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DIFFERENTIAL NATURAL KILLER CELL REACTIVITY AGAINST T CELL LYMPHOMAS BY CELLS FROM NORMAL OR STIMULATED MICE[1]

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One of the major problems confronting the study of NK cell populations concerns their potential recognition patterns and specificity. In this study three T cell lymphomas that differ markedly in their susceptibility to NK-mediated lysis from normal mice are shown to bear quantitatively but not qualitatively different degrees of NK target structures. The ability of these tumors to be lysed by NK cells can be drastically changed by augmentation of NK activity by LCM virus, but in a manner that differs relatively for the different targets. Comparison of the growth of these tumors *in vivo* strongly suggests an *in vivo* action for NK cells.

Natural killer cells (NK)[4] have come to represent one of the effector immune cell types that may play a major role in immune surveillance and protection against tumor growth (1, 2). One of the main problems concerning the NK cell population concerns its potential specificity. It has been noted in many studies that different tumor lines show considerable variability in their sensitivity to NK-mediated lysis, and although it was initially thought that viral associated lymphomas were the predominantly susceptible group of tumor targets (3–6), it is now apparent that other tumor types can also be susceptible to NK-mediated lysis (7, 8), and furthermore that certain normal or viral-infected cell types may be lysed by NK cells (1, 2, 9). This "broadening" of the potential target cell types for NK recognition has particularly become evident with the use of various agents that augment NK activity, such as infection with various agents like LCM virus, which may act by elevating interferon levels (10–12).

In this present study, we have concentrated on several aspects of this question of susceptibility of lysis by NK cells and on the related questions of specificity by using three T cell lymphomas that are all quite sensitive to T cell-mediated lysis, but that differ markedly in their reactivity with NK cell populations. The results demonstrate that augmentation of NK activity can change the ability to recognize various tumor targets, and yet no evidence can be found from cold target inhibition studies to suggest that different, i.e., "antigen-specific," target structures exist on these same tumor cell lines.

MATERIALS AND METHODS

Cell lines and tissue culture media. Eagle's minimal essential medium (MEMF) with nonessential amino acids (Grand Island Biological Co., Grand Island, N. Y., Cat. No. F/15) was used for the *in vitro* induction technique described later. Unless other wise stated, it was supplemented with 5% fetal calf serum (FCS), 100 units/ml of penicillin, 100 μg/ml of streptomycin, and buffered with sodium bicarbonate. The medium was prepared fresh each day, and 2-mercaptoethanol (2-ME) was added to a final concentration of 10^{-4} M before used. Dulbecco's modified Eagle's medium (GIBCO) supplemented with 10% FCS (DMEF) was used for all cytotoxicity assays and for tissue culture cell lines. T cell lymphomas WEHI-7 and WEHI-22 (13) of BALB/c origin and YAC-1 of A/Sn origin (3) were maintained as tissue culture cell lines.

Mice. All mice used were of 6 to 8 weeks of age and included BALB/c ByJ from The Jackson Laboratory, BALB/c WEHI and CBA/J from the Walter and Eliza Hall Institute colonies. Athymic BALB/c.nu and CBA.nu were from the Hall Institute colonies, and CBA.nu were also from Dr. R. E. Anderson, University of New Mexico.

LCM treatment. Mice were injected i.p. with 0.1 ml of a suspension of lymphocytic choriomeningitis virus (LCM) (kindly provided by Dr. R. Welsh, Scripps Clinic, La Jolla, California), and spleens were removed from 1 to 7 days later.

In vitro induction of cytotoxic T cells (T_c). The T_c were induced by an *in vitro* method previously described in detail (14). Briefly, alloimmune T_c were induced by mixing 15×10^6 BALB/c.H-2^b or CBA viable spleen cells with 1.5×10^6 irradiated (5000 rads) BALB/c spleen cells in compartments of a tissue culture tray (Sterilin Ltd., Richmond, Surrey, U. K., Cat. No. 306V) containing 4 ml MEMF. These trays were then incubated for 5 days at 37°C in a CO_2 humidified incubator. At the end of that period the cells were harvested and pooled, and the viability was determined by eosin dye exclusion. The *in vitro* allograft reaction produces T_c that are highly active

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[4] Abbreviations used in this paper: NK, natural killer cells; LCM, lymphocytic choriomeningitis virus; MEMF, Eagle's minimal essential medium with fetal calf serum; 2-ME, 2-mercaptoethanol; DMEF, Dulbecco's modified Eagle's medium with fetal calf serum; B/T, blocker-to-target ratio; E/T, effector-to-target ratio; T_c, cytotoxic T cells; TAA, tumor-associated antigens; CL, nucleated effector cells; CL/T, CL-to-target ratio; EDTA, ethylenediaminetetraacetic acid.

against the immunizing alloantigens (15), in this case BALB/c.H-2^b T_c reactive of H-2^d alloantigens, and CBA T_c reactive against BALB/c alloantigens. T_c induced *in vitro* to tumor-associated antigens (TAA) were prepared by using techniques previously described in detail (14, 16). In outline, the method involved co-culturing (BALB/c × C57BL)F_1 hybrid spleen cells (15×10^6) with irradiated WEHI-22 tumor cells (7.5×10^5) under the same conditions as were described for the allogeneic cultures. At the end of the 5-day culture period the cells were harvested, washed, and preincubated in fresh tissue culture medium for 18 hr before assay on day 6. This technique results in T_c that are directed against T lymphoma TAA (16).

