Differential Induction of Cytolytic Susceptibility by E1A, *myc*, and *ras* Oncogenes in Immortalized Cells

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The E1A oncogene of adenovirus serotypes 2 and 5 induces susceptibility to the cytolytic effects of natural killer lymphocytes and activated macrophages when expressed in infected and transformed mammalian cells (cytolysis-susceptible phenotype). E1A and the oncogenes *v-myc*, long-terminal-repeat-promoted *c-myc*, and activated *c-ras* share the ability to immortalize transfected low-passage rodent cells. The cytolytic phenotypes of well-characterized rodent cell lines immortalized by these three oncogenes were defined. In contrast to target cells expressing the intact E1A gene, *myc*- and *ras*-expressing, immortalized primary transfectants were resistant to lysis by both types of killer cell populations. The same patterns of susceptibility (E1A) and resistance (*myc* and *ras*) to cytolysis were observed in oncogene-transfected continuous rat (REF52) and mouse (NIH 3T3) cell lines, indicating that differences in the cytolytic phenotypes associated with expression of these oncogenes are not due to cell selection during immortalization. The results suggest that the E1A oncogene may possess a functional domain that is different from those of other oncogenes, such as *myc* and *ras*, and that the activity linked to this postulated domain is dissociable from the process of immortalization.

An increasing number of oncogenes have been associated with the process of neoplastic cell transformation. Most of the information generated during studies of the activities of these genes relates to possible mechanisms by which oncogene products induce conversion of normal cells to cells exhibiting in vitro morphologies and growth properties commonly associated with neoplastic cells. This approach to evaluating the roles of oncogenes in neoplasia assumes that acquisition of these in vitro traits is synonymous with tumorigenicity. However, cell lines may exhibit fully transformed phenotypes in vitro and yet be unable to induce tumors in immunocompetent animals. These observations suggest the existence of oncogene-associated traits that determine the success or failure of transformed cells in establishing progressive malignancies when confronted with the potentially destructive effects of the antineoplastic immunological response of the host.

Natural killer lymphocytes (NK cells) and activated macrophages are putative components of the cellular immune response to transformed cells that may provide an early barrier to the establishment of primary or metastatic neoplasms in immunocompetent hosts. Differences exist in the abilities of DNA tumor virus oncogenes to render immortalized (partially transformed) or completely transformed cells susceptible or resistant to the cytolytic mechanisms of these two host killer cell populations. Expression of the E1A oncogene of human adenovirus serotypes 2 and 5 (Ad2/5) in transformed hamster, rat, and mouse cells induces susceptibility of these cells to lysis by NK cells and activated macrophages (cytolytic susceptibility) (6, 31; J. L. Cook, D. L. May, B. A. Wilson, B. Holskin, M.-J. Chen, D. Shalloway, and T. A. Walker, *J. Immunol.*, in press). This E1A oncogene-induced cytolytic susceptibility correlates with the rejection of these transformed cells by immunocompetent animals (21). In contrast, expression of the analogous E1A oncogene of Ad12 in transformed cells is not associated

with efficient induction of cytolytic susceptibility, and these cytolysis-resistant, Ad12 E1A-expressing cells are highly oncogenic in immunocompetent animals (21, 31). Studies of regulation of NK cell cytolytic susceptibility by the oncogene encoding the large T antigen of simian virus 40 (SV40) have shown that oncogene induction of cytolytic susceptibility in transformed cells may also be host cell species specific. For example, SV40 T-antigen-expressing, transformed mouse cell lines are susceptible to lysis by NK cells and activated macrophages, whereas SV40 T-antigen-expressing transformed hamster cell lines are resistant to lysis by both types of host effector cells (2, 3, 10). As observed with E1A oncogene-expressing transformed cells, there is also a correlation between the cytolytic phenotypes and tumorigenic phenotypes of SV40 T-antigen-expressing mouse (usually nontumorigenic) and hamster (highly tumorigenic) cells (21, 35).

