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E1A oncogene induction of cytolytic susceptibility eliminates sarcoma cell tumorigenicity

(neoplastic transformation/adenovirus/natural killer cell/tumor immunity)

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ABSTRACT **The manner in which oncogenes influence tumorigenicity beyond their ability to immortalize cells is uncertain. We tested the hypothesis that, in addition to subverting cellular growth controls, oncogenes can actively determine tumor-inducing capacity by affecting neoplastic cell susceptibility to destruction by the host cellular immune response. The adenovirus type 5 *E1A* oncogene, which induces susceptibility to lysis by natural killer cells and encodes epitopes recognized by cytotoxic T lymphocytes, was transfected into highly tumorigenic sarcoma cells. *E1A* expression in these sarcoma cells eliminated their tumorigenicity in recipients with natural killer cell activity that was competent to lyse these *E1A*-positive targets. Thymus-dependent responses were not required for tumor rejection. These results indicate that oncogene-regulated cellular pathways that affect neoplastic cell susceptibility to natural killer cell lytic mechanisms may influence tumor development in the immunocompetent host.**

One objective in the study of the role of oncogenes in cancer is to define the oncogene activities that determine whether neoplastic cells will or will not cause progressive tumors. Oncogenes are defined primarily by their abilities to transform normal cells into neoplastic cells *in vitro*. However, factors other than those commonly associated with morphological transformation also appear to be important in determining tumor development.

Studies of rodent cells transformed by nononcogenic adenovirus types 2 and 5 (Ad2/5) that express conditionally nontumorigenic phenotypes (i.e., nontumorigenic in immunocompetent animals, but tumorigenic in immunocompromised animals) suggest that the Ad2/5 *E1A* oncogene actively induces susceptibility of transformed cells to killing by host natural killer lymphocytes (NK cells) (1). NK cell susceptibility is also induced in normal cells expressing the Ad2/5 *E1A* oncogene during acute viral infection (2). *E1A* also encodes cell-surface epitopes that are recognized by thymus-dependent, cytotoxic T lymphocytes (CTLs) (3, 4). Therefore, both immunologically nonspecific (NK cells) and immunologically specific (CTLs) components of the cellular immune response provide theoretical defenses against E1A-expressing transformed cells. It has been proposed that *E1A* oncogene-induced susceptibility to rejection by these host defenses explains the absence of tumorigenicity of Ad2/5-transformed rodent cells in immunocompetent animals (1, 4, 5). However, Ad2/5 conversion of nontumorigenic primary cells to nontumorigenic transformed cells limits one's ability to attribute the lack of tumorigenicity to an active process induced by the *E1A* oncogene.

As a different test of the active involvement of *E1A* in inducing the nontumorigenic phenotype, we asked whether expression of the Ad5 *E1A* oncogene could alter the tumorigenicity of spontaneously transformed, highly tumorigenic sarcoma cells. The relative roles of NK cells and CTLs in the host response to E1A-expressing tumor cells were also examined. We report that E1A expression can eliminate sarcoma cell tumor-inducing capacity by activating susceptibility to killing by host NK cells in the absence of thymus-dependent cellular immune responses.

MATERIALS AND METHODS

Sarcoma Cell Transfection and Characterization. Syrian hamster BHK-21(C-13) sarcoma cells (American Type Culture Collection; CCL 10) were maintained in Dulbecco's modified Eagle's medium containing penicillin G (100 units/ml) and streptomycin (100 μg/ml), supplemented with glucose (final concentration, 15 mM) and 5% defined, supplemented bovine calf serum (Sterile Systems, Logan, UT) (complete medium). BHK-21 clone 13.8 cells, derived by subcloning BHK-21(C-13) cells, were used for all transfection studies. The plasmids, p5XhoI-C (6), which contains the *E1* region of Ad5, and pMLneo, which contains the simian virus 40 promoted bacterial *neo*^{r} gene that conveys resistance to the antibiotic G418, were used to transfect sarcoma cells by electroporation (7). Transfected cells were selected in G418 (800 μg/ml in complete medium) and cloned by limiting dilution. Transfected clones and tumor cell lines were tested for E1A protein expression by either immunofluorescence or quantitative immunoprecipitation by the E1A-specific monoclonal antibody M73 (8). For indirect immunofluorescence studies, acetone-fixed cells were incubated with M73 as the primary antibody and fluoresceinated goat anti-mouse IgG antibody (Fisher Scientific) as the secondary antibody (9). Quantitative immunoprecipitation of E1A proteins from lysates of cells labeled with $H_3{}^{32}PO_4$ was performed as described (10).

Animal Studies. Random bred golden Syrian hamsters were obtained from the National Jewish Center Animal Care Facility. Congenitally athymic (nude) mice (athymic NCr-nu) and rats (Cr:NIH-rnu) were obtained from the Frederick Cancer Research Facility. Quantitative tumor induction studies were performed with adult (6–8 wk old) animals, as described (11), by inoculating animals subcutaneously with serial 1:10 dilutions of cell suspensions (at least three animals per dilution). Animals were sacrificed when tumors measured >30 mm (mean diameter) or at the end of a 12-wk period of

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Abbreviations: Ad2/5, human adenovirus types 2 and 5; TPD_{50}, dose of neoplastic cells required to produce tumors in 50% of challenged animals (50% end point); CTL, cytotoxic T lymphocyte.

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observation. The 50% end points for tumor production (TPD_{50}) were estimated by the method of Karber (12). The amplitude of the change in the tumorigenicity of transfected clones compared to BHK-21 13.8 cells was calculated by comparing TPD_{50} values.

For immunosuppression studies, adult male hamsters were treated i.p. with 0.5 ml of rabbit anti-hamster lymphocyte antiserum (Accurate Chemicals, Westbury, NY) 1 wk before and at the time of tumor challenge and 0.2 ml of antiserum (i.p.) twice weekly thereafter. Untreated, age-matched male hamsters were used as controls. The effect of *in vivo* depletion of asialo-GM1-positive NK cells on tumor susceptibility of nude rats was also tested by treating weanling (4 wk old) nude rats with i.p. injections (4 μl per g of body weight) of a 1:20 dilution in balanced salt solution of the NK cell-reactive antibody anti-asialo-GM1 (Wako Chemicals, Osaka) every 3–5 days beginning 1 wk before tumor challenge and continuing for 6 wk (13). Untreated, age-matched nude rats were used as controls. Such low dose *in vivo* treatment with anti-asialo-GM1 antibody has little or no effect on rat macrophage numbers or cytolytic activity (14).

For studies of age-related resistance to tumor induction, newborn (<4 days old) and adult nude rats were compared for susceptibility to tumor challenge. Animals of both ages were challenged subcutaneously with 10^7 E1A-expressing sarcoma cells from the same cell suspension and were observed for tumor development for 12–16 wk.

Cytolysis Assays. NK cell assays were performed as described (13) with spleen cells from adult (2–4 months old) hamsters, nude mice, or nude rats as the sources of NK cells. Target cells were labeled with ^{51}Cr (100 μCi/ml for 1 hr; 1 Ci = 37 GBq) for 6-hr cytolysis assays or with [^{3}H]thymidine (specific activity, 2 Ci/mmol; 0.6 μCi/ml for 18 hr) for 48-hr cytolysis assays. The spontaneous release (mean ± SEM) of radiolabel from target cells ranged from 24.3% ± 3.8% for BHK-E1 to 35.7% ± 3.2% for Ad2HE3 in ^{51}Cr release assays and from 9.3% ± 2.5% for BHK-21 parental cells to 24.0% ± 3.1% for BHK-A3 in [^{3}H]thymidine release assays. Various numbers of spleen cells were incubated with fixed numbers of target cells (10^4 targets in 300 μl in 96-well plates for ^{51}Cr release assays or 3×10^4 targets in 1.0 ml in 48-well plates for [^{3}H]thymidine release assays) to create spleen cell/target cell ratios ranging from 25:1 to 400:1. Target cell killing was estimated by calculating the percentage of NK cell-induced release of radiolabel as described (13). The significance of the differences in the susceptibilities of control and E1A-transfected sarcoma cells to NK cell-mediated lysis was estimated by Student's *t* test.

To determine the effect of depleting asialo-GM1-positive NK cells on cytolytic activity, spleen cell populations were preincubated for 30 min in a 1:100 dilution of anti-asialo-GM1 antibody (Wako Chemicals) and then for 30 min at 37°C in a 1:10 dilution of guinea pig complement (Pel-Freez Biologicals). These conditions reduced the concentrations of asialo-GM1-positive cells in nonadherent spleen cell populations by ≈50% as assessed by fluorescence-activated cell sorting analysis. To quantitate the reduction in NK cell lytic activity induced by depletion of asialo-GM1-positive NK cells, the numbers of lytic units (LU_{20}) per 10^7 spleen cells was calculated (13) for aliquots of spleen cells treated with either anti-asialo-GM1 antibody plus complement or complement alone. The significance of the differences between the lytic activities of control and antibody-depleted spleen cell populations was estimated by Student's *t* test.

RESULTS AND DISCUSSION

The hamster fibrosarcoma cell line BHK-21 was chosen for these studies due to its tumorigenicity in immunocompetent animals, its inherent resistance to lysis by NK cells, and its suitability for transfection. The Ad5 *E1* gene region that was transfected into BHK-21 contains the *E1A* oncogene and a second gene, *E1B*, that enhances E1A expression (15). E1A expression is sufficient for induction of susceptibility to NK cell killing in transformed primary cells, whereas E1B expression is not required (1). Three clones expressing high levels of E1A proteins—BHK-A3, BHK-D5, and BHK-E1 (Fig. 1)—were tested in tumor induction studies and were used as target cells in cytolysis assays. To control for the effects of transfection and G418 selection, a G418-resistant cell line, BHK-neo, was established by transfecting BHK-21 13.8 cells with the pMLneo plasmid. There were no major differences in the *in vitro* growth curves of these E1A-expressing clones compared to parental BHK-21 cells (data not shown).

To determine the effects of *E1A* oncogene expression on the tumorigenicity of BHK-21 sarcoma cells in the context of the host cellular immune response, three types of recipient animals were tested: adult golden Syrian hamsters in which BHK-21 tumorigenicity was defined originally, congenitally athymic (nude) mice, and nude rats. In addition to normal T-lymphocyte activity (16), adult hamsters have NK cells that efficiently lyse Ad2/5 E1A-expressing target cells (1). When the three E1A-expressing BHK-21 clones were compared to BHK-21 13.8 and BHK-neo cells, a marked difference in tumorigenicity was observed in immunocompetent hamsters. There was at least a 30,000-fold reduction in the tumor-inducing capacities of the E1A-expressing BHK clones A3, D5, and E1 (Table 1). These clones produced no tumors at the highest cell challenge dose tested (10^7 cells). BHK-21 transfection with the *neo^r* gene alone (BHK-neo) had no effect on tumorigenicity (Table 1).

In contrast to immunocompetent hamsters, adult hamsters immunosuppressed with rabbit anti-hamster lymphocyte antiserum developed progressively enlarging BHK-D5 tumors when challenged with 10^6 or 10^7 cells. Untreated hamsters developed no tumors when challenged with 10^7 BHK-D5 cells from the same suspension. Anti-lymphocyte antiserum treatment also resulted in depletion of hamster NK cell activity. For example, in four [^{3}H]thymidine release assays, the percentage (mean ± SEM) of spleen cell-induced lysis of YAC-1 cells at optimal 100:1 spleen cell/target cell ratios was 35.3% ± 5.3% for untreated donors compared to 2.7% ± 2.7% for anti-lymphocyte antiserum-treated donors ($P = 0.011$). Comparable reductions in NK cell activity of treated animals were observed in 6-hr and 18-hr ^{51}Cr release assays (data not shown). This development of BHK-D5 tumors in hamsters immunosuppressed with anti-hamster lymphocyte antiserum suggested the importance of NK cells in rejecting these E1A-expressing sarcoma cells.

In addition to NK cells, CTLs can also lyse target cells expressing *E1A* oncogene products (3, 4). Since immunosuppression with anti-lymphocyte antiserum would also deplete T lymphocytes in tumor challenge recipients, the possibility that CTL responses were also important in rejection of E1A-expressing sarcoma cells was considered. To evaluate the relative importance of NK cell and CTL responses in rejecting these E1A-expressing sarcoma cells, tumor challenges were repeated using congenitally athymic nude mice and nude rats (Table 1). Nude mice and nude rats both lack the ability to mount normal T-cell responses but have high levels of NK cell activity (17, 18). However, there is a key difference in the cytolytic competence of NK cells from nude mice and nude rats that was useful for this analysis. Nude mouse NK cells are unable to lyse E1A-expressing, xenogeneic (e.g., hamster) target cells, whereas nude rat NK cells efficiently lyse such targets (13). Tumor induction studies with control and transfected BHK lines tested the predictions suggested by these patterns of cytolytic activity. BHK-21 13.8 and BHK-neo cells induced tumors in both nude mice and nude rats. However, while E1A-expressing BHK-21

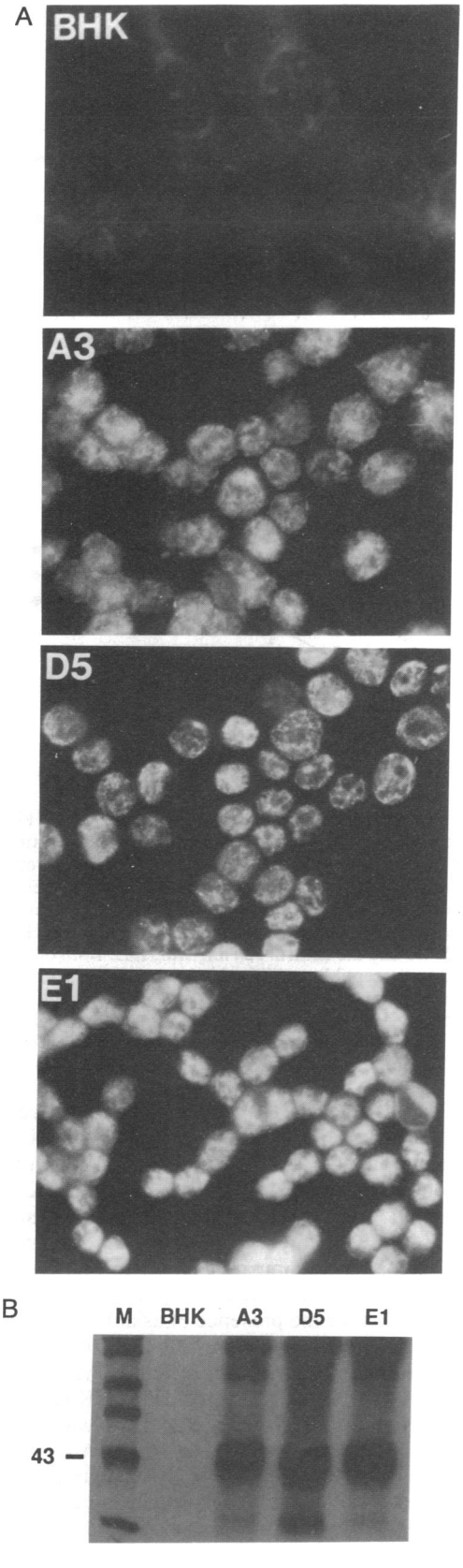

FIG. 1. E1A protein expression by transfected BHK-21 clones. (*A*) Cells from clones A3, D5, and E1 were uniformly (>95%) positive for E1A-specific nuclear immunofluorescence; BHK-21 13.8 parental cells (BHK) were negative. (*B*) The same transfected clones contained large amounts of E1A phosphoproteins detected by quantitative immunoprecipitation (10) with M73 antibody. Lane M, size markers (kDa).

Table 1. Tumor-inducing capacities of transfectants compared to parental BHK-21 cells in immunocompetent hamsters and in congenitally athymic (nude) mice and rats

Cell line	No. of Cells 10^3	10^4	10^5	10^6	10^7	TPD_{50}	Amplitude of decrease in tumorigenicity
			Adult hamsters				
BHK-21	2/4	4/4	4/4	4/4	4/4	3.0	—
BHK-neo	2/3	3/3	3/3	3/3	3/3	2.8	0
BHK-A3	—	—	0/3	0/3	0/6	≥7.5	≥30,000
BHK-D5	—	—	0/3	0/3	0/9	≥7.5	≥30,000
BHK-E1	—	—	0/3	0/3	0/6	≥7.5	≥30,000
			Nude mice				
BHK-21	3/4	4/4	4/4	4/4	4/4	2.8	—
BHK-neo	2/3	3/3	3/3	3/3	3/3	2.8	0
BHK-A3	0/3	2/3	6/6	6/6	3/3	3.8	10
BHK-D5	0/3	0/3	9/10	7/7	3/3	4.6	63
BHK-E1	0/3	0/3	4/5	6/6	3/3	4.7	79
			Nude rats				
BHK-neo	0/3	0/3	3/3	3/3	3/3	4.5	—
BHK-D5	—	—	0/3	0/3	0/3	≥7.5	≥1,000

Hamsters, nude mice, and nude rats (6–8 wk old) were challenged subcutaneously with the indicated numbers of cells and observed for 12 wk for tumor development. Tumor incidence = no. of animals with progressive tumors (>30 mm) ÷ no. of animals challenged. TPD_{50} = the logarithm of the number of cells required to cause progressively enlarging tumors in 50% of animals calculated by the method of Karber (12). The amplitude of the decrease in tumorigenicity of transfected clones is the antilogarithm of the difference in the TPD_{50} values of the transfectant and BHK-21 parental cells. For example, BHK-A3 is at least 4.5 logs or 30,000-fold less tumorigenic than BHK-21 parental cells in adult hamsters as evidenced by the increased numbers of tumor cells required to reach a 50% endpoint of tumor induction. The reduction, but not elimination, of the tumor-inducing capacity of two of the three E1A-positive BHK clones in nude mice (BHK-D5 and BHK-E1) may be explained by the presence of T-cell-independent defenses against tumor challenge other than NK cells, as suggested by studies of the cytolytic activity of nude mouse-activated macrophages for these targets. BHK-D5 and BHK-E1 cells were significantly more susceptible to lysis by BCG-activated, nude mouse macrophages (1) than were parental BHK-21 cells, whereas BHK-A3 cells were not. In four 48-hr [^{3}H]thymidine release macrophage cytolysis assays, the percentage killing (mean ± SEM) of these target cells was as follows: BHK-21, 3.4% ± 1.5%; BHK-D5, 31.9% ± 3.6% (P = 0.018); BHK-E1, 28.2% ± 3.1% (P = 0.036); BHK-A3, 12.3% ± 2.5% (P = 0.092).

clones remained tumorigenic in nude mice, they were nontumorigenic in nude rats (Table 1). This rejection of BHK-D5 cells by nude rats demonstrates that normal, thymus-dependent responses are not required for rejection of E1A-expressing sarcoma cells. Furthermore, the comparison of nude rats to nude mice as tumor challenge recipients suggests that a NK cell response that is competent to lyse E1A-expressing sarcoma cells is involved in this tumor rejection process. To test this hypothesis further, weanling nude rats whose NK cell responses were compromised by treatment with the NK cell-reactive antibody anti-asialo-GM1 were compared to untreated controls for susceptibility to challenge with BHK-D5. Seven of eight (87.5%) anti-asialo-GM1 antibody-treated nude rats developed subcutaneous sarcomas within 4–6 wk after challenge with 10^7 BHK-D5 cells, whereas none of 10 control nude rats challenged with the same inocula developed tumors during a 16-wk period of observation. A tumor from a random, NK cell-depleted nude rat challenged with BHK-D5 cells was established in tissue culture and tested for E1A protein expression and NK cell susceptibility to control for the *in vivo* emergence of E1A-negative or NK-resistant subpopulations from the BHK-D5 clone. These cells resembled BHK-D5 cells morphologically, uniformly expressed bright E1A-specific immunofluores-

cence, and were highly susceptible to lysis by anti-asialo-GM1 antibody-inhibitable nude rat NK cell lytic activity (data not shown). This observation that NK cell depletion of nude rats decreased their resistance to tumor development with E1A-positive sarcoma cells supports the conclusion that NK cells play a direct role in the *in vivo* rejection of these *E1A* oncogene-expressing tumor cells.

The correlation between E1A-induced elimination of sarcoma cell tumorigenicity and E1A-induced susceptibility to NK cell killing among these transfected sarcoma cell lines was tested directly by comparing the tumor susceptibilities of these three types of animals with the *in vitro* cytolytic activities of their NK cells against these same E1A-expressing sarcoma cells (Figs. 2 and 3). The E1A-expressing sarcoma cell lines BHK-A3, BHK-D5, and BHK-E1 were all highly susceptible to hamster NK cell-mediated lysis in both short-term (6 hr) and long-term (48 hr) cytolysis assays, whereas parental BHK-21 13.8 cells and the BHK-neo control transfectant were highly resistant to hamster NK cell lysis irrespective of the length of the assay (Fig. 2). In contrast to the pattern seen in hamster NK cell assays, nude mouse NK cells could not efficiently lyse E1A-expressing BHK-21 clones even in 48-hr assays (Fig. 3*A*). This observation, combined with the susceptibility of nude mice to tumor induction by the E1A-expressing BHK-21 clones (Table 1), supports the conclusion that competence of host NK

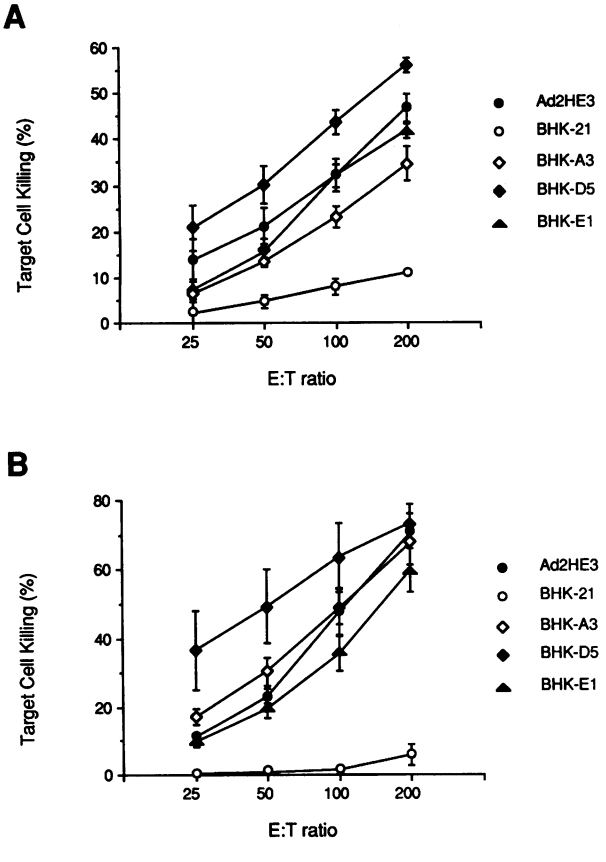

FIG. 2. Hamster NK cell killing of E1A-expressing BHK-21 clones. Clones A3, D5, and E1 were tested for susceptibility to NK cell-mediated lysis in 6-hr ^{51}Cr release assays (*A*) and 48-hr [^{3}H]thymidine release assays (*B*). Ad2HE3, NK-susceptible control cell hamster line (13); BHK-21, NK-resistant parental cell line. Killing curves represent the results (mean ± SEM) of four experiments. All E1A-expressing clones were significantly more susceptible to NK cell killing than BHK-21 parental cells in both short-term [$P < 0.03$ for effector/target cell ratios (E:T) ranging from 50:1 to 200:1] and long-term [$P < 0.04$ for E:T ranging from 25:1 to 200:1] assays. BHK-neo target cells were no more susceptible to NK cell-mediated lysis than BHK-21 parental cells (data not shown).

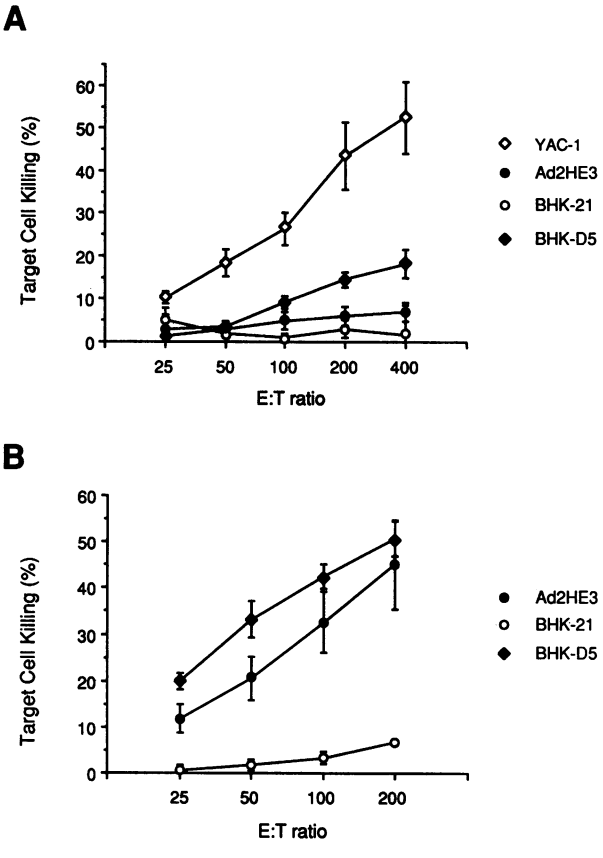

FIG. 3. Comparison of nude mouse and nude rat NK killing of the E1A-expressing clone BHK-D5. YAC-1, NK-susceptible control cells for nude mouse NK cell assays. Killing curves represent the results (mean ± SEM) of four (nude mouse) and three (nude rat) experiments. (*A*) Nude mouse NK cells (48-hr assay) were inefficient in killing two E1A-expressing hamster targets—the BHK transfectant BHK-D5 and the control cell line Ad2HE3. Both of these targets were highly susceptible to killing by hamster NK cells (see Fig. 2). (*B*) In contrast, nude rat NK cells (6-hr assay) efficiently lysed both BHK-D5 and Ad2HE3 cells. BHK-21 parental cells and BHK-neo cells (data not shown) were highly resistant to lysis by nude rat NK cells. E:T ratio, effector/target cell ratio.

cells to destroy these E1A-expressing sarcoma cells is necessary for tumor rejection. This conclusion is reinforced by the observation that NK cells from nude rats (highly resistant to challenge with the E1A-expressing clone BHK-D5; Table 1) exhibited high level cytolytic activity against E1A-expressing BHK-21 targets similar to that observed in hamster NK cell assays (compare Fig. 3*B* to Fig. 2*A*).

The results of *in vivo* NK cell depletion studies suggested the requirement for the presence of asialo-GM1-positive NK cells for efficient rejection of E1A-expressing sarcoma cells by weanling nude rats. This observation was supported by the results of studies of *in vitro* nude rat NK cell activity. In six 6-hr ^{51}Cr release NK assays, there was a significant reduction in lytic activity against the E1A-expressing sarcoma target cell BHK-D5 (mean lytic units per 10^7 spleen cells ± SEM; ref. 13) in nude rat spleen cells treated with anti-asialo-GM1 antibody plus complement (6.9 ± 3.1 LU_{20} per 10^7 cells) compared to the same spleen cells treated with complement alone (24.8 ± 8.9 LU_{20} per 10^7 cells; $P = 0.031$).

The results of comparative NK cell assays and tumor induction studies in newborn (<4 days old) and adult (>6 weeks old) nude rats provided further evidence that *E1A* oncogene-induced susceptibility to NK cell killing is a key mechanism of E1A-induced abrogation of sarcoma cell virulence. Newborn nude rats lack detectable NK cell activity

against either E1A-positive BHK-D5 cells or YAC-1 cells (mean ± SEM percentage target cell killing at 100:1, 50:1, 25:1, and 12:1 spleen cell/target cell ratios, respectively, in three 18-hr ^{51}Cr release assays): (*i*) BHK-D5 target cells: newborn NK cell activity = 10.4% ± 2.7%, 5.3% ± 1.1%, 4.6% ± 1.3%, 4.4% ± 0.9%; adult NK cell activity = 72.9% ± 5.0%, 61.7% ± 8.0%, 43.2% ± 8.8%, 26.8% ± 6.3% (P = <0.001–0.024); (*ii*) YAC-1 target cells: newborn NK cell activity = 11.2% ± 3.4%, 8.7% ± 1.2%, 6.1% ± 1.7%, 2.7% ± 1.2%; adult NK cell activity = 84.2% ± 2.1%, 80.5% ± 3.6%, 60.9% ± 8.7%, 39.9% ± 7.6% (P = <0.001–0.008). As an *in vivo* corollary for these NK cell assays, newborn and adult nude rats were challenged with E1A-expressing BHK-D5 sarcoma cells. Newborn nude rats challenged with 10^7 BHK-D5 cells developed progressively enlarging subcutaneous tumors consisting of E1A-positive cells by immunofluorescence, whereas adult nude rats rejected challenge with the same BHK-D5 inoculum. These data suggest that maturation of the NK cell response in athymic rats is required for rejection of these E1A-expressing tumor cells.

These data provide a model for future genetic analysis of Ad2/5 *E1A* oncogene control of induction of NK cell cytolytic susceptibility *in vitro* and of NK cell-dependent rejection of E1A-expressing tumor cells *in vivo*. Data from previous *E1A* mapping studies provide a framework for this analysis. DNA and amino acid sequence analyses have shown that E1A transforming activity involves a gene region (E1A domain 2) that is highly conserved among adenovirus serotypes and that has marked homology with transforming regions of other nuclear oncogenes, including myc, p53, and the large tumor antigen-encoding regions of papovaviruses (19–21). Another conserved *E1A* region (domain 3) is dispensable for transformation but is required for activation of viral gene transcription (22–25). Other *E1A* regions appear to be sufficient for activation of cellular gene transcription (25, 26). Therefore, *E1A*, like other nuclear oncogenes (e.g., myc; see ref. 27), is composed of multiple domains that can function independently in regulating expression of a variety of cellular traits. Using the format provided by this study, it may be possible to map regulation of induction of NK cell susceptibility and associated tumor rejection to one or more of the known *E1A* domains or to a region of the *E1A* oncogene that has not previously been assigned a function.

Studies of the roles of other oncogenes in inducing susceptibility of neoplastic cells to NK cell killing have yielded conflicting results. These data suggest that the type of cell in which the oncogene is expressed and the degree of NK cell activation determine whether oncogene expression will or will not induce NK cell susceptibility, NK cell resistance, or no change in cytolytic susceptibility compared to control cells (28–34). Since *E1A* oncogene expression has been observed to induce susceptibility to NK cell killing in a variety of cell types from different species (1, 5, 10), it appears that this *E1A*-regulated cellular phenotype may be less dependent on the experimental model being studied than the cytolytic susceptibility phenotypes induced by other oncogenes. This consistency of *E1A* oncogene activity may be important for future studies of structure–function relationships through which oncogenes determine tumor cell virulence in the context of the host NK cell response.

Analysis of the mechanisms by which E1A induces cytolytic susceptibility in tumor cells may also allow definition of a cellular pathway(s) through which this tumor cell phenotype is regulated. The observation that *E1A* oncogene expression can induce increased NK cell cytolytic susceptibility in normal cells (2), virally transformed cells (1), and spontaneously transformed sarcoma cells suggests that this hypothetical pathway may lie dormant in many, if not all, cell types, including highly tumorigenic cells. Identification of the mechanisms through which oncogenes control NK cell cytolytic susceptibility using the Ad2/5 *E1A* oncogene model may provide a basis for development of strategies to increase the usefulness of immunotherapy in the control of neoplastic disease.

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