In vitro assays: ^{51}Cr release. Nude or normal spleen cells from nonimmune mice were prepared as described previously (6) and tested *in vitro* for NK cell activity on ^{51}Cr-labeled tumor targets, by using a 4-hour lysis assay. T_c induced *in vitro* as described above were also assayed by this technique. The assays were always performed over a range of nucleated effector cell-(CL) to-target (CL/T) ratios, as indicated in the text. Results were calculated as:

$$\text{Percent specific lysis} = \frac{\text{Test count} - \text{background count}}{\text{Maximal count} - \text{background count}} \times 100,$$

as described elsewhere (6), and are presented as the mean ± S.E.M. of quadruplicate determinations.

Cellular competitive inhibition assay. This assay has been described in detail elsewhere (17), and only the essential details are included here. The CL or NK containing spleen preparations were dispensed into the wells of the microtiter tray in 50 μl of DMEF. The viable unlabeled tumor or normal spleen (blocker) cells were added in 50 μl of DMEF. Finally the ^{51}Cr-labeled target cells were added in 100 μl of DMEF, and the contents of each well were mixed. The trays were then incubated at 37°C for 4 to 6 hr. The CL number, target cell number, and hence the CL/T ratio were kept constant, and only the blocker cell number, and hence, the blocker/target (B/T) ratio, was varied. Background and maximal lysis of ^{51}Cr tumor target cells were determined as described previously (6). A control set of four wells, in which the blockers were omitted, was set up to determine lysis in absence of competitive inhibition. The results were calculated for each B/T ratio as:

$$\text{Percent inhibition of cytotoxicity (lysis)} = \frac{\text{mean percent control lysis} - \text{mean percent test lysis} \times 100}{\text{Mean percent control lysis}}$$

In order to adjust this assay for maximum sensitivity, and to minimize nonspecific blocking effects due to the volume of the blocker cells, the conditions were adjusted to produce control levels of lysis in the range 20 to 50%, and blocker cells were added at B/T ratios of 30/1 and less (17).

In vivo tumor growth. Groups of 8 BALB/c and BALB/c.nu mice were injected with 10^6 viable WEHI-7 or WEHI-22 tumor cells (the minimal number that will produce tumors in either nude or normal BALB/c mice). The mice were examined three times a week, and tumor growth was measured as the mean of two diameters at right angles. The results were expressed as the mean ± S.E.M. of these diameters for each group of mice.

Purification of extracellular virus from lymphoma cell lines. Virus was purified from three lymphoma lines according to the procedure used by Ihle *et al.* (18). Cells were grown in a 100-ml spinner culture flask in DMEF until cell density reached 1.5×10^6/ml, at which time the contents were transferred into a 1-liter flask and cells were allowed to attain a density of 1×10^6 cells/ml. One-tenth milliliter of 1×10^{-6} M hydrocortisone and 5 μCi/ml of (^3H)-uridine (Amersham Corp., Ill.) were added, and the final volume was adjusted to 1000 ml and allowed to incubate at 37°C for 24 hr. The medium was clarified by spinning contents in the Sorvall GSA rotor at 5000 rpm for 20 min at 4°C. Virus from clarified medium was pelleted by layering supernatant on top of 5 ml 25% sucrose in PNE buffer (0.05 M sodium phosphate, pH 7.0, 0.1 M NaCl, 0.001 M EDTA[4]) in polycarbonate tubes and spinning in a Beckman SW27 rotor at 25,000 rpm for 1 hr at 4°C. Pellets were resuspended in 4 ml of PNE buffer. Resuspended virus was layered onto 15 to 50% linear sucrose gradients made up in PNE buffer and allowed to spin for 12 hr at 25,000 rpm at 4°C. One-milliliter fractions were collected by dripping from the bottom of the gradients. Refractive index was determined, and the virus band at 36% sucrose was saved. Virus from the band was resuspended in PNE and spun at 25,000 rpm in the SW27 rotor for 1 hr at 4°C. Concentrated virus was stored at −70°C until used.

Velocity sedimentation. Splenic lymphoid cells (8 to 10×10^8 cells), depleted of erythrocytes in 30 ml of phosphate-buffered saline (PBS) with 0.5% bovine serum albumin (BSA; Sigma Chemical Co.), were loaded into the sedimentation chamber of the Staput apparatus (John Scientific, Toronto, Canada) and allowed to sediment at 4°C for 3½ hr through a 1000-ml gradient of 1 to 2% BSA. A cone volume of 350 ml was collected and discarded, followed by the collection of 13 or 14 50-ml fractions. The fractions were spun and washed with DMEF. Cell numbers and viability were determined. Various fractions were pooled as indicated and used in cytotoxicity assays by using an effector-to-target (E/T) ratio of 50:1.

Nylon wool column fractionation. A modified procedure as described by Julius *et al.* was used (19). Scrubbed fibrous nylon (0.85 g) (Fenwal lab., Deerfield, Ill.) was packed into a 20-ml syringe to a final volume of 15 ml. The column was autoclaved for 20 min. Columns were equilibrated with DMEF (at room temperature or at 37°C) for 1 hr before cell loading. 4×10^8 cells in 4 to 5 ml were loaded onto the column, the medium was allowed to run through, and column and cells were incubated for 30 min further. The cells were eluted at a flow rate of 1 ml/min through a 23-gauge needle. Thirty milliliters were collected as a first fraction. The needle was removed and a further 50-ml run through was collected as fraction 2. Finally, a 50-ml ringette fraction was collected by compressing medium through the nylon wool. Cells were washed with DMEF, counted, and set up for assay.

RESULTS

Susceptibility of BALB/c T lymphomas to NK cell-mediated lysis. WEHI-22 and WEHI-7 are both radiation-induced BALB/c T lymphomas, which are known to share certain TAA (16) and to produce abundant C type virus. The tumors markedly differed, however, in susceptibility to NK cell-rich suspensions derived from the spleens of BALB/c and CBA nude and normal mice, in that less lysis of WEHI-22 tumor cells occurred than with the WEHI-7 tumor target when tested in the ^{51}Cr release assay (Table I). This difference was most marked with BALB/c or BALB/c.nu effectors.

Comparison of WEHI-7 and WEHI-22 for susceptibility to T-mediated lysis. Since different cell lines may release ^{51}Cr at different rates after damage (20), experiments were performed with T_c induced *in vitro* to alloantigens and T lymphoma TAA in order to determine whether WEHI-22 was inherently more resistant to cell-mediated *in vitro* lysis than WEHI-7. The

TABLE I

Comparison of natural cytotoxicity of nucleated spleen cells from two mouse strains on two BALB/c T lymphomas

Strain	No. of Expts.	Mean % Specific Lysis (WEHI-7)	No. of Expts.	Mean % Specific Lysis (WEHI-22)
BALB/c.nu	20	40[a]	6	6
BALB/c	8	22	2	4
CBA.nu	6	36	2	25
CBA	3	10	2	5

[a] Values are the mean specific lysis for the indicated number of experiments performed at CL/T of 50/1.

activity of these T_c was then compared to that of NK cells derived from BALB/c and CBA nude spleen in ^{51}Cr release assays performed simultaneously with both ^{51}Cr-labeled WEHI-7 and WEHI-22 as targets.

The results demonstrated that ^{51}Cr-labeled WEHI-7 and WEHI-22 were equally sensitive to T_c induced *in vitro* to alloantigens (Fig. 1). When Figures 1*A* and 1*B* are compared, it can be seen that CBA T_c induced *in vitro* to BALB/c alloantigens (CBA anti BALB/c) and the BALB/c.H-2^b T_c induced *in vitro* to H-2^d alloantigens (BALB/c.H-$2^b\alpha$BALB/c) mediated comparable levels of lysis of both targets over the same CL/T ratio range. A similar result was obtained with (BALB/c × C57BL)F_1 T_c induced *in vitro* to WEHI-22 TAA by *in vitro* coculture of (BALB/c × C57BL)F_1 spleen cells with irradiated WEHI-22 cells. The T_c lysed both ^{51}Cr-labeled tumor targets WEHI-22 and WEHI-7 equally (Fig. 1*C*). However, in the same experiment the susceptibility of the same targets to NK cell-mediated lysis was very different. It can be seen that WEHI-7 was susceptible to NK cell-mediated lysis by BALB/c nude spleen cells, whereas WEHI-22 was totally resistant (Fig. 1*D*).

Kinetics of LCM virus induction of NK activity against T lymphomas. The ability of splenic NK cells to lyse T lymphomas could be augmented by the i.p. injection of LCM virus. Spleens from LCM virus-infected and control mice were tested for NK activity against several targets. As shown in Figure 2, left, YAC-1 tumor is highly susceptible to normal splenic NK cells of BALB/c ByJ, WEHI-7 was moderately susceptible, and WEHI-22 was completely nonsusceptible. The level of NK activity was clearly augmented by LCM virus injection as early as day 1 post-injection (Fig. 2, center). Spleens from mice that were given LCM virus 3 days before testing (Fig. 2, right) demonstrated extremely high levels of lysis against all three lymphomas, with now almost identical CL/T titration curves. In other experiments using LCM virus to augment lysis, the optimal enhancement was observed to be at day 3 or 4 post-injection, with enhanced activity declining by day 7.

Velocity sedimentation separation of NK activity. Velocity sedimentation has been shown to separate cells principally with respect to cell size. Because of the lability of NK cells at 37°C, the sedimentation procedure was carried out at 4°C. The profiles of total cell distribution from normal BALB/c ByJ spleen cells (Fig. 3*A*) and of LCM injected BALB/c ByJ spleen cells (Fig. 3*B*) were similar and reproducible in at least four different experiments, with the peak cell recovery appearing at fraction 8. The NK activity of the different fractions was tested by pooling several fractions and by using different ^{51}Cr-labeled targets. The results in Figure 3*C* and Figure 3*D* clearly show that the normal splenic NK activity is preferentially found in cells of small to medium size range, but is quite heterogeneous and spread over a wide range. Spleen cells from mice that have been treated with LCM virus maintained a similar total cell sedimentation profile, but have been shown to be capable of killing a much greater range of targets (*cf.* virtually no killing of

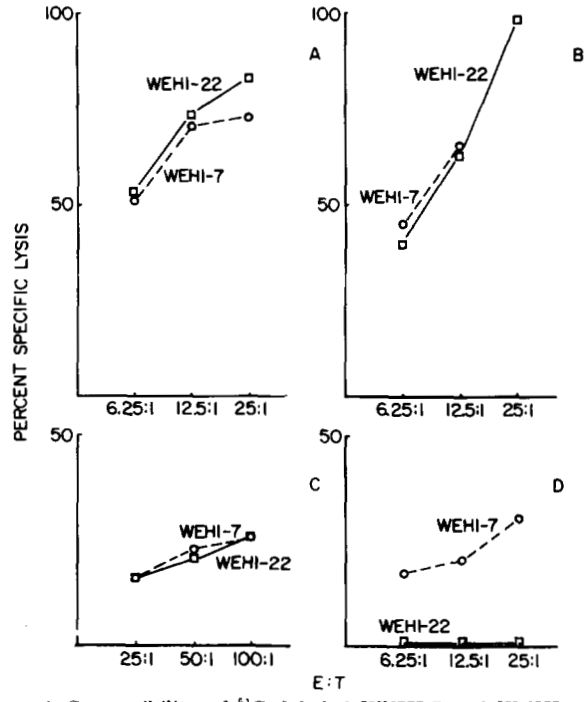

Figure 1. Susceptibility of ^{51}Cr-labeled WEHI-7 and WEHI-22 to alloimmune and tumor T_c populations, in contrast to NK containing populations. *A*, effector population BALB/c.H-2^b anti-BALB/c T_c culture; *B*, effector population CBA anti-BALB/c T_c; *C*, effector population (BALB/c × C57)F_1 anti-WEHI-22 T_c population (1b); *D*, effector population BALB/c.nu spleen cells as NK source.

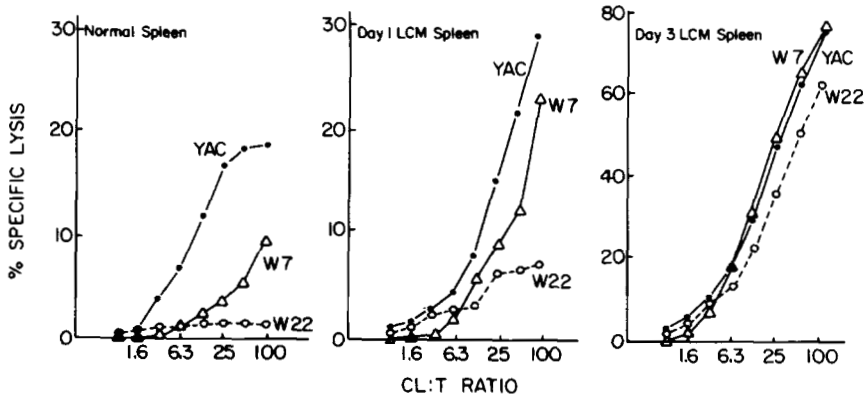

Figure 2. Enhancement of NK activity in spleen cell populations from BALB/c mice pretreated (1 or 3 days previously) with LCM virus. Percent lytic activity against ^{51}Cr-labeled targets is shown for various E/T ratios.

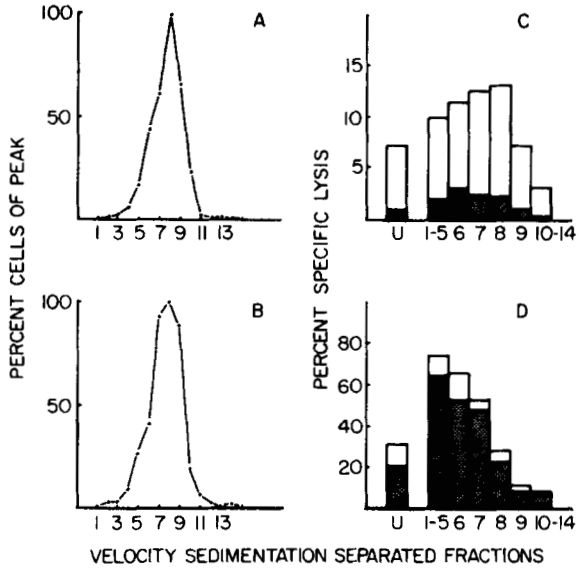

Figure 3. Velocity sedimentation separation of NK activity. *A*, total cell profile of normal spleen; *B*, total cell profile of LCM-treated spleen; *C*, lytic activity of each fraction from normal cell separation against ^{51}Cr-labeled YAC-1 (*open*) or WEHI-22 (*shaded*) by using an E/T ratio of 50:1; *D*, same as for *C* with spleen cell fraction from LCM-treated mice.

WEHI-22 by fractions from normal spleen, Fig. 3*C*). When LCM spleen cells were sedimented and fractions were tested for NK activity on WEHI-22 and YAC-1 (Fig. 3*D*), distinctly different cytotoxicity profiles were obtained compared with those obtained from normal spleen cells (Fig. 3*C*). NK activity was still spread over a wide size range, but the larger cell fractions (1 through 5) now contained the greatest degree of NK activity for both targets.

Cellular competitive inhibition studies. The specificity of NK cell lysis was examined by the cellular competitive inhibition assay by using spleen cells from CBA/.nu mice and various cell lines as ^{51}Cr-labeled targets and blockers. As shown in Figure 4*A–C*, the three T cell lymphomas were susceptible to CBA/nu spleen cells to varying degrees, and all three cell lines appeared to be capable of inhibiting the lysis of the three targets. With all three targets tested, YAC-1 was the most effective blocker, although some specificity was perhaps observed with WEHI-7 and WEHI-22, in that when ^{51}Cr-labeled WEHI-22 was used as target, unlabeled WEHI-7 was less effective than WEHI-22 in blocking the lysis. In over six experiments performed by using the three T lymphomas as blockers, the relative blocking abilities of the three tumors remains unchanged, YAC-1 always blocking best, whereas WEHI-7 and WEHI-22 were similar in their ability to block. At the ratios used in these assays, nonspecific inhibition of lysis has usually not been observed (17). Since BALB/c.nu spleen cells were able to lyse WEHI-7 but not WEHI-22, we also tested the ability of WEHI-7 and WEHI-22 to block the lysis of ^{51}Cr-labeled WEHI-7 by using BALB/c.nu spleen cells. As shown in Figure 4*D*, both lines were capable of mediating such inhibition. The control BALB/c normal thymus or fetal liver cells did not inhibit lysis.

The results of blocking experiments that utilized spleen cells from mice injected with LCM virus are shown in Figure 5*A–C*. The same trends were observed for the three tumor lines tested, in that YAC-1 was the most effective blocker with all targets, with little difference between WEHI-7 and WEHI-22. In studies

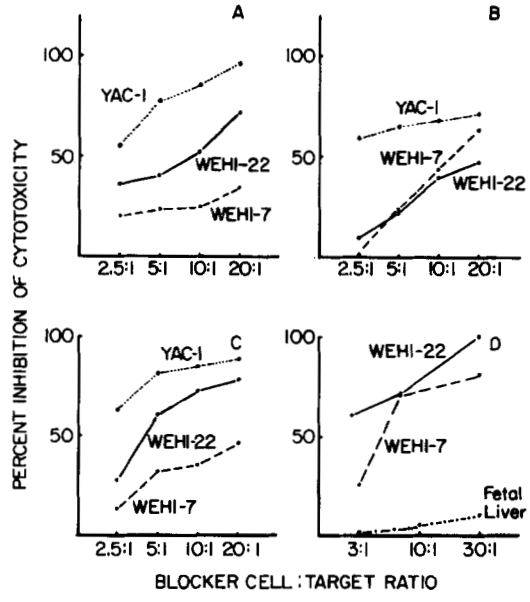

Figure 4. Inhibition of nude splenic NK-mediated lysis by T cell lymphomas. Each graph shows the cold target inhibition assay expressed as percent inhibition of lysis of the indicated ^{51}Cr-labeled target by the three (unlabeled) T cell lymphoma lines used at various B/T. *A–C*, involve CBA.nu spleen cells as the NK source with ^{51}Cr-targets: YAC-1 (*A*), WEHI-7 (*B*), and WEHI-22 (*C*). *D*, BALB/c.nu spleen cells are used as NK source with WEHI-7 target cells. Control percent lysis by CBA.nu spleen cells for WEHI-7, WEHI-22, and YAC-1 were 24.1 ± 1.9, 13.8 ± 0.5, 29.7 ± 1.5, respectively. Control percent lysis by BALB/c.nu spleens for WEHI-7 was 31.0 ± 2.0.

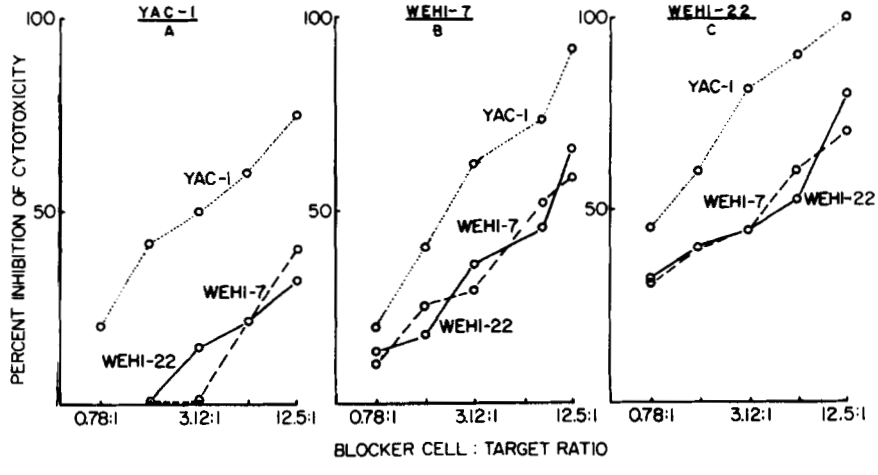

Figure 5. Inhibition of LCM virus augmented NK-mediated lysis by T cell lymphomas. Spleen cells from BALB/c ByJ mice that were given LCM virus i.p. 3 days previously were used as effector cells. Cold target inhibition of the lysis of ^{51}Cr-labeled YAC-1 (*A*), WEHI-7 (*B*), and WEHI-22 (*C*) by unlabeled YAC, WEHI-7, and WEHI-22 are shown. An E/T of 50:1 was used.

to be reported elsewhere (Tai, A. and Warner, N. L.), we have observed that passage of NK-containing spleen cell suspensions over nylon wool columns held at room temperature can result in a relative enrichment of NK activity in the adherent/eluted ringette fraction. The use of these cells in blocking assays helps to reduce nonspecificity, since a smaller (but enriched) number of effector cells can be used. Results of inhibition experiments by using such a population derived from fractionation of LCM-activated spleens are shown in Figure 6*A–C*. All three T lymphomas were lysed to a similar extent by the effector cell population. However, again YAC-1 was by far the most efficient blocker, and WEHI-7 and -22 were similar in their ability to block lysis.

The results of these various inhibition experiments are summarized in Table II, which clearly shows that regardless of the NK population used as effector and regardless of the ^{51}Cr-labeled tumor target used, YAC-1 has considerably greater inhibitory activity, and WEHI-7 and WEHI-22 are virtually identical, regardless of their different susceptibilities to lysis by certain effector populations.

Virus blocking assays. Since WEHI-7 and WEHI-22 are known to both produce abundant extracellular RNA virus, it was thought pertinent to examine whether viral determinants are involved in the recognition of these targets by the NK effector cells. Extracellular virus was purified from the medium of log-phage cells by using the procedure of Ihle *et al.* (18) as described in *Materials and Methods*, and was banded by velocity sedimentation followed by equilibrium density centrifugation. The ability of V-WEHI-7 (virus from WEHI-7), V-WEHI-22, and V-YAC to act as blockers for activity of LCM spleen cells was examined. In no case were any of the viruses able to negate the activity of the spleen cells on any of the three targets. Although we cannot detail the absolute viral content of these fractions, it is to be stressed that the viral preparations used would be equivalent to the virus production from tumor cells if used as inhibitors, at a ratio of 100:1 B/T, if all virus produced by those cells were recovered in our fractionation scheme.

Growth rate of WEHI-7 and WEHI-22 in nude and normal BALB/c mice. In vivo experiments were conducted in which 10^6 viable WEHI-7 or WEHI-22 tumor cells were inoculated into BALB/c and BALB/c.nu mice. The results demonstrated

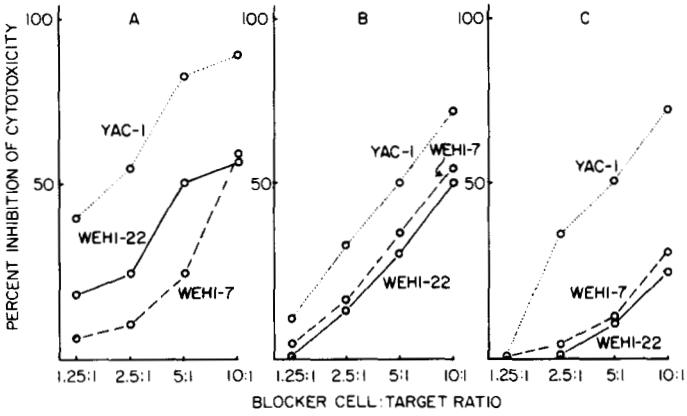

Figure 6. Inhibition of nylon wool fractionated splenic NK-mediated lysis by T cell lymphomas. Spleen cells from BALB/c ByJ mice treated 3 days previously with LCM virus were fractionated on nylon wool column and cells eluted as fraction 3 were used as effector cells. Inhibition of the lysis of ^{51}Cr-labeled YAC-1 (*A*), WEHI-7 (*B*), and WEHI-22 (*C*) by unlabeled blockers YAC-1, WEHI-7, and WEHI-22 were compared by using an E/T of 20:1.

TABLE II

Inhibitory activity of T cell lymphomas in various NK lytic assays

^{51}Cr-Labeled Tumor Target	NK Effector Population	No. of Cells for 50% Inhibition[a] (× 10^4)		
		YAC-1	WEHI-7	WEHI-22
YAC-1	LCM spleen	5	20 (25%)	13 (40%)
WEHI-7	LCM spleen	13	~50 (26%)	~50 (26%)
WEHI-22	LCM spleen	13	25 (50%)	25 (50%)
YAC-1	Nylon adherence fraction	3	18 (16%)	25 (12%)
WEHI-7	Nylon adherence fraction	2	7 (28%)	7 (28%)
WEHI-22	Nylon adherence fraction	1	5 (20%)	5 (20%)

[a] Data calculated from Figures 5 and 6. Values shown in brackets represent the percent inhibitory activity of WEHI-7 and -22 as contrasted with YAC-1.

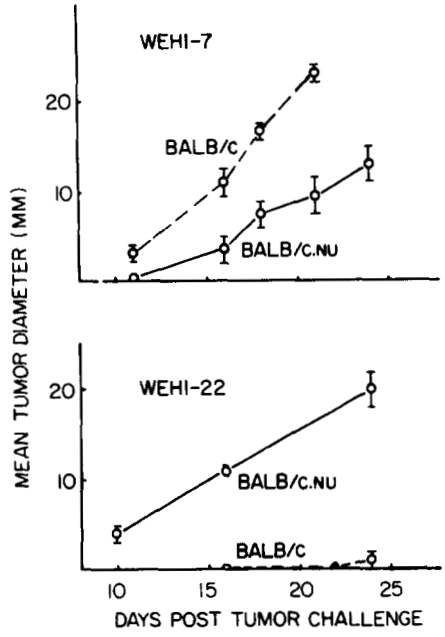

Figure 7. Comparison of the growth of WEHI-7 and WEHI-22 in normal and nude BALB/c mice. *A*, growth of WEHI-7. The lines, O-----O, (BALB/c); and O——O, (BALB/c.nu), are the growth curves of WEHI-7 in normal and homozygous nude BALB/c mice after a s.c. injection of 10^6 tumor cells. *B*, growth of WEHI-22. The lines, O-----O, (BALB/c); and O——O, (BALB/c.nu), are the growth curves of WEHI-22 in normal and homozygous nude BALB/c mice after a s.c. injection of 10^6 tumor cells.

that WEHI-7 grew significantly better in BALB/c than in BALB/c.nu mice (Fig. 7*A*), and by day 25, only two out of eight nude mice had died from tumor growth, whereas six out of eight controls were dead. However, for WEHI-22 the converse applied. This tumor grows extremely poorly in normal mice by using an inoculum of 10^6 cells, and successful growth usually requires a greater number of cells. However in BALB/c.nu mice, the inoculum of 10^6 cells results in fatal tumor growth in all mice at a rapid growth rate (Fig. 7*B*).

DISCUSSION

Two BALB/c radiation-induced, C-type RNA viral-producing thymic (T) lymphomas, WEHI-7 and WEHI-22, were examined for their susceptibility to *in vitro* lysis by NK cells and their growth in syngeneic homozygous nude and normal BALB/c mice. Both tumors were equally susceptible to T_c-mediated

killing in the ^{51}Cr release assay when the antigens involved were major histocompatibility antigens or TAA. There were, however, marked differences in their susceptibility to killing by NK cells by using normal and nude spleen cell suspension. WEHI-7 was susceptible to lysis by NK cell populations to which WEHI-22 was relatively resistant. This distinction was most clearly evident with BALB/c-derived spleen cell suspensions, whereas CBA spleen cells were capable of mediating some lysis of WEHI-22 cells. The strain dependence of NK cell-mediated lysis has also been observed with other tumors (6), and current studies are attempting to determine whether there is a specific genetic control involved.

The different susceptibility of WEHI-7 and WEHI-22 to BALB/c.nu-derived NK lysis may indicate: i) that the determinant expressed on WEHI-7 and recognized by NK cells is not present on WEHI-22; ii) that both tumors express this determinant but its manner of presentation on WEHI-22 is such as to exclude cell-mediated lysis; or iii) that both tumors express the determinant in a similar fashion, but that these cell lines differ in their ability to be lysed by NK cells. However, their almost identical sensitivity to T_c-mediated lysis suggests that resistance to cell-mediated lysis *per se* is not the explanation. These two T cell lymphomas were then further studied in comparison with YAC-1, a widely used NK susceptible cell target (1), and compared with NK cell populations obtained from mice where activity was augmented with LCM virus. The third possibility listed above was further negated by the striking susceptibility of WEHI-22 to LCM-augmented NK-mediated lysis. Over the 3-day period post-injection, although the overall activity of NK lysis against all three tumors increased, a striking relative change in susceptibility of lysis of YAC-1 *vs* WEHI-22 occurred.

This observation poses the question as to whether a distinct new subset of NK cells emerges after LCM activation, or whether a relative change in the activity of a single subpopulation occurred. The data with velocity sedimentation certainly indicated a relative shift in the size profile of the NK population after LCM activation. This may reflect a "blastogenic" type response in the NK population as a result of interferon-mediated activation. The specificity of NK cell-mediated lysis was then extensively studied by using the cold target cellular competitive inhibition assay. The results obtained with all sources of NK cell populations demonstrated that all three T cell lymphomas could block lysis regardless of the target tumor used. This included combinations with effector populations that could not even lyse the WEHI-22 tumor, e.g., BALB/c.nu. In all instances, the YAC-1 tumor showed a greater efficiency to inhibit lysis, in that fewer YAC-1 cells were required for inhibition of lysis than for the other tumors. However, within the limits of this assay (17), we would not conclude that YAC-1 had both "unique" antigen(s) and "common" antigen(s) (with WEHI-7 and WEHI-22). Rather, since YAC-1 was more efficient in inhibition even when a WEHI tumor was the target, we would suggest that YAC-1 possesses more of the target cell structure per cell that is "recognized" by the NK population. The efficiency of blocking at relatively low B/T ratios, such as with the nylon adherence/eluted fraction of NK cells (Fig. 6), is well above the level of "nonspecific" blocking in this assay system (17).

Analogous findings have been obtained in a study of the NK cell phenomenon by Kiessling *et al.* (21), who reported that YLI, a C57L Moloney lymphoma, could completely inhibit the lysis of ^{51}Cr-labeled YAC-1 when added to an assay of CBA spleen cells and ^{51}Cr YAC-1. However, this same tumor line was totally resistant to lysis when labeled with ^{51}Cr and mixed with CBA spleen cells in a cytotoxicity assay.

Accordingly, we propose that the inhibition experiments probably indicate a real presence of a similar determinant on WEHI-7, WEHI-22, and YAC-1 cells that can be "recognized" by NK cells. Whatever the determinant(s) is that NK cells recognize, it is not present on normal adult or fetal lymphoid cells. The possible role of lymphoma-associated viral components, however, must be considered. WEHI-22 produces relatively large amounts of C-type viral particles, whereas WEHI-7, by comparison, produces only relatively small amounts (T. Mandel, personal communication). It is therefore possible that when WEHI-22 is used as a labeled target, the released virus blocks the "receptor" sites on the NK cells, thus preventing direct lysis of the intact WEHI-22 cell. In the cellular competitive inhibition assay, WEHI-22 would thus be expected to compete in the same way. However, this would not explain the susceptibility of WEHI-22 to NK cells from LCM-treated mice. Furthermore, no direct evidence supporting a role for extracellular viral structures as target sites for NK cells could be found in this study, in that isolated C-type viral particles completely failed to inhibit NK lysis. This result thus does not lend support to other claims of viral components acting as NK targets (22).

Our present interpretation would thus favor the view that there is but one common NK target structure, which, however, requires a rather specific "fit" with the NK cell to permit lysis to occur. This infers that in NK-mediated lysis, as in T_c-mediated lysis (23), at least two events occur. First, there is an interaction between NK cells and the target cell, and then an event mediated by the NK cell that initiates lysis of the target. This concept suggests that the first of these two events can occur without the second (i.e., blocking of NK by an insusceptible target), and that changes in either the effector NK cell type or "avidity" changes in the one NK population can alter the efficiency of the second event, i.e., as in the ability of LCM activated NK cells to now lyse WEHI-22.

Clearly this concept requires further evidence that the same NK population is involved when derived from either normal or LCM-augmented mice, and the present recognition of several NK specific alloantigens[5] (24) provides an approach to the further characterization of the NK populations.

Another major issue of relevance to NK studies concerns the potential *in vivo* role of NK cell populations. The present *in vivo* studies provide further support for the propositions put forward on the basis of previous studies of the growth of lymphoid tumors in nude and normal mice (25). The WEHI-7 tumor cell line, which is susceptible to *in vitro* lysis by nude spleen cells, grew at a significantly slower rate in BALB/c nude mice compared with BALB/c normal mice. The converse, however, also held, in that WEHI-22, which was resistant to *in vitro* lysis with BALB/c spleen cells, grew better in the BALB/c nude than in the normal mice. Since a similar phenomenon has also been demonstrated for a BALB/c Abelson virus-induced myeloid leukemia, WEHI-265 (25), it is probable that NK cell-mediated *in vitro* lysis is a real measure of an *in vivo* effector mechanism against at least some tumors. The results with WEHI-22 are those anticipated on the basis of a T cell-mediated immune surveillance concept (26), whereas the results with WEHI-7 indicate the possible importance of NK cells in immune surveillance.

[5] Burton, R. C., and H. J. Winn. The natural killer (NK) cell phenomenon. An analysis of murine lymphoid NK, solid NK, and cultured NK cells by antigenic markers. Submitted for publication.

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