The available studies of the effects of other oncogenes on the susceptibility of transformed cells to destruction by NK cells suggest the existence of oncogene-related and host-cell-specific differences in the cytolytic phenotypes of oncogene-transformed cells that are analogous to those observed with DNA virus oncogenes (12, 20, 36). However, all reported studies have used continuous cell lines that already express many of the traits commonly associated with cell transformation as the parental cells for oncogene transformation. Using this approach, one cannot exclude the possibility that combined interactions between transfected oncogenes and undefined activities expressed in continuous parental cells will affect the interpretation of the effects of the transfected oncogene on cytolytic susceptibility assays. A more direct analysis of oncogene effects on target cell cytolytic susceptibility is provided by studying target cells derived by oncogene transfection of primary, nontransformed cells. In this investigation, three oncogenes that share the ability to immortalize primary cells, E1A, *myc*, and *ras*, were used to study the NK cell and macrophage cytolytic susceptibilities of primary oncogene transfectants.

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MATERIALS AND METHODS

Target cells. Primary Fischer rat embryo cells (REC) were derived as previously described (4) by trypsinization of tissue fragments from near-term embryos. A2T2C4 is an Ad2-transformed Hooded Lister strain rat embryo fibroblast cell line (11) that has been shown to be highly susceptible to lysis by NK cells (5). MT1A is a Fischer strain rat cell line derived by immortalization of primary baby rat kidney (BRK) cells by transfection with the Ad5 E1A-containing plasmid pMTE1A, in which the E1A gene is controlled by the promoter of the mouse metallothionein I gene (38). E1A2 and E1A3 (formal names, BRKnE1A2 and BRKnE1A3, respectively) are Fischer strain cell lines derived by immortalization of secondary BRK cells by transfection with the Ad5 E1A-containing plasmid p1A, in which the E1A gene is controlled by the adenovirus promoter (16). HrA2 is a Fischer strain cell line derived by immortalization of primary BRK cells by transfection with plasmid pHrA, which contains the E1A region from the Ad5 host range mutant H5hr440 (38). The H5hr440 mutation results in synthesis of a truncated E1A polypeptide encoded by sequences in the first (amino-terminal) E1A exon that are common to the E1A 13S and 12S mRNAs (34); second E1A exon sequences are not expressed. myc3 is a Fischer strain cell line derived by immortalization of primary BRK cells by transfection with plasmid pv-myc, which contains a portion of the MC29 avian myelocytomatosis virus that encodes the recombinant p110^{gag-myc} molecule under control of the native virus promoter, the 5' long terminal repeat (LTR) (29). hmyc1 and hmyc3 (formal names, BRKhmyc1 and BRKhmyc3, respectively) are Fischer strain cell lines derived by immortalization of secondary BRK cells by transfection with plasmid pLTRhmyc, which contains the normal human *c-myc* gene controlled by a retrovirus LTR (16). ras2, ras3, and ras5 (formal names, BRKnras2, BRKnras3, and BRKnras5, respectively) are Fischer strain cell lines derived by immortalization of secondary BRK cells by transfection with plasmid pEJras, containing an activated human *ras* gene (Gly to Val, residue 12) derived from a bladder carcinoma (16).

REF52 is a spontaneously immortalized, clonal fibroblast cell line derived from a Fischer REC culture (23). The following cell lines were derived by transfection of REF52 cells with the indicated plasmids. 1A3 is a cell line transfected with the Ad5 E1A-containing plasmid p1Aneo, in which the E1A gene is controlled by the adenovirus promoter (17). Mmyc-1 and Mmyc-4 are cell lines transfected with the murine *c-myc*-containing plasmid pSVMmycMoneo, in which the *myc* gene is controlled by the SV40 promoter (17). Mmyc1 expresses the exogenous *myc* gene at approximately 2 times the level of endogenous *c-myc*, whereas Mmyc4 expresses exogenous *myc* at approximately 10 times endogenous *c-myc* levels (17). RN12, RN13, and RN14 are cell lines transfected with the pT24 Ha-*ras* plasmid containing the activated (Val-12) human *ras* oncogene (9).

ras1N, ras3N, ras4N, and ras6N were derived from G418-resistant foci of murine NIH 3T3 cells after transfection with the plasmid pT24neo, in which the gene encoding the activated (Val-12) human *ras* gene is linked to the G418 resistance marker gene, *SVaph* (9). PE1A (formal name, NIH[pE1Awt/pSV2neo/cos]A; 1) is an E1A oncogene-expressing NIH 3T3 cell line derived by cotransfection of parental cells with an E1A-containing plasmid and the *neo* gene and by selection in G418 (GIBCO Laboratories).

