined by fluorescent staining (using rabbit anti-rat Ig [Nordic Immunological Laboratories, Tilburg, The Netherlands] and fluorescein-labelled sheep anti-rabbit Ig [Wellcome Reagents Ltd, Beckenham, Kent]).

Cytotoxicity assay. The target cells used in this assay were the K562 human-myeloid cell line (obtained from the Karolinska Institute, Stockholm) and a W/Not rat cell line (Mc40) derived from a methylcholanthrene-induced fibrosarcoma Mc40A. Both cell lines were grown in RPMI 1640 medium + 10% Foetal calf serum (RPMI–FCS), K562 in suspension and Mc40 as an adherent monolayer (harvested using 0·1% trypsin).

Target cells ($3–5 \times 10^6$) were labelled for 90 min at 37°C using 100 μC ^{51}Cr Sodium Chromate (Radiochemical Centre, Amersham) and then washed four times in RPMI. Labelled target cells were resuspended in RPMI–FCS and 0·2 ml samples containing 10^4 cells were added to 2·5 ml plastic tubes. Effector cells were added to the tubes in a further 0·2 ml volume of RPMI–FCS and effector to target cell ratios of 40:1, 20:1, 10:1 and 5:1 were used routinely. Control tubes containing target cells only were included to give the background isotope release and maximum release was determined by adding Triton X100 ($\frac{1}{100}$ dilution) to target cells. All tests were set up in triplicate and incubated for 18 hr at 37°C in an atmosphere of 95% air and 5% CO_2. At the end of the incubation period the tubes were spun at 1200 rev/min for 10 min, 0·2 ml samples of the supernatant were removed from each tube and both aliquots counted on a Searle 1185 gamma counter. The percentage ^{51}Cr release was determined for each tube and using the mean value of the triplicate tubes the percentage cytotoxicity was calculated according to the following formula:

$$\text{Percentage cytotoxicity} = \frac{(\%\ ^{51}\text{Cr release in sample} - \%\ ^{51}\text{Cr release in control})}{(\%\ ^{51}\text{Cr release in Triton} - \%\ ^{51}\text{Cr release in control})} \times 100)$$

The background isotope release was 15–25% for K562 and 20–35% for Mc40 and the maximum isotope release was 85–98% for both cell lines. An individual experiment consisted of a cytotoxicity test performed at one time using a single batch of target cells and effector cells.

The cytotoxic activity of cells from different lymphoid organs are shown as dose response curves and also expressed as lytic units/10^6 cells. (One lytic unit being defined as the number of effector cells required to produce 20% cytotoxicity above the base line.)

Statistical analysis of data. Experiments were repeated at least four times and the mean values determined. (Mean values were representative since there was in general little variation between experiments and results were symmetrically distributed.)

To determine the relative effects of the different methods of erythrocyte lysis on spontaneous cytotoxicity (Fig. 1), a two-factor analysis of variance (ANOVA) was carried out.

In the remaining experiments, with the exception of those where individual values are recorded, a logit transformation was used first to stabilize the variance. As the profiles of the graphs (Figs 2 and 4) were not parallel, a one-factor ANOVA was carried out on each of the ratios individually. If the ANOVA indicated a significant difference between the groups then Duncan's tests were used to identify which of the individual means were significantly different from each other. In legends to the figures, group means are ordered from low to high. If a set of means is underlined it denotes that there is no statistically significant difference between them.

RESULTS

Methods of red cell lysis

In preliminary experiments the effects of treating lymphoid cell suspensions with distilled water or

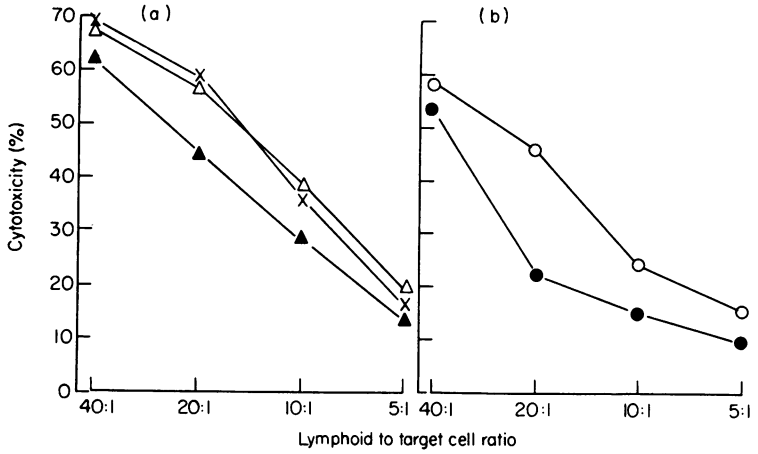

FIG. 1. The effect of erythrocyte lysis on spontaneous cytotoxicity of W/Not rat blood and spleen cells against K562 target cells. Ficoll-Triosil blood lymphocytes were tested before erythrocyte lysis (×) and following lysis with distilled water (△) and Tris-NH_4Cl (▲). Spleen cells were tested after lysis with distilled water (○) and Tris-NH_4Cl (●). The results represent the mean of four experiments.

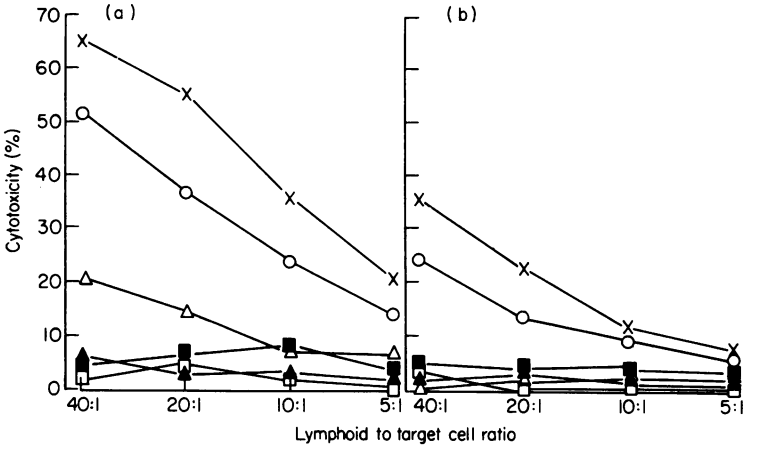

FIG. 2. Cytotoxicity of lymphoid cells from W/Not and PVG/c rats against K562 target cells at ratios of 40:1 to 5:1. Lymphoid cells tested were from blood (×) (B), spleen (○) (S), peripheral lymph nodes (▲) (PL), mesenteric lymph nodes, (△) (ML), thymus (□) (T) and bone marrow (■) (BM). The results represent the mean of five experiments.

Ratio	W/Not	$F_{5,24}$	P	PVG/c	$F_{5,24}$	P
40:1	T BM PL ML S B	23·04	< 0·001	ML PL BM T S B	14·24	< 0·001
20:1	PL T BM ML S B	12·26	< 0·001	ML T PL BM S B	9·34	< 0·001
10:1	T PL ML BM S B	7·03	< 0·001	PL T ML BM S B	8·64	< 0·001
5:1	T PL ML BM S B	6·55	< 0·001	T PL ML BM S B	8·46	< 0·001

Tris-NH_4Cl to lyse contaminating erythrocytes were examined. W/Not rat spleen and blood lymphoid cell preparations were subjected to distilled water or Tris-NH_4Cl treatment and their NC against K562 measured.

Untreated and distilled water treated blood lymphocytes had a similar high level of cytotoxicity but Tris-NH_4Cl-treated cells had significantly lower activity at all four cell ratios tested ($F_{2,36}$, = 11·44,

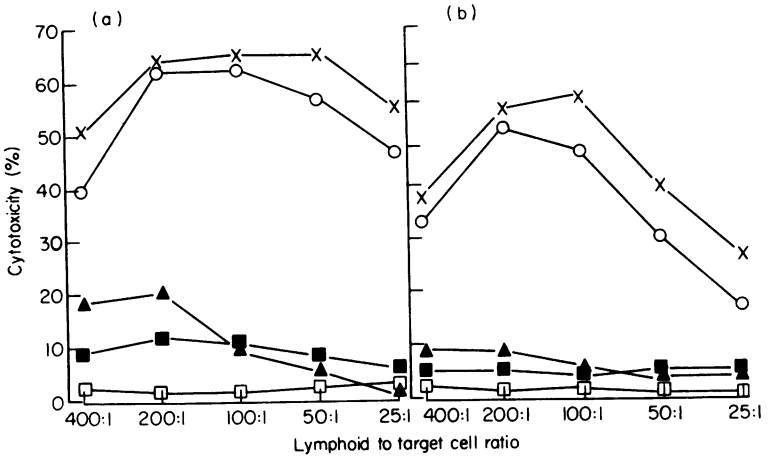

FIG. 3. Cytotoxicity of lymphoid cells from W/Not and PVG/c rats against K562 target cells at ratios of 400:1 to 25:1. Lymphoid cells tested were from blood (×), spleen (○), peripheral lymph nodes (▲), thymus (□) and bone marrow (■). The results represent a typical experiment.

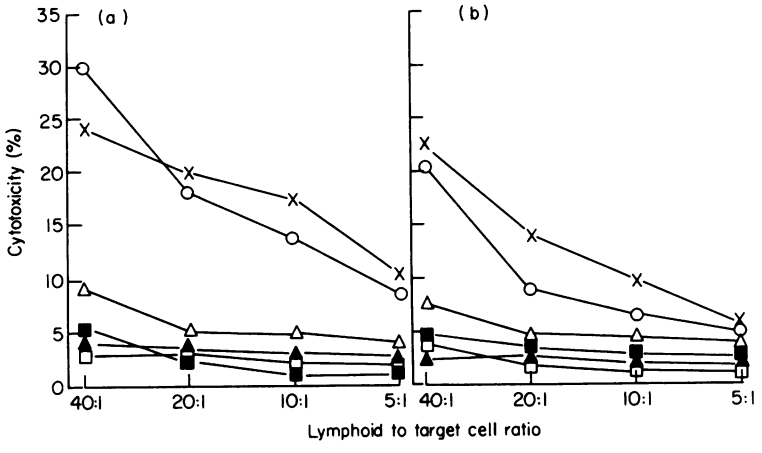

FIG. 4. Cytotoxicity of lymphoid cells from W/Not and PVG/c rats against a rat fibrosarcoma target cell (Mc40) at ratios of 40:1 to 5:1. Lymphoid cells tested were from blood (×) (B), spleen (○) (S), peripheral lymph nodes (▲) (PL), mesenteric lymph nodes (△) (ML), thymus (□) (T) and bone marrow (■) (BM). The results represent the mean of four experiments.

Ratio	W/Not	$F_{5,18}$	P	PVG/c	$F_{5,18}$	P
40:1	T PL BM ML B S	43·99	< 0·001	PL T BM ML S B	29·67	< 0·001
20:1	PL BM T ML S B	7·84	< 0·001	PL T BM ML S B	21·87	< 0·001
10:1	BM T ML PL S B	12·17	< 0·001	PL BM T ML S B	8·19	< 0·001
5:1	BM PL T ML S B	11·75	< 0·001	PL BM T S ML B	1·42	> 0·05

$P<0·001$) (Fig. 1a). In the case of spleen cells, Tris-NH_4Cl treatment resulted in lower cytotoxic activity compared with distilled water treatment (Fig. 1b), although this difference was not significant ($F_{1,4}$ = 2·17, $P>0·05$). In subsequent experiments all cell preparations were treated with distilled water to lyse contaminating erythrocytes.

Organ distribution of spontaneous cytotoxic activity

The percentage cytotoxicity produced by different lymphoid cell populations from W/Not and PVG/c rats when tested against K562 target cells was measured at effector to target cell ratios of 40:1 to 5:1. Fig. 2 shows the average values obtained in five experiments.

In the case of W/Not rats blood lymphocytes produced the highest levels of cytotoxicity and spleen cells also gave high cytotoxicity (Fig. 2a). Mesenteric lymph node cells gave low levels of cytotoxicity at ratios of 40:1 and 20:1 while peripheral lymph node cells, thymus cells and bone-marrow cells produced very low levels of cytotoxicity at all four cell ratios, with values only just above the background. When expressed as lytic units/10^6 cells blood and spleen contained 20·0 and 12·5 units respectively, mesenteric lymph nodes 2·5 and peripheral lymph nodes, thymus and bone marrow less than 1 (Table 1). The results obtained using lymphoid cell populations from PVG/c rats were similar but the levels of cytotoxicity produced were lower than the corresponding values for W/Not rats (Fig. 2b & Table 1). Blood and spleen cell populations again produced the highest cytotoxicity (representing 5·6 and 3·2 lytic units respectively) and cells from peripheral lymph nodes, mesenteric lymph nodes, thymus and bone marrow showed virtually no activity (all giving <1 lytic unit).

TABLE 1. Cytotoxicity of K562 and Mc40 target cells by W/Not and PVG/c rat lymphoid cells, expressed as lytic units/10^6 cells. (Calculated from dose response curves representing the mean values of at least four experiments)

	Lytic units/10^6 cells			
	W/Not effector cells Target cell		PVG/c effector cells Target cell	
Effector cells	K562	Mc40	K562	Mc40
Blood	20·0	5·0	5·6	2·9
Spleen	12·5	4·3	3·2	2·6
Peripheral lymph node	<1	<1	<1	<1
Mesenteric lymph node	2·5	<1	<1	<1
Thymus	<1	<1	<1	<1
Bone marrow	<1	<1	<1	<1

In order to determine if the cell populations with very low levels of cytotoxicity could produce significant cytotoxicity at higher effector to target cell ratios, peripheral lymph node cells, thymus cells and bone-marrow cells from W/Not and PVG/c rats were tested against K562 at ratios of 400:1 to 25:1. Blood and spleen cell preparations were also tested as control populations with cytotoxic activity. Fig. 3 shows an example of such an experiment. In the case of W/Not rats blood and spleen populations again produced high cytotoxicity but activity reached a peak at ratios of 200:1 to 50:1 and declined at the highest ratio (400:1). Peripheral lymph node cells and bone-marrow cells produced slightly more cytotoxicity than was seen at the lower cell ratios with maximum values occurring at a ratio of 200:1, but the levels were still low (maximum values 20% and 12% respectively). Thymus cells again produced no significant cytotoxicity even at the highest ratios. Lymphoid cell populations from PVG/c rats produced similar results but the levels of cytotoxicity were again lower than for the corresponding W/Not populations (Fig. 3b). Blood and spleen cell populations produced maximum cytotoxicity at ratios of 200:1 and 100:1. Peripheral lymph node cells and bone-marrow cells produced very low levels of cytotoxicity and thymus cells virtually no activity.

To determine the NC of rat lymphoid cells against a second cell line tests were performed using the rat tumour-derived Mc40 cells as targets. Lymph node (peripheral and mesenteric) spleen, blood, thymus and bone-marrow lymphoid cell preparations from W/Not and PVG/c rats were tested. The

organ distribution of NC was similar to that seen with K562 target cells and lymphoid cells from W/Not rats again showed higher activity than cells from PVG/c rats (Fig. 4 & Table 1). For both strains of rat the highest levels of NC were produced by blood and spleen-cell populations, mesenteric lymph node cells produced low activity and peripheral lymph node cells and bone-marrow cells almost no activity.

Spontaneous cytotoxic activity of mixed cell populations

In order to determine if lymph node cells, thymus cells or bone-marrow cells, which all produced very little cytotoxicity, were capable of suppressing the high level of activity expressed by spleen cells, cytotoxicity tests were performed using mixed populations of effector cells. Variable numbers of W/Not peripheral lymph node cells, thymus cells or bone-marrow cells were mixed with a constant number of spleen cells and added to K562 target cells. Each effector cell population was also tested alone. Spleen cells were used at an effector to target cell ratio of 40:1 and this population alone produced 50·6% cytotoxicity. When lymph node cells, thymus cells or bone-marrow cells were added to target cells in addition to spleen cells they did not significantly alter the cytotoxicity produced (Table 2). Over the range of lymph node, thymus and bone-marrow cells used (40:1 to 5:1) the cytotoxicity produced in combination with spleen cells was similar to that produced by spleen cells alone (range of values 48·3–53·9% cytotoxicity). Lymph node cells, thymus cells or bone-marrow cells alone produced virtually no cytotoxicity.

TABLE 2. Cytotoxicity of K562 target cells by mixed populations of W/Not rat effector cells

Effector cells	Percentage cytotoxicity			
	4×10^5	2×10^5	10^5	5×10^4
Lymph node+spleen (4×10^5)	48·6	48·9	52·5	53·9
Thymus+spleen (4×10^5)	50·1	49·6	48·9	49·0
Bone marrow+spleen (4×10^5)	48·3	52·8	54·3	49·6
Lymph node only	2·8	0·8	0·5	0·2
Thymus only	1·6	1·0	0·3	0
Bone marrow only	5·3	7·4	5·9	2·3
Spleen only	50·6	—	—	—

Lymph node, thymus and bone-marrow cells, at the concentration shown, were mixed with spleen cells (4×10^5) and added to target cells (10^4).

Spontaneous cytotoxicity following depletion of adherent or phagocytic cells

The effects of adherent and phagocytic cells on NC were examined by testing the cytotoxic activity of depleted W/Not spleen cell populations. Spleen cell populations were depleted of cells adhering to nylon fibre by column filtration or depleted of cells adhering to plastic by incubation in tissue-culture flasks. Phagocytic cells were depleted by removing cells ingesting carbonyl iron using a magnet. Untreated and depleted spleen cell populations were tested for cytotoxic activity using K562 target cells. Table 3 shows the results of a typical experiment. Nylon-fibre-column filtered cells retained their cytotoxic activity and gave values slightly higher than control untreated cells. Depletion by plastic adherence or carbonyl-iron uptake also gave populations which retained a high cytotoxic activity with values equal to, or slightly greater than, untreated cells.

DISCUSSION

This study demonstrates that the K562 human myeloid cell line is highly sensitive to NC by rat lymphoid

TABLE 3. Cytotoxicity of K562 target cells by W/Not rat-spleen cells depleted by nylon-column filtration, plastic adherence or carbonyl-iron uptake

Depletion procedure	Percentage cytotoxicity			
	Effect : Target cell ratio			
	40:1	20:1	10:1	5:1
None	50·9	39·7	23·6	15·0
Nylon column	55·8	44·3	30·1	19·8
Plastic adherence	54·8	43·6	27·2	17·0
Carbonyl iron	50·8	41·3	26·7	14·8

cells in addition to its previously reported susceptibility to killing by human blood lymphocytes (Jondal & Pross, 1975; Pross & Baines, 1976; West *et al.*, 1977) and mouse spleen cells (Haller *et al.*, 1977).

The two rat strains showed different levels of cytotoxicity against K562 target cells, with W/Not rats consistently giving more activity than PVG/c rats. Strain differences in NC have been reported in other systems using mouse (Greenberg & Playfair, 1974; Herberman *et al.*, 1975a; Kiessling *et al.*, 1975a; Sendo *et al.*, 1975; Zarling, Nowinski & Bach, 1975; Haller *et al.*, 1977) and rat (Shellam & Hogg, 1977) lymphoid cells.

The NC produced by cell populations from different lymphoid tissues of the rat showed considerable variation which was most apparent in the case of W/Not rats due to their relatively greater activity, in comparison with the PVG/c strain. Blood lymphocytes and spleen cells gave high levels of cytotoxicity against K562 which were detectable down to a low effector to target cell ratio (5:1). Mesenteric lymph node cells produced a low level of cytotoxicity at ratios above 20:1, while peripheral lymph node cells had even lower activity with significant cytotoxicity at only the highest ratios (200:1 & 400:1). Bone-marrow cells produced a very low level of cytotoxicity which did not increase at high cell ratios and thymus cells gave no significant cytotoxicity at any of the ratios tested (400:1 to 5:1). PVG/c rats showed a similar organ distribution of activity but with lower levels of cytotoxicity. The cytotoxic activity of rat lymphoid populations against rat tumour targets (Mc40), in syngeneic or allogeneic combination was similar to that seen using K562 target cells but with lower levels of cytotoxicity. The rat fibrosarcoma cell line thus showed considerably less susceptibility to NC than the K562 cell line. In comparative studies using cell lines of widely different histogenic derivation it has been found that T-leukaemia cell lines and myeloid-leukaemia cell lines (e.g. K562) are particularly susceptible to this effect. (Jondal & Pross, 1975; Haller *et al.*, 1977).

The cytotoxic activity of cells from various lymphoid organs described in this study show some differences to those reported by other workers using different target cells. In general it has been found that spleen cells have high activity and thymus and bone-marrow cells little or no activity but there are conflicting reports concerning the activity of blood lymphocytes and lymph node cells. High activity was found in mouse lymph node and blood using a Rauscher virus-induced-tumour target cell (Herberman *et al.*, 1975a) and low activity in rat lymph node and blood using a Gross virus-induced-tumour target cell (Shellam & Hogg, 1977).

Low activity has also been reported in mouse lymph node using a Moloney virus-induced-tumour target cell (Kiessling *et al.*, 1975b) and in lymph nodes from cancer patients using K562 as target cell (Vose *et al.*, 1977).

In our study blood lymphocytes consistently produced high cytotoxicity against K562 and Mc40 target cells and lymph node cells low cytotoxicity, with mesenteric lymph node cells showing somewhat more activity than peripheral lymph-node cells.

The experiments using mixed effector cell populations demonstrated that the high cytotoxic activity of spleen cells could not be suppressed by adding lymph node, thymus or bone-marrow cells. This suggests that lymph node, thymus and bone marrow which show low cytotoxicity do not contain a suppressor

element. The low cytotoxicity of these tissues may be due to the fact that the active cell population is either absent from these tissues or present in an inactive form. However, since the migration, lifespan and state of activation of natural killer cells remain to be determined, the significance of these low values is uncertain. It also remains to be determined whether cells from lymph nodes undergoing an active immune response have low NC.

The active cell population in our system was shown to be non-adherent to nylon fibre or plastic surfaces and non-phagocytic. Since nylon-column filtration is known to deplete surface immunoglobulin-positive lymphocytes, as well as monocytes and polymorphs (Julius, Simpson & Herzenberg, 1973; Greaves & Brown, 1974; Potter & Moore, 1977), the results suggest that the active cell is a lymphocyte without B-cell characteristics. In addition the organ distribution of NC does not correspond to the known organ distribution of T lymphocytes. These results are in agreement with several other reports indicating that natural killer cells are lymphocytes which lack the characteristics of T or B cells (Greenberg & Playfair, 1974; Herberman *et al.*, 1975b; Jondal & Pross, 1975; Kiessling *et al.*, 1975b; Kiuchi & Takasugi, 1976; Nunn *et al.*, 1976; Bakács *et al.*, 1977; Haller *et al.*, 1977; Shellam, 1977). It is clear, therefore, that natural cytotoxic cells with similar characteristics are present in human and experimental animals and that these cells have the potential to kill cells of syngeneic, allogeneic and xenogeneic origin, in particular certain cell lines. However, the *in vivo* function of these cytotoxic cells remains to be determined and also their relationship to other cytotoxic and immune mechanisms.

Note added in proof

Since these data were submitted, Oehler *et al.* (1978) have reported the tissue and strain distribution of rat cells with NC for a Gross virus-induced rat lymphoma cell line.

This work was supported by grants from the Medical Research Council and the Cancer Research Campaign. We are indebted to Miss Wendy J. White for skilled technical assistance. Mr M. Palmer and Miss Sheila Hull kindly performed the statistical analyses.

REFERENCES

Bakács, T., Gergely, P., Cornain, S. & Klein, E. (1977) Characterization of human lymphocyte subpopulations for cytotoxicity against tumor-derived monolayer cultures. *Int. J. Cancer*, **19**, 441.

Greaves, M.F. & Brown, G. (1974) Purification of human T and B lymphocytes. *J. Immunol.* **112**, 420.

Greenberg, A.H. & Playfair, J.H.L. (1974) Spontaneously arising cytotoxicity to the P-815-Y mastocytoma in NZB mice. *Clin. exp. Immunol.* **16**, 99.

Haller, O., Kiessling, R., Örn, A., Kärre, K., Nilsson, K. & Wigzell, H. (1977) Natural cytotoxicity to human leukemia mediated by mouse non-T cells. *Int. J. Cancer*, **20**, 93.

Herberman, R.B., Nunn, M.E., Holden, H.T. & Lavrin, D.H. (1975b) Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells. *Int. J. Cancer*, **16**, 230.

Herberman, R.B., Nunn, M.E. & Lavrin, D.H. (1975a) Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. I. Distribution of reactivity and specificity. *Int. J. Cancer*, **16**, 216.

Herberman, R.B., Nunn, M.E., Lavrin, D.H. & Asofsky, R. (1973) Effect of antibody to θ antigen on cell-mediated immunity in syngeneic mice by murine sarcoma virus. *J. Nat. Cancer Inst.* **51**, 1509.

Jondal, M. & Pross, H.F. (1975) Surface markers on human B and T lymphocytes. VI. Cytotoxicity against cell lines as a functional marker for lymphocyte subpopulations. *Int. J. Cancer*, **15**, 596.

Julius, M.H., Simpson, E. & Herzenberg, L.A. (1973) A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Europ. J. Immunol.* **3**, 645.

Kiessling, R., Klein, E., Pross, H. & Wigzell, H. (1975b) 'Natural' killer cells in the Mouse. II. Cytotoxic cells with specificity for mouse moloney leukemia cells. Characteristics of the killer cell. *Europ. J. Immunol.* **5**, 117.

Kiessling, R., Klein, E. & Wigzell, H. (1975a) 'Natural' killer cells in the mouse. I. Cytotoxicity cells with specificity for mouse moloney leukemia cells. Specificity and distribution according to genotype. *Europ. J. Immunol.* **5**, 112.

Kiuchi, M. & Takasugi, M. (1976) The Nonselective cytotoxic cell (N cell). *J. Nat. Cancer Inst.* **56**, 575.

Nunn, M.E., Djeu, J.Y., Glaser, M., Lavrin, D.H. & Herberman, R.B. (1976) Natural cytotoxic reactivity of rat lymphocytes against syngeneic gross virus-induced lymphoma. *J. Nat. Cancer Inst.* **56**, 393.

Oehler, J.R., Lindsay, L.R., Nunn, M.E. & Herberman, R.B. (1978) Natural cell-mediated cytotoxicity in rats. I. Tissue and strain distribution and demonstration of a membrane receptor for the Fc portion of IgG. *Int. J. Cancer*, **21**, 204.

Potter, M.R. & Moore, M. (1975) PHA stimulation of separated human lymphocyte populations. *Clin. exp. Immunol.* **21**, 456.

Potter, M.R. & Moore, M. (1977) The effect of adherent and phagocytic cells on human lymphocyte PHA responsiveness. *Clin. exp. Immunol.* **27**, 159.

Pross, H.F. & Baines, M.G. (1976) Spontaneous human lymphocyte-mediated cytotoxicity against tumour target

cells. I. The effect of malignant disease. *Int. J. Cancer*, **18**, 593.

PROSS, H.F. & JONDAL, M. (1975) Cytotoxic lymphocytes from normal donors. A functional marker of human non-T lymphocytes. *Clin. exp. Immunol.* **21**, 226.

ROSENBERG, E.B., MCCOY, J.L., GREEN, S.S., DONNELLY, F.C., SIWARSKI, D.F., LEVINE, P.H. & HERBERMAN, R.B. (1974) Destruction of human lymphoid tissue-culture cell lines by human peripheral lymphocytes in ^{51}Cr-release cellular cytotoxicity assays. *J. Nat. Cancer Inst.* **52**, 345.

SENDO, F., AOKI, T., BOYSE, E.A. & BUAFO, C.K. (1975) Natural occurrence of lymphocytes showing cytotoxic activity to BALB/c Radiation-induced leukemia RL♂1 cells. *J. Nat. Cancer Inst.* **55**, 603.

SHELLAM, G.R. (1977) Gross-virus-induced lymphoma in the rat. V. Natural cytotoxic cells are non-T cells. *Int. J. Cancer*, **19**, 225.

SHELLAM, G.R. & HOGG, N. (1977) Gross-virus-induced lymphoma in the rat. IV. Cytotoxic cells in normal rats. *Int. J. Cancer*, **19**, 212.

TAKASUGI, M., MICKEY, M.R. & TERASAKI, P.I. (1973) Reactivity of lymphocytes from normal persons on cultured tumor cells. *Cancer Res.* **33**, 2898.

VOSE, B.M., VÁNKY, F., ARGOV, S. & KLEIN, E. (1977) Natural cytotoxicity in man: activity of lymph node and tumor infiltrating lymphocytes. *Europ. J. Immunol.* **7**, 753.

WEST, W.H., CANNON, G.B., KAY, H.D., BONNARD, G.D. & HERBERMAN, R.B. (1977) Natural cytotoxic reactivity of human lymphocytes against a myeloid cell line: Characterization of effector cells. *J. Immunol.* **118**, 355.

ZARLING, J.M., NOWINSKI, R.C. & BACH, F.H. (1975) Lysis of leukemia cells by spleen cells of normal mice. *Proc. Nat. Acad. Sci. (Wash.)*, **72**, 2780.