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Immunological Surveillance against DNA Virus-Transformed Cells: Correlations between Natural Killer Cell Cytolytic Competence and Tumor Susceptibility of Athymic Rodents

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Adenovirus type 2 (Ad2)-transformed hamster and rat cells are susceptible to lysis by natural killer (NK) cells from the host of origin and are nontumorigenic in immunocompetent hamsters and rats, respectively. These NK-cell-susceptible, virus-transformed cells are, however, highly tumorigenic in athymic (nude) mice—animals with intact NK-cell responses. In vitro lysis of these xenogeneic, Ad2-transformed cells by nude-mouse NK cells was found to be defective. In contrast, Ad2-transformed hamster and rat cells were highly susceptible to lysis by nude-rat NK cells. Furthermore, xenogeneic, Ad2-transformed hamster cells were nontumorigenic in nude rats unless the NK-cell responses of the challenged animals were compromised. The results of the nude-rat studies show that thymus-dependent, cytotoxic T-lymphocyte-mediated, host cellular immune responses are not essential for rejection of xenogeneic cells transformed by nononcogenic Ad2. The data suggest instead that immunologically nonspecific host cellular immune responses, such as those mediated by NK cells, are sufficient for rejection of Ad2-transformed cells. These results indicate that biologically important differences exist in the NK-cell-mediated defenses mounted by nude mice and nude rats against transformed cells that may account for the different patterns of tumor induction by various neoplastic cell types in these athymic animals.

As an appreciation of the role of cell-mediated immunity in the host response to neoplastic cells has evolved, the complexity of this response has also become apparent. The host appears to be able to generate two categorically different types of cellular immune response to neoplastically transformed cells: early-appearing, immunologically nonspecific responses and relatively late-appearing, immunologically specific responses (13). Natural killer (NK) lymphocytes and macrophages are the main components of the early, nonspecific response, and cytotoxic, thymus-derived lymphocytes (CTL) are the effectors of the immunologically specific component of the antineoplastic cellular immune response of the host. To evaluate the interactions between these components of the host cellular immune response and tumor target cells, both in vitro cytolysis assays and correlative tumor induction studies are required.

DNA tumor virus systems have proved to be no exception to the problem of defining the critical interactions between transformed cells and the host cellular immune response. Because of the ability of DNA viruses and the cells they transform to induce virus-specific protection against a subsequent challenge of the immunized host with virus-transformed cells, it was originally assumed that immunologically specific host responses (e.g., CTL) directed against transformed cells bearing surface, virus-specific transplantation antigens were the main determinants of tumor rejection in vivo. Support for a possible role of CTL in determining the tumorigenicity of DNA viruses was provided by recent reports indicating that highly oncogenic adenovirus type 12 (Ad12), but not nononcogenic Ad5, causes a reduction in the expression of class I major histocompatibility complex antigen (MHC; required for recognition of target cells by CTL) on transformed cell surfaces and an associated reduction in Ad12-transformed cell susceptibility to lysis by alloimmune CTL (sensitized only against class I antigen) (1, 7, 22). However, several other experimental observations have indicated that immunologically nonspecific cellular immune responses, and not CTL, may be the critical determinants of progression or rejection of adenovirus-transformed cell-induced tumors in immunologically naive hosts. For example, it has been reported that rodent cells transformed by either highly oncogenic or nononcogenic DNA viruses are highly immunogenic in bioassays (9, 20) and are equally susceptible to lysis by syngeneic CTL sensitized to the relevant, virus-transformed cell type (21). Thus, induction of and susceptibility to syngeneic CTL may not necessarily discriminate between adenovirus-transformed cells with greatly different tumor-inducing capacities. Conversely, the results of our studies and those of others indicate a strong correlation between the susceptibility of DNA virus-transformed hamster and rat cells to the lytic effects of NK cells and activated macrophages and the tumor-inducing capacities of such cells in immunocompetent, nonimmunized hosts (4, 21, 23). The purpose of the present study was to evaluate the significance of interactions between host NK lymphocytes and DNA virus-transformed cells in the absence of thymus-dependent cellular immune responses such as CTL by using congenitally athymic rodents. The results presented show that NK-cell cytolytic competence against DNA virus-transformed cells may be an independent vari-

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able in the determination of the tumor-inducing capacity of such neoplastic cells.

MATERIALS AND METHODS

Cells and cell lines. YAC-1, a nonadherent lymphoma cell line, is the prototype NK-cell-susceptible murine target cell (16). F9 is an adherent, undifferentiated murine teratocarcinoma cell line that we have found to be sensitive to mouse NK-cell-mediated lysis (2). Ad2HE1, Ad2HE3, Ad2HE7, and Ad2HE9 are clonally derived Ad2-transformed LSH Syrian hamster embryo cell lines that are highly susceptible to lysis by hamster NK cells (4, 20). A2T2C4 is an Ad2-transformed Hooded Lister rat embryo cell line that is highly susceptible to lysis by both rat and hamster NK cells (5, 8). F4 is an Ad2-transformed AS strain rat embryo cell line (10). SV40HE1 is a simian virus 40 (SV40)-transformed LSH Syrian hamster embryo cell line that is highly resistant to lysis by hamster and rat NK cells and activated macrophages (4, 20). Ad12HE4 is an Ad12-transformed LSH Syrian hamster embryo cell line that is resistant to lysis by hamster NK cells (4, 20). All cell lines were maintained in Dulbecco modified Eagle medium containing antibiotics and supplemented with 5% calf serum. All lines were tested for mycoplasma and were negative.

Cytolysis assays. Adult (2- to 5-month-old) animals were used as spleen cell donors. Swiss Webster nude mice or Rowett nude rats were maintained in laminar airflow units. Random-bred Golden Syrian hamsters, Sprague-Dawley rats, and CBA/J mice were obtained from the National Jewish Center Animal Care Facility. NK-cell assays were done with unselected spleen cells or with spleen cell populations that had been depleted of adherent cells by using nylon wool columns as described previously (4). NK-cell depletion from nonadherent spleen cell populations was accomplished by treatment with the NK-cell-reactive antibody, anti-asialo GM1 (Wako Chemicals, Dallas, Tex.), plus guinea pig complement (15). Target cells were labeled with [^{3}H]thymidine (0.5 μCi/ml overnight) or with ^{51}Cr (100 μCi/ml for 45 min) as indicated, washed to remove unincorporated label, and cocultured with spleen cells at spleen cell to target cell ratios ranging from 25:1 to 400:1 for 6 to 48 h at 37°C. Our previous studies in which the cytolytic activity of hamster lymphoid cells for DNA virus-transformed cells was characterized showed that optimal killing occurred between 24 and 48 h of cocultivation of spleen cells and [^{3}H]thymidine-labeled target cells (4). In the present study, different periods of cocultivation of spleen cells with target cells labeled with either ^{51}Cr or [^{3}H]thymidine were used to evaluate the effects of assay conditions on experimental results. The average spontaneous release of [^{3}H]thymidine during the 48-h incubation period ranged from 11.1% for A2T2C4 to 27.7% for F9. The average spontaneous release of ^{51}Cr during the 24-h incubation period was 38.0% for YAC-1 and 36.6% for Ad2HE3. Data are presented as the percentage of total radiolabel released from target cells caused by NK-cell-cell-induced lysis, calculated as described previously (4). Quantitation of the number of NK-cell lytic units per 10^7 spleen cells was done by the method of Bloom and Korn (3).

Tumor induction studies. Quantitative tumor induction studies were done as described previously (18). Briefly, serial 10-fold dilutions of the indicated transformed cell types in 0.2 ml of Eagle medium were inoculated into the subcutaneous tissues between the scapulae (three to five animals per dilution). Tumor incidence is expressed as the number of animals that developed progressively enlarging tumors during a 10- to 12-week observation period. The 50% endpoint of tumor induction was calculated by the method of Karber (14). Nude rats treated with the NK-cell-reactive antibody, anti-asialo GM1, received 0.5 ml of a 1:10 dilution of the antibody intraperitoneally every 3 to 5 days beginning 1 week before challenge and continuing for 6 weeks.

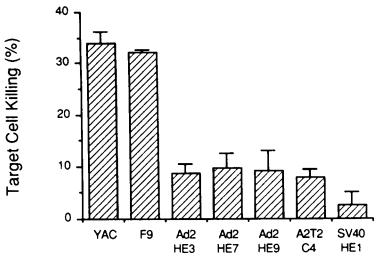

FIG. 1. Cytolytic activity of nude-mouse spleen cells against xenogeneic, DNA virus-transformed cells. Lysis of three clonally derived, Ad2-transformed LSH strain Syrian hamster cell lines (Ad2HE3, Ad2HE7, and Ad2HE9), an Ad2-transformed Hooded Lister rat embryo cell line (A2T2C4), and an SV40-transformed LSH hamster cell line (SV40HE1) by spleen cells from specific-pathogen-free NIH Swiss Webster nude mice was compared. The cytolytic-susceptible murine lymphoma, YAC-1, and teratocarcinoma, F9, were used as positive control target cells in these experiments. Target cells were labeled with [^{3}H]thymidine and cocultured with spleen cells at spleen cell to target cell ratios of 25:1 to 400:1 for 48 h. Bars represent the results (mean ± standard error of the mean) of four experiments at the 200:1 spleen cell to target cell ratio. Similar patterns of target-cell killing were observed at 50:1, 100:1, and 400:1 ratios. The Ad2- and SV40-transformed hamster and rat cell lines were significantly less susceptible to lysis by nude-mouse spleen cells than were the murine target cells ($P < 0.001$) as estimated by Student's t test.

RESULTS

Defective nude-mouse NK-cell activity against Ad2-transformed cells. The current study was stimulated by a paradoxical observation. Ad2-transformed hamster and rat cell lines that did not induce tumors in syngeneic newborn animals and that were highly susceptible to lysis by NK cells from the same species were also highly tumorigenic in nude mice, which have intact NK-cell responses (4, 8, 9, 20, 21). Subtle differences have been reported in the lytic efficiency of NK cells against target cells from different species, with homologous effector cell-target cell interactions generally functioning better than heterologous interactions (11). However, in other instances, NK-cell killing of transformed cells from other species may be quite efficient. To evaluate the possibility that the reason for the tumorigenicity of Ad2-transformed hamster and rat cells in nude mice involved a defect in NK cell-target cell interactions, in vitro cytolysis assays with nude-mouse spleen NK cells and transformed cells from different species were done (Fig. 1). Nonadherent YAC-1 mouse lymphoma target cells and adherent F9 mouse teratocarcinoma cells were found to be equally susceptible to lysis by nude-mouse NK cells, whereas Ad2-transformed hamster and rat cells were found to be almost as resistant to lysis as the highly cytolytic-resistant SV40-transformed hamster cell line, SV40HE1 (4, 20).

Comparison of anti-Ad2-transformed cell lytic activity of NK cells from euthymic mice, hamsters, and rats. The above results raised questions about the apparent defect in nude-mouse NK-cell killing of xenogeneic, Ad2-transformed ham-

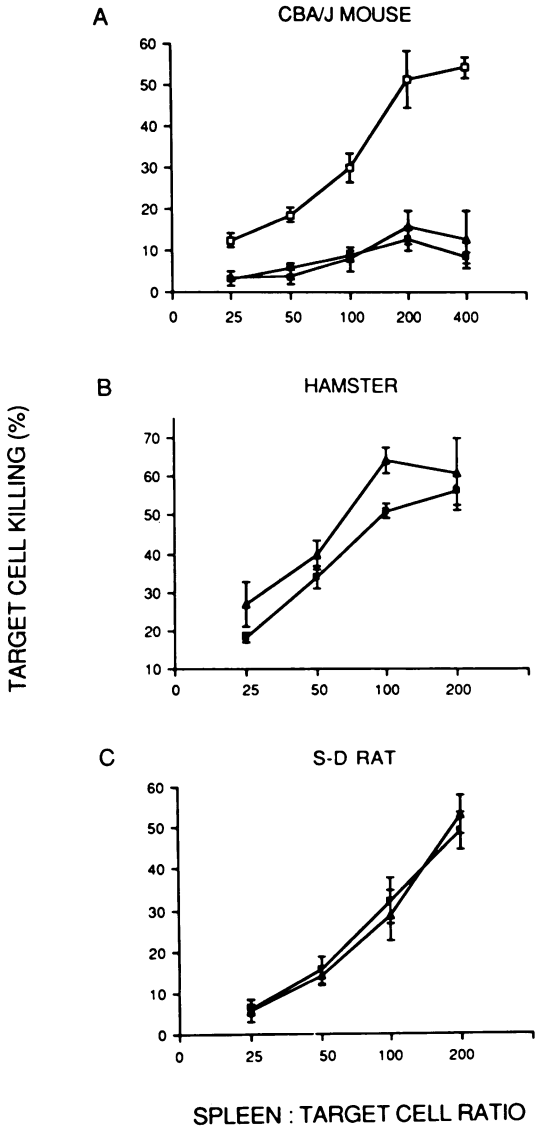

FIG. 2. Cytolytic activity of euthymic mouse, hamster, and rat spleen cells against Ad2-transformed hamster (Ad2HE3, ▲) and rat (A2T2C4, ■) cells. The murine cell line, YAC-1 (□), which is susceptible to lysis by mouse NK cells, was used as a positive control in the mouse NK-cell cytolysis assays. These target cells were tested for susceptibility to killing by spleen NK cells from adult CBA/J mice, random-bred Golden Syrian hamsters, or Sprague-Dawley rats in 48-h [^{3}H]thymidine-release assays. The range of spleen cell to target cell ratios used in the cytolysis assays (25:1 to 400:1 in mouse NK-cell assays and 25:1 to 200:1 in rat and hamster NK-cell assays) is shown on the abscissas. Points represent the results (mean ± standard error of the mean) of three (mouse) and four (hamster and rat) experiments.

ster and rat cells. Would such defective killing be observed with NK cells from other mouse strains? Would such inefficient lysis of Ad2-transformed cells be observed with other xenogeneic NK cell-target cell combinations? To evaluate these questions, killing of Ad2-transformed hamster (Ad2HE3) and rat (A2T2C4) cells was quantitated by using high-NK-cell strain CBA/J mice (12), random-bred Golden Syrian hamsters, and Sprague-Dawley rats as NK-cell donors (Fig. 2). As was observed with nude-mouse NK-cell donors, spleen cells from euthymic CBA/J mice were defective for killing of xenogeneic Ad2-transformed hamster and rat cells even at very high spleen cell to target cell ratios (Fig. 2A). The same defect in CBA/J NK-cell lytic activity was observed with three other Ad2-transformed hamster cell lines (Ad2HE1, Ad2HE7, and Ad2HE9) and one other Ad2-transformed rat cell line, F4 (percent target cell killing at an optimal 200:1 spleen cell to target cell ratio ranged from 9.3 ± 3.5% for Ad2HE1 to 16.5 ± 1.8% for Ad2HE7 compared with 54.2 ± 8.4% for YAC-1; mean ± standard error of the mean of four experiments). Thus, the inability of mouse NK cells to efficiently kill xenogeneic Ad2-transformed rodent cells is not a peculiarity of nude mice. The ability of hamster and rat NK-cell populations to kill Ad2-transformed cells from the opposite species as well as they kill target cells from the same species (Fig. 2B and C, respectively) indicates that the inefficient killing of xenogeneic Ad2-transformed cells observed with murine NK cells does not represent a general species restriction of cytolytic activity against adenovirus-transformed cells. One possibility that is not excluded by these data is that mouse NK cells are simply unable to recognize and destroy adenovirus-transformed cells, irrespective of the species of origin of the target cells, including adenovirus-transformed mouse cells. However, the results of our preliminary studies indicate that this is not the case, as an Ad5-transformed, BALB/c mouse embryo fibroblast cell line, Ad5ME2, has been found to be as susceptible to lysis by CBA/J NK cells as are YAC-1 target cells (unpublished data).

Cytolytic competence of nude-rat NK cells for xenogeneic, Ad2-transformed cells. To determine whether the defect in nude-mouse NK-cell cytolytic activity for xenogeneic, Ad2-transformed cells would also be observed with other athymic rodents with high NK-cell activity, studies were done with congenitally athymic nude rats as NK-cell donors. Nude rats, like nude mice, are deficient in T-cell function and have relatively high NK-cell activity compared with euthymic rats (6). When the same battery of Ad2- and SV40-transformed target cells was tested against nude-rat NK cells, a different pattern of cytolytic susceptibility was obtained (Fig. 3) compared with that observed with nude-mouse NK-effector

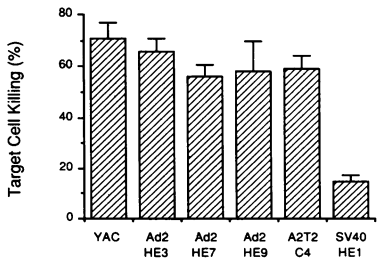

FIG. 3. Cytolytic activity of nude-rat spleen cells against xenogeneic, DNA virus-transformed cells. The same battery of target cells used in the experiments described in the legend to Fig. 1 (with the exception of F9) was tested against spleen effector cells from specific-pathogen-free Rowett strain nude rats in 48-h-[^{3}H]thymidine-release assays. Bars represent the results (mean ± standard error of the mean) of four experiments at the 200:1 spleen cell to target cell ratio. Similar patterns of target cell killing were observed at the 50:1 and 100:1 ratios. There were no significant differences in the killing of xenogeneic, Ad2-transformed hamster and rat cell lines ($P > 0.05$); however, the SV40HE1 cell line, which is highly resistant to lysis by hamster NK cells (4, 5), was resistant to lysis by nude-rat spleen cells compared with the murine YAC-1 cell line or the Ad2-transformed hamster or rat cell lines ($P < 0.001$).

cells (Fig. 1). Although nude-mouse NK cells had not been efficient in lysing Ad2-transformed hamster cells, nude-rat NK cells killed the three different hamster cell lines, Ad2HE3, Ad2HE7, and Ad2HE9, and the Ad2-transformed rat cell line, A2T2C4, with equal efficiency. The hamster NK-cell-resistant SV40HE1 cell line was also resistant to lysis by nude-rat NK cells. Since differences in cytolysis assay conditions, such as the duration of cocultivation of NK cells and target cells, might theoretically affect patterns of target-cell cytolytic susceptibility, comparisons of nude-mouse and nude-rat NK-cell lytic activity against the xenogeneic Ad2HE3 cell line under different culture conditions were done (Fig. 4). The same pattern of efficient killing by nude-rat NK cells and inefficient killing by nude-mouse NK cells was observed irrespective of the radiolabel (^{51}Cr or [^{3}H]thymidine) or the duration of the cytolysis assay (6, 24, or 48 h). Further studies supported the conclusion that NK cells were responsible for the observed cytolytic activity in nude-rat spleen cell suspensions (Table 1). Thus, the cytolytic activity against xenogeneic Ad2HE3 cells was not caused by activated macrophages as it persisted in the nylon wool-nonadherent spleen cell fraction (Table 1, series 1) (12), and cytolytic activity was depleted by pretreatment of the nonadherent spleen cell population with the NK-cell-reactive antibody, anti-asialo GM1 (Table 1, series 2) (15) plus complement. In addition to the qualitative differences in nude-mouse and nude-rat NK-cell cytolytic activity against Ad2-transformed hamster cells, there also were differences in the amplitudes of killing by NK cells from these species against the susceptible YAC-1 target cell (Fig. 1 and 3). The results of comparative titrations of nude-rat versus nude-mouse spleen cells against YAC-1 target cells in three different assays indicated that nude-rat spleen cell populations contained significantly more lytic activity than did nude-mouse spleen cell populations (Table 1, series 3). Thus, there is a quantitative, as well as a qualitative, difference in the NK-cell cytolytic activity of lymphoid cell populations from these two nude-rodent species.

Correlations between NK-cell cytolytic competence and tumor susceptibility of nude mice and nude rats. To evaluate the in vivo relevance of the observed differences in NK-cell cytolytic competence of nude-mouse and nude-rat cells for xenogeneic DNA virus-transformed cells, comparative quantitative tumor induction studies were done with Ad2-, Ad12-, and SV40-transformed hamster cell lines to challenge these animals (Table 2). Nude mice were found to be susceptible to tumor induction by all three types of cell lines, whereas nude rats were highly resistant to tumor challenge with Ad2-transformed hamster cell lines (either no tumors were observed, or small tumor nodules appeared and then regressed after a challenge with 10^8 cells) but were susceptible to tumor induction by Ad12- and SV40-transformed hamster cell lines that were resistant to lysis by nude-rat NK cells. The results of these comparisons of nude-mouse and nude-rat NK-cell cytolytic competence in vitro and susceptibility to tumor cell challenge provide the first direct evidence that immunologically specific host responses mediated by CTL are not required for rejection of Ad2-transformed cells and support the conclusion that immunologically nonspecific host effector cells, such as NK cells, may be the key host cellular immune defenses in this system. Further support for the conclusion that nude-rat NK-cell activity caused rejection of xenogeneic, Ad2-transformed hamster cells in these studies was provided by the results of tumor induction studies in nude rats treated with the NK-cell-reactive antibody, anti-asialo GM1 (15). Two of three antibody-treated animals developed progressive tumors after a challenge with

TABLE 1. Characteristics of nude-rat NK-cell activity against xenogeneic, virus-transformed target cells and quantitative comparison of nude-rat and nude-mouse NK-cell cytolytic activity

Series (no. of expts)	NK-cell source and assay (spleen cell to target cell ratio)[a]	Target cell[b]	Results[c]	
			% Killing	Lytic units
1 (2)	Untreated NR spleen cells (100:1)	Ad2HE3	40.6 ± 2.3	
	Nylon wool-depleted NR spleen cells (100:1)	Ad2HE3	38.7 ± 0.4	
2 (4)	Complement-treated NR spleen cells (50:1)	Ad2HE3	23.9 ± 2.7	
	Anti-asialo GM1-complement-treated NR spleen cells (50:1)	Ad2HE3	6.0 ± 2.0	
3 (3)	6-h ^{51}Cr assay	YAC-1		
	NR spleen cells			25.3 ± 5.7
	NM spleen cells			3.9 ± 0.8
	24-h ^{51}Cr assay	YAC-1		
	NR spleen cells			110.9 ± 3.2
	NM spleen cells			10.8 ± 0.8
	48-h [^{3}H]thymidine assay	YAC-1		
	NR spleen cells			129.5 ± 26.7
	NM spleen cells			7.0 ± 2.5

[a] In series 1, killing of Ad2HE3 target cells by unseparated nude-rat (NR) spleen cells was compared with killing by the same spleen cell population after selection for nonadherence to nylon wool columns. In series 2, samples of a nude-rat spleen cell population were treated with either complement alone or anti-asialo GM1 antibody followed by complement before use in cytolysis assays.

[b] Unless otherwise indicated, target cells were prelabeled with [^{3}H]thymidine and cocultured with appropriately treated spleen cells at various spleen cell to target cell ratios for 48 h, at which time spleen cell-induced killing of target cells was determined on the basis of radiolabel release.

[c] Results represent the mean ± standard error of the mean of either the percent target cell killing (series 1 and 2) or the number of lytic units per 10^7 spleen cells (series 3). Nude-rat NK-cell activity was significantly reduced by pretreatment of the spleen cell population with anti-asialo GM1 antibody plus complement ($P < 0.001$) as estimated by Student's t test. The anti-YAC-1 NK-cell activity of nude-rat spleen cells was significantly greater than that of nude-mouse (NM) spleen cells in all three types of cytolysis assays done ($P < 0.001$).

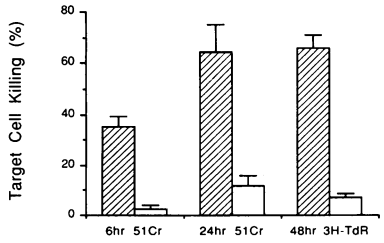

FIG. 4. Comparison of nude-rat (▨) and nude-mouse (□) spleen-cell killing of the xenogeneic, Ad2-transformed hamster cell line, Ad2HE3, in short- (6 h), intermediate- (24 h), and long- (48 h) term radiorelease assays. Bars represent the results (mean ± standard error of the mean) of three different experiments at the 200:1 spleen cell to target cell ratio. Nude-rat spleen cells were significantly more efficient in killing xenogeneic Ad2HE3 cells than were nude-mouse spleen cells in all three types of assay ($P < 0.001$).

TABLE 2. Nude-mouse and nude-rat susceptibility to DNA virus-transformed-cell challenge compared with transformed-cell-specific NK-cell activity

Animal and transformed-cell line[a]	No. of animals developing tumors/total no. surviving at dose[b]:							TPD_{50}[c]	NK-cell lysis[d]
	8	7	6	5	4	3	2		
Nude mouse									
Ad2HE1		5/5	9/9	9/9	5/9	0/5	0/5	4.1	8.7 ± 1.9
Ad2HE3		4/4	12/12	11/12	0/8	0/4	NT	4.5	6.1 ± 1.4
Ad12HE4		NT	8/8	5/8	3/4	0/4	NT	4.5	1.0 ± 0.9
SV40HE1		5/5	5/5	5/5	5/5	4/5	0/5	2.7	2.6 ± 1.8
Nude rat									
Ad2HE1	0/3	0/3	NT	NT	NT	NT		≥8.5[e]	52.1 ± 2.8
Ad2HE3	0/5	0/5	0/5	0/2	0/2	NT		≥8.5[e]	62.3 ± 4.0
Ad12HE4	3/3	3/3	1/3	0/3	0/3	0/3		6.2	17.7 ± 2.3
SV40HE1	NT	3/3	3/3	0/3	0/3	0/3		5.5	15.4 ± 2.7

[a] Cell lines are hamster embryo cell lines transformed by Ad2, Ad12, or SV40, as indicated in cell line designations.

[b] Tumor incidence is expressed as the number of animals that developed progressively enlarging tumors (>40-mm diameter) during a 10- to 12-week observation period. Dose is given as number (log_{10}) of transformed cells inoculated subcutaneously per animal. NT, Not tested.

[c] TPD_{50}, Logarithm of the number of transformed cells required to induce progressive tumors in 50% of surviving animals.

[d] Percent target cell killing by spleen cells (NK-cell lysis) at a spleen cell to target cell ratio of 200:1 in 48-h [^{3}H]thymidine-release assays (mean ± standard error of the mean of two to five assays). Ad12HE4 and SV40HE1 were significantly less sensitive to the cytolytic effects of nude-rat spleen NK cells than were the two Ad2-transformed cell lines, Ad2HE1 and Ad2HE3 ($P < 0.001$). Nude-mouse spleen NK-cell killing of the cytolytic susceptible target cell, YAC-1, was 41.7 ± 4.9% in these experiments.

[e] No tumors developed after a challenge with 10^8 transformed cells, or small tumors appeared and regressed during the period of observation.

10^8 Ad2HE3 cells, whereas all three untreated animals rejected a challenge with the same Ad2HE3 cell inoculum.

DISCUSSION

Although both nononcogenic (e.g., Ad2) and highly oncogenic (e.g., Ad12) adenoviruses can transform rodent cells in vitro, only cells transformed by highly oncogenic adenovirus can induce tumors in immunocompetent animals (for a review, see reference 20). The results of several studies have suggested that host cellular immune responses cause the rejection of Ad2-transformed cells and are insufficient to cause rejection of Ad12-transformed cells (for a review, see reference 19). It has not previously been clear, however, whether thymus-independent host cellular immune responses such as NK cells and activated macrophages or thymus-dependent responses such as CTL could independently mediate Ad2-transformed cell rejection or whether both host responses are required. The previous observations that Ad2-transformed hamster and rat cells are highly tumorigenic in congenitally athymic nude mice (8, 20) which have intact NK-cell responses (for a review, see reference 17) would appear to suggest that thymus-dependent host responses are required for Ad2-transformed cell rejection and that NK-cell responses are ineffective in this rejection process. The data presented in this report showing that nude-mouse NK cells are defective for cytolytic activity against xenogeneic, Ad2-transformed cells provide an alternative explanation for the susceptibility of nude mice to tumor induction by these cells. Thus, one cannot assume that the absence of T-lymphocyte responses in nude mice is the sole reason for the increased susceptibility of these animals to tumor induction by Ad2-transformed cells. That the same defect in NK-cell killing of xenogeneic, Ad2-transformed cells seen with nude-mouse spleen donors was also seen with euthymic-mouse spleen cell donors, but not with hamster or rat donors, suggests the existence of a species-related restriction in the recognition and destruction of xenogeneic Ad2-transformed cells by murine NK cells. When considering the significance of NK-cell activity in vivo to susceptibility to tumor challenge, this murine NK-cell defect might be critical in nude mice since they lack the potent T-cell-mediated xenograft response available to euthymic mice and would presumably be dependent on NK cells and other such immunologically nonspecific antineoplastic cellular immune responses for rejection of a transformed-cell challenge.

The results presented here on nude-rat NK-cell activity and tumor resistance show that host T-cell-mediated immune responses are not required for rejection of Ad2-transformed cells and suggest that host NK-cell responses (and perhaps other such immunologically nonspecific host cellular immune responses) may be sufficient to mediate this rejection process. In contrast to their resistance to tumor induction by Ad2-transformed hamster cells, nude rats were susceptible to tumor induction by nude-rat NK-cell-resistant Ad12- and SV40-transformed hamster cells (Table 2). These cytolytic and tumorigenic phenotypes of Ad2-transformed hamster cells compared with Ad12- and SV40-transformed hamster cells are identical to those observed in hamster NK-cell assays and in tumor induction studies in immunocompetent, syngeneic adult hamsters (i.e., Ad2-transformed cells are NK cell susceptible and nontumorigenic, whereas Ad12- and SV40-transformed cells are NK cell resistant and tumorigenic; 4, 20). Therefore, these nude-rat data provide further experimental support for the concepts that (i) the thymus-independent host cellular immune response of which NK cells are a component can function as a barrier to tumor induction by certain types of neoplastic cells (e.g., hamster or rat cells transformed by nononcogenic adenovirus) independent of thymus-dependent cellular immune responses (e.g., CTL) and that (ii) such NK-cell-mediated host defenses are ineffective against cytolytic-resistant neoplastic cell populations (e.g., Ad12- or SV40-transformed hamster cells).

The results of the present study also support an alternative explanation for the differences in the tumor-inducing capacities of cells transformed by nononcogenic adenovirus compared with Ad12 that does not depend on the differences in transformed-cell-surface class I MHC expression that have been proposed as a mechanism of escape from CTL killing by Ad12-transformed rat cells. NK-cell-mediated target-cell killing, unlike CTL killing, is not restricted by or dependent on expression of class I MHC on the target-cell surface (11,

24). Therefore, the differences in the nude-rat NK-cell susceptibilities and in the tumor-inducing capacities of Ad2- compared with Ad12-transformed hamster cells in T-cell-deficient nude rats cannot be explained by differences in E1A gene-mediated regulation of class I MHC expression on these cells. The results of our recently reported study do indicate, however, that expression of the E1A gene in Ad2- or Ad5-transformed hamster and rat cells is sufficient, in the absence of other early viral gene function, to induce susceptibility of transformed cells to the lytic effects of NK cells and activated macrophages (5). These results along with those in the present study provide a different perspective about a possible role of adenovirus E1A in the regulation of transformed-cell susceptibility to destruction by host cellular immune defenses and the related virulence of such cells in vivo. In the class I MHC antigen-CTL model of Bernards et al. (1), it is proposed that Ad12 E1A actively suppresses cell-surface class I MHC expression, resulting in decreased transformed-cell susceptibility to CTL-mediated killing, whereas Ad5 E1A has little or no effect on class I MHC expression. In this adenovirus, E1A-NK cell/macrophage model, Ad2 or Ad5 E1A appears to actively induce transformed cell susceptibility to killing by these two types of effector cells in a manner that is unrelated to class I MHC expression. The data available on Ad12-transformed cell resistance to NK-cell-mediated cytolysis are insufficient to allow a clear definition of the mechanism of resistance. We speculate that Ad12 E1A simply may be inefficient in inducing the cytolytic-susceptible transformed-cell phenotype. It cannot be excluded, however, that the cytolytic resistance of Ad12-transformed hamster cells in this model is an active E1A-regulated process.

The data presented here also show that the cytolytic-susceptible Ad2-transformed cell phenotype depends on the cytolytic competence of the killer-cell population. Thus, expression of the E1A gene in Ad2-transformed hamster and rat cell lines does not guarantee lysis of these cells by nude-mouse NK cells. Furthermore, these data indicate that there are potential pitfalls in the interpretation of the immunological significance of tumor induction studies done only with nude mice. For example, these results show that one cannot necessarily conclude that NK cells are not a factor in tumor rejection in the host of origin on the basis of progression of xenogeneic tumors in nude mice with intact NK-cell responses, when the presence of NK-cell activity is gauged by lytic activity for a known susceptible target cell such as YAC-1. Theoretically, additional defects, such as alterations in effector cell chemotaxis or amplification, could exist in NK-cell responses and other thymus-independent inflammatory cell responses that might render nude mice unusually susceptible to challenge with certain types of xenogeneic neoplastic cells whether or not such host cellular immune defenses play an important role in the biology of tumor rejection in hosts from which the transformed cell line or tumor was originally derived.

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