Cell lines were maintained by weekly passage in Dulbecco modified Eagle medium containing antibiotics and 5 to 10%

calf serum. Cell lines transfected with the *neo* gene were maintained in the same medium containing 200 µg of G418 per ml. All cell lines were tested periodically for contamination with mycoplasma by using the Mycotect (Bethesda Research Laboratories, Inc.) assay and were negative.

Cytotoxicity assays. Assays to evaluate the susceptibilities of target cells to the cytolytic effects of NK cells and activated macrophages were performed essentially as described previously (2, 3), with minor modifications. Animals of either sex were obtained from the National Jewish Center Animal Care Facility (random-bred golden Syrian hamsters), from Jackson Laboratory (CBA/J mice), or from Banton-Kingman Laboratory (outbred Sprague-Dawley rats). Suspensions of single cells from normal spleens obtained from unprimed adult (6- to 12-week-old mice and 2- to 6-month-old rats and hamsters) donor animals were used as the source of NK cell activity. Spleen cells and labeled target cells were added at various ratios (as indicated in the figure legends) to flat-bottom 96-well plates (Costar 3596) for periods of cocultivation ranging from 18 to 48 h in a volume of 300 µl. Target cells were prelabeled with ⁵¹Cr (100 µCi/ml for 45 min at 37°C) for 18-h assays or with [³H]thymidine (0.2 µCi/ml, overnight at 37°C) for 48-h assays. After the period of cocultivation, the amount of target cell radiolabel released into the supernatant was used to calculate spleen cell-induced target cell killing as described previously (2). Assays of activated macrophage killing of xenogeneic, oncogene-transfected target cells were performed as described previously (2), using monolayers of hamster macrophages activated in vivo by donor infection with *Mycobacterium bovis* BCG (bacillus Calmette-Guérin). Such activated macrophage monolayers contained >95% macrophages, as evidenced by histochemical staining and phagocytosis assays. Target cells labeled with [³H]thymidine were incubated on activated macrophage monolayers for 48 h, and macrophage-induced target cell killing was quantitated as described for NK cell assays. Activated macrophage-to-target cell ratios were approximately 33:1 in these assays. All cytotoxicity assays were performed in Dulbecco modified Eagle medium containing antibiotics and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (20 mM) supplemented with glucose (final concentration, 15 mM) and 10% low-endotoxin (<1 ng/ml) fetal bovine serum (Sterile Systems). The significance of the differences in the levels of killer cell-induced cytotoxicity between target cell lines was estimated by Student's *t* test.

RESULTS

Comparisons of E1A-, *myc*-, and *ras*-immortalized primary transfectants for susceptibility to NK cell and activated macrophage killing. Transformation experiments using single oncogenes have shown that normal primary cells may be rendered immortal as a result of the expression of certain transfecting oncogenes but may not exhibit many of the traits of cells transformed by combinations of oncogenes (14, 16, 29, 38). To dissect the effects of expression of single oncogenes on target cell cytolytic phenotypes, it was useful to contrast this series of well-characterized cell lines derived after single-oncogene immortalization of early-passage rodent cells (Table 1).

The isolated expression of the E1A oncogene, under the control of the strong heterologous metallothionein promoter (e.g., MT1A), is sufficient to induce susceptibility of rat cell primary transformants to NK cell- and macrophage-mediated lysis of target cells that is comparable to the cytolytic

TABLE 1. Oncogene-transfected cell lines

Cell line	Parental cell type	Virus or oncogene ^a	Reference
T2C4	Rat embryo fibroblasts	Ad2	11
MT1A	Baby rat kidney cells	Ad2/5 E1A (MT)	36
E1A2			
E1A3	Baby rat kidney cells	Ad2/5 E1A	15
HrA2	Baby rat kidney cells	E1A, ~1st exon	36
myc3	Baby rat kidney cells	v-myc	28
hmyc1			
hmyc3	Baby rat kidney cells	Human-c-myc (LTR)	15
ras2			
ras3			
ras5	Baby rat kidney cells	Activated <i>ras</i> (Val-12)	15
1A3	REF52	Ad2/5 E1A	16
Mmyc1			
Mmyc4	REF52	Mouse-c-myc (SV)	16
RN12			
RN13			
RN14	REF52	Activated <i>ras</i> (Val-12)	9
PE1A	NIH 3T3	Ad2/5 E1A	1
ras1N			
ras3N			
ras4N			
ras6N	NIH 3T3	Activated <i>ras</i> (Val-12)	— ^b

^a See Results for description of plasmids used for transfection. MT, LTR, or SV indicates that the oncogene is controlled by a heterologous promoter (MT, mouse metallothionein promoter; LTR, retrovirus promoter [long terminal repeat]; SV, SV40 promoter). Otherwise, the oncogenes are controlled by their native promoters.

^b Derivation is described in Materials and Methods.

susceptibility seen with Ad2-transformed rat cells (e.g., T2C4; 5). These two E1A-expressing cell types were used interchangeably as cytolysis-susceptible controls for these studies. The *myc* and *ras* oncogenes, like E1A, have also been shown to immortalize low-passage rat cells in vitro (16, 29). Figure 1 shows the NK cell susceptibilities of target cells derived after immortalization of rat cells with these three oncogenes. E1A2 and E1A3 (Fig. 1A) are rat cell lines immortalized by the E1A gene under control of the adenovirus promoter. Both of these cell lines were as susceptible as the positive control cell lines to lysis by rat NK cells and

were significantly more susceptible to lysis than were nontransfected REC. These two target cells and the positive control cell lines, T2C4 and MT1A, express both exons of the E1A oncogene. In contrast, HrA2 cells, immortalized by expression of only the first E1A exon, are NK cell resistant (6; Fig. 1). These data show that the E1A oncogene induces cytolytic susceptibility in immortalized primary cells at the level of expression induced by the E1A promoter (Fig. 1, E1A2 and E1A3) and suggest that E1A oncogene-induced cytolytic susceptibility may be contingent on expression of the second E1A exon (Fig. 1, HrA2). The NK cell and macrophage cytolysis-resistant phenotype of the HrA2 cell line is supported by the observation that three other cell lines derived by transfection with the pHRa plasmid plus a complementing oncogene are also highly resistant to NK cell and macrophage lysis (J. L. Cook, unpublished data).

NK susceptibilities of *myc*- and *ras*-immortalized rat cells were compared with those observed with E1A-immortalized target cells (Fig. 1B and C). These target cells, immortalized by either the viral *myc* gene (*myc3*), the LTR-promoted human *c-myc* gene (*hmyc1* and *hmyc3*), or the activated human *ras* gene (*ras2*, *ras3*, and *ras5*), were highly resistant to NK cell-mediated lysis. When representative E1A-, *myc*-, and *ras*-expressing BRK cell lines were tested in cytolysis assays using xenogeneic (hamster) NK cells and activated macrophages rather than rat NK cells, the same patterns of cytolytic susceptibility and resistance were seen (Fig. 2), indicating that killer cell recognition of these oncogene-associated differences in the cytolytic susceptibilities of target cells is not species restricted or NK cell specific.

Differences in E1A, *myc*, and *ras* oncogene-induced cytolytic susceptibility of host cells are not due to cell selection during immortalization. The oncogene-immortalized cell lines used as targets in the NK cell assays shown in Fig. 1 were all derived by oncogene transfection of uncloned populations of primary or secondary REC. Since certain embryonic cell types are susceptible to NK cell-mediated lysis (30), one possibility to consider is that E1A transfection selects among the heterogeneous embryonic cell population for immortalized cells that are inherently more NK susceptible than are cells selected during immortalization by either *myc* or *ras*. To address this question, comparative NK cell cytolysis

FIG. 1. Comparative NK cell susceptibilities of BRK cell lines immortalized by transfection with the E1A (A), *myc* (B), or *ras* (C) oncogene. Bars represent the results (mean $\pm$ standard error) of at least four rat NK cell cytolysis assays. Target cell lines are described in Table 1 and Materials and Methods. REC, Nontransfected rat embryo cells. Rat spleen NK cell assays were performed with spleen cell-to-target cell ratios of 200:1, 100:1, 50:1, and 25:1. Spleen cells and target cells were cocultured for 48 h to optimize killing of resistant target cells; identical patterns of killing were seen in cytolysis assays of 6 and 24 h, using T2C4, *hmyc3*, and *ras3* target cells. Only results at the optimal 200:1 ratio are shown; identical patterns of killing were observed at ratios of 100:1 and 50:1. The four target cell types expressing the intact E1A gene, T2C4, MT1A, E1A2, and E1A3, were significantly more susceptible to rat NK cell lysis than were nontransformed REC ($P < 0.001$). The target cell expressing only the first E1A exon, HrA2, and the *myc*- and *ras*-immortalized cell lines were not more susceptible than nontransfected REC to NK cell-mediated lysis ($P > 0.05$).

FIG. 2. Comparative susceptibilities of E1A-, *myc*-, and *ras*-immortalized BRK cell lines to lysis by xenogeneic (hamster) NK cells (A) and activated macrophages (B). Bars represent the results (mean $\pm$ standard error) of seven NK cell or five macrophage cytotoxicity assays, as indicated. Target cell lines are described in Table 1 and Materials and Methods. REC, Nontransfected rat embryo cells. Hamster spleen NK cell assays were performed with spleen cell-to-target cell ratios of 100:1, 50:1, 25:1, and 12:1. Only the results at the optimal 100:1 ratio are shown; identical patterns of killing were observed at ratios of 50:1 and 25:1. Macrophage cytotoxicity assays were performed by incubating prelabeled target cells on monolayers of *M. bovis* BCG-activated hamster peritoneal macrophages at an approximately 33:1 macrophage-to-target cell ratio. Target cells were cocultured with killer cells for 48 h in both NK cell and macrophage cytotoxicity assays to optimize killing of resistant target cells. Only the E1A-expressing target cell, T2C4, was significantly more susceptible than nontransfected REC to lysis by xenogeneic hamster NK cells ($P < 0.001$) and activated macrophages ($P < 0.005$).

assays were performed, using target cell lines derived after oncogene transfection of the clonal Fischer rat cell line REF52 (Fig. 3). Nontransfected REF52 cells were shown in preliminary experiments to be highly resistant to lysis by rat NK cells. The E1A oncogene-expressing REF52 cell line, 1A3, was highly susceptible to lysis by rat NK cells at a level of killing comparable to that seen with cytotoxicity-susceptible control target cells, T2C4 (Fig. 3A). In contrast, two REF52 cell lines that express different levels of the murine *c-myc* gene controlled by the SV40 promoter (Fig. 3B; 17) and three *ras*-transfected REF52 cell lines that express the activated *ras* oncogene (Fig. 3C; 9) were no more susceptible to NK cell-mediated lysis than were nontransfected REF52 control target cells (Fig. 3A). The identity of these patterns of NK cell susceptibility and resistance obtained by using oncogene-transfected REF52 target cells to the pattern observed with oncogene-transfected REC shown in Fig. 1 argues against selection of inherently NK-susceptible cells during E1A, but not *myc* or *ras*, gene transfection. Therefore, these results support the conclusion that the products of the E1A oncogene expressed during cell immortalization are uniquely

active in induction of susceptibility of the host cell to NK cell killing, whereas the products of the *myc* and *ras* oncogenes are inefficient at inducing this immortalized cell phenotype.

Oncogene-induced differences in target cell morphology do not explain differences in cytolytic susceptibility. Examination of the morphologies of the target cells used in these assays revealed differences between E1A-transfected target cells and targets immortalized by *myc* or *ras*. E1A-immortalized REC and REF52 cells exhibited a clear morphological transformation (smaller epithelioid cells similar in morphology to the E1A-transfected NIH 3T3 cells [Fig. 4b]) compared with the parental cells from which they had been derived, whereas *myc*- and *ras*-immortalized cells exhibited minimal morphological differences compared with the cells of origin (i.e., they remained more fibroblastic). The neoplastic cell characteristics that render target cells recognizable for killing by NK cells and activated macrophages are not well defined. However, it has been proposed that such effector cells can recognize and be triggered by target cell membrane changes that are associated with morphological transforma-

FIG. 3. Comparative NK cell susceptibilities of REF52 cell lines expressing the E1A (A), *myc* (B), or *ras* (C) oncogene. Bars represent the results (mean $\pm$ standard error) of two to five rat NK cell cytotoxicity assays. Target cell lines are described in Table 1 and Materials and Methods. REF52, Nontransfected parental cells. Spleen cells and target cells were cocultured at the optimal 200:1 spleen cell-to-target cell ratio for 48 h to optimize killing of resistant target cells. The target cells expressing the E1A gene, T2C4 and 1A3, were significantly more susceptible than parental cells to NK cell-mediated lysis ($P < 0.005$); however, the *myc*- and *ras*-expressing cell lines were not ($P > 0.05$).

FIG. 4. Morphologies of NIH 3T3 cell lines transfected with either the E1A or *ras* oncogene. (a) Nontransfected NIH 3T3 parental cells; (b) PE1A cells transfected with the Ad5 E1A oncogene (1); (c) ras1N; (d) ras3N; (e) ras4N; (f) ras6N. Panels c to f show the four morphologies obtained by transfection of NIH 3T3 cells with plasmid pT24neo, containing the activated human *ras* oncogene. Magnification, $\times 100$.

tion. For example, BALB/c 3T3 cells that spontaneously undergo morphological transformation to 3T12 cells simultaneously change from cytolysis-resistant to highly cytolysis-susceptible target cells in macrophage cytolysis assays (13). Therefore, one question that derives from the experiments presented here is whether *myc* and *ras* are simply unable to induce the membrane abnormalities associated with morphological transformation that are adequate to trigger effector cell recognition and lytic activity. Comparisons of the transformed morphologies and cytolytic susceptibilities of a series of *ras*-transfected NIH 3T3 mouse cell lines provided data relevant to this question.

Previous studies have shown that NIH 3T3 cells are resistant to killing by murine NK cells and activated macrophages and that E1A oncogene expression in transfected NIH 3T3 cells renders them highly susceptible to NK cell killing (Cook et al., in press). Upon transfection of NIH 3T3 cells with the activated *ras* oncogene, a variety of transformed morphologies of *ras*-expressing clones was observed, ranging from clones that were minimally transformed in comparison with parental NIH 3T3 cells (for example, Fig. 4d and e compared with Fig. 4a) to clones that were markedly transformed as a result of *ras* transfection (for example, Fig. 4c and f compared with Fig. 4a). This

FIG. 5. Comparative NK cell susceptibilities of NIH 3T3 cells transfected with either the E1A or *ras* oncogene. Bars represent the results (mean $\pm$ standard error) of at least six mouse NK cell cytotoxicity assays. 3T3, Nontransfected NIH 3T3 parental cells. Target cell lines are described in Table 1 and Materials and Methods, and target cell morphologies are shown in Fig. 4. Spleen cells and target cells were cocultured for 18 h at spleen cell-to-target cell ratios of 400:1, 200:1, 100:1, 50:1, and 25:1. Only results at the optimal 400:1 ratio are shown; identical patterns of killing were observed at ratios of 200:1 and 100:1. Only the E1A-expressing target cell line, PE1A, was significantly more susceptible than the nontransfected NIH 3T3 parental cells to NK cