VULNERABILITY OF CULTURED CANINE LUNG TUMOR CELLS TO NK CELL-MEDIATED CYTOLYSIS

PRINCIPAL INVESTIGATORS

P. J. Haley

J. M. Kohr

G. Kelly

B. A. Muggenburg

R. A. Guilmette

Abstract — Five cell lines, designated as canine lung epithelial cell (CLEP), derived from radiation-induced canine lung tumors and canine thyroid adenocarcinoma (CTAC) cells were compared for their susceptibility to NK cell-mediated cytolysis using peripheral blood lymphocytes from normal, healthy Beagle dogs as effector cells. Effector cells and chromium-51 radiolabeled target cells were incubated for 16 h at ratios of 12.5:1, 25:1, 50:1, and 100:1. Increasing cytolysis was observed for all cell lines as the effector-to-target-cell ratios increased from 12.5:1 to 100:1. The percent cytotoxicity was significantly less for all lung tumor cell lines as compared to CTAC at the 100:1 ratio. One lung tumor cell line, CLEP-9, had 85% of the lytic vulnerability of the CTAC cell line and significantly greater susceptibility to NK cell-mediated lysis than all of the other lung tumor cell lines. Susceptibility to NK cell cytolysis did not correlate with in vivo malignant behavior of the original tumor. These data suggest that cultured canine lung tumor cells are susceptible to NK cell cytolytic activity in vitro and that at least one of these cell lines (CLEP-9) is a candidate for substitution of the standard canine NK cell target, CTAC, in NK cell assays. The use of lung tumor cells in NK cell assays may provide greater insight into the control of lung tumors by immune mechanisms.

Natural killer (NK) cells are large granular lymphocytes that mediate nonspecifically, and without immunologic memory, the cytolysis of a wide range of target cells that include tumor cells, virus-infected cells, lymphoblasts and hematopoietic primordia.[1] These cells may represent the first line of defense in immunosurveillance against neoplastic cells. However, susceptibility of tumor cells to NK cell-mediated cytotoxicity varies among tumor cell clones in vitro and is suspected to occur in vivo also. Invulnerability of tumor cells to cytotoxic mechanisms may contribute to the malignant behavior of a given tumor. Thus, during the development of a neoplasm, tumor cells that are vulnerable to immune mechanisms, specific or nonspecific, may be eliminated leaving subpopulations of cells that are resistant to anti-tumor immune responses.

The purpose of this study was to evaluate the susceptibility of five cell lines derived from radionuclide-induced dog lung tumors to NK cell-mediated cytolysis and compare the results to the susceptibility of a recognized canine NK target cell line, canine thyroid adenocarcinoma (CTAC).[2]

MATERIALS AND METHODS

Six normal, unexposed male Beagle dogs, 1.5 to 2 yr of age were used as a source of peripheral blood lymphocytes. Approximately 16 mL of peripheral blood were obtained by venipuncture of the jugular vein and collected in tubes with EDTA anticoagulant. Dogs used for blood collection were maintained in their kennels, not in cages, and food was not withheld before blood collection. Dogs used for this study were derived from unassigned dogs in the LRI colony.

Whole blood was layered on lymphocyte separation medium (Isolymph) and centrifuged at 1500 rpm for 30 min. Lymphocytes were collected from a dense band at the interface. Cells were washed

twice in incomplete medium (RPMI 1640) followed by counting and resuspension at a concentration of 1×10^7 cells/mL of complete medium (RPMI 1640 supplemented with 10% fetal bovine serum).

NK cell cytolytic activity was evaluated using the following assay. Target cells were maintained by continual cell culture in complete medium and harvested three days after passage for use in the assay. A total of 2×10^6 cells were incubated with 100 μCi $Na_2\,^{51}CrO4$ for 1 h, washed twice in incomplete medium and resuspended in 20 mL of complete medium. Radiolabeled target cells were incubated with effector cells in wells of a 96-well microtiter plate at effector-to-target-cell ratios of 100:1, 50:1, 25:1, and 12.5:1 in a total volume of 0.2 mL per well. Each sample was run in quadruplicate. Spontaneous release wells contained 0.1 mL of radiolabeled target cells and 0.1 mL of complete medium. Total release wells contained 0.1 mL of radiolabeled target cells to which was added 0.1 mL of 1% Triton X 100 at the end of the incubation period. The 96-well plates were centrifuged at 300 rpm for 3 min and then incubated for 16 h at 37°C/5% CO_2. The supernatant was harvested after incubation using a Skatron supernatant harvester and the samples were counted on a Beckman Model 5500 gamma counter. The percent cytotoxicity was calculated as follows:

$$\% \text{ Cytotoxicity} = \frac{\text{CPM Sample} - \text{CPM Spontaneous Release}}{\text{CPM Total Release} - \text{CPM Spontaneous Release}} \times 100$$

Background CPM was subtracted from each sample before calculations were made.

Individual lung tumor cell lines (CLEP) were numbered sequentially. Each time a single tumor cell line was tested, canine thyroid adenocarcinoma (CTAC) cells were tested in parallel. The CTAC cell line is a well-recognized target cell that is used as the standard target in NK cell assays of dog lymphocytes.[2] The passage number (p) of each cell line before its use in the cytolytic assay was as follows: CLEP-4 - p14, CLEP-6 - p4, CLEP-9 - p4, CM-2 - p3, and CLEP-10 - p3. The tumor cell line designated CM varies from the others by being of mesenchymal origin.

RESULTS

We noted a clear dose response of cytolytic activity as the effector-to-target-cell ratio was increased from 12.5:1 to 100:1 for all target cells tested (Fig. 1). Maximum cytolytic activity observed at the 100:1 ratio ranged from approximately 41 to 65%. At this effector-to-target-cell ratio, all values for the lung tumor cell lines were significantly less than the values for the CTAC cell line ($p < 0.05$). When the cell lines were compared among themselves, it was found that cell line CLEP-9 was statistically different from each of the other cell lines and CLEP-10 was statistically different from CM-2 and CLEP-4 as well.

The percent cytotoxicity of each cell line was also evaluated as a percentage of the CTAC response by using only results of the 100:1 effector-to-target-cell ratio (Fig. 2). Lung tumor cell line CLEP-9 was found to be statistically different from each of the other cell lines and had approximately 85% of the lytic susceptibility of the CTAC cell line.

DISCUSSION

These data indicate that while cultured canine lung tumor cells are susceptible to NK cell-mediated lysis, they are less sensitive than the standard target cell, CTAC, typically used in canine NK cell assays.[2] Nevertheless, the overall percent cytotoxicity range of 41 to 65% for lung tumor cells suggests that these tumor cell lines might be useful and more relevant than the CTAC cell line when investigating specific immune responses against lung tumors. In particular,

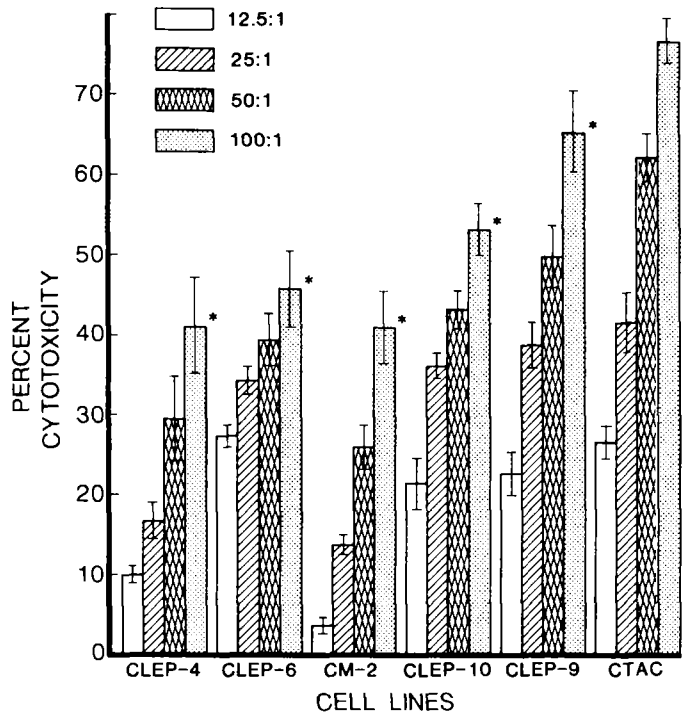

Figure 1. Percent cytotoxicity for individual lung tumor cell lines and canine thyroid adenocarcinoma (CTAC) cells. Each target cell was tested at four effector (lymphocyte)-to-target-cell ratios: 12.5:1, 25:1, 50:1, and 100:1. N = 6. * = The response for the CTAC cell line was significantly different than those of all other lung tumor cell lines ($p < 0.05$). Bars = standard error.

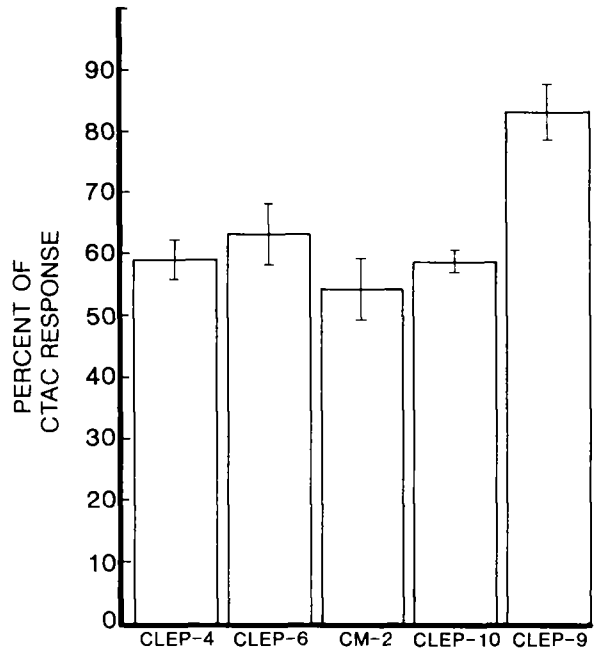

Figure 2. Percent cytotoxicity for individual lung tumor cell lines reported as a percentage of the percent cytotoxicity of canine thyroid adenocarcinoma (CTAC) cells. N = 6. Cell line CLEP-9 was found to be statistically different from each of the other cell lines ($p < 0.05$). Bars = standard error.

tumor cell line CLEP-9 had 85% of the susceptibility of the CTAC cell line to NK cell-mediated lysis, indicating that it is a good candidate for substitution for CTAC in this assay.

The observation that the percent cytolysis increased with increased concentration of lymphocytes indicates that lysis of the tumor cells was a product of the lymphocytes present. These results were consistent findings for each cell line tested and confirm the reliability of their use in this assay.

Susceptibility to NK cell-mediated lysis was not associated with the histologic diagnosis of the original tumor (Table 1). Although the tumors from which the cell lines CLEP-6, CLEP-10, and

Table 1

Diagnosis and Metastatic Qualities of Original Lung Tumors With Vulnerability to NK Cell-Mediated Cytolysis of Derived Lung Tumor Cell Lines

Lung Tumor Cell Line	Diagnosis	Metastasis Outside the Lung	Percent Cytotoxicity[a]
CLEP-4	Large cell, anaplastic adenocarcinoma	+++	40.9 (± 6)[b]
CLEP-6	Adenosquamous carcinoma	+	45.6 (± 5)
CM-2	Undifferentiated sarcoma	-	40.8 (± 4)
CLEP-10	Adenosquamous carcinoma	-	52.9 (± 3)
CLEP-9	Adenosquamous carcinoma	+	65.0 (± 5)

[a]Percent cytotoxicity at the 100:1 effector-to-target-cell ratio.

[b]Standard error.

CLEP-9 were derived were all originally diagnosed as adenosquamous carcinomas, tumor line CLEP-9 was significantly more susceptible to lysis than were the other tumor cell lines. Interestingly, the tumor generating the cell line CLEP-4 was diagnosed as a large cell anaplastic adenocarcinoma that metastasized widely. NK cells are suspected to be most effective against metastatic tumor cells,[3] thus the relatively low vulnerability to NK cell lytic activity (41% at 100:1) of this tumor may have contributed to its successful dissemination throughout the body. However, another tumor cell line, CM-2, with similar low vulnerability to NK cell activity was derived from a tumor that did not metastasize, indicating that in vitro susceptibility to NK cell lysis does not necessarily correlate with in vivo malignant behavior. Furthermore, because cultured cell lines were used in this assay rather than fresh tumor tissue, it is unknown if the original tumor cells had comparable degrees of vulnerability to NK cell-mediated lysis. It is not clear if the increased number of passages for tumor cell line CLEP-4 (14 as compared to 3-4 for all other cell lines) conferred on that cell line a particular advantage in terms of resistance to NK cell cytolysis.

In summary, cultured cells from radiation-induced lung tumors are vulnerable to NK cell-mediated lysis, but the degree of sensitivity to cytolysis is variable. One of the tumor cell lines tested, CLEP-9, is sensitive enough to NK cell lytic activity that it could be used to study immune defenses against lung tumors in lieu of the CTAC cell line typically used to assess canine NK cell activity.

REFERENCES

1. Trinchieri, G. and B. Perussia. Human Natural Killer Cells: Biologic and Pathologic Aspects, Lab. Invest. 50: 489-513, 1984.

2. Krakowka, S. Natural Killer Cell Activity in Adult Gnotobiotic Dogs, Am. J. Vet. Res. 44(4): 635-638, 1983.

3. Hanna, N. and R. C. Burton. Definitive Evidence that Natural Killer (NK) Cells Inhibit Experimental Metastasis In Vivo, J. Immunol. 127(5): 1754-1758, 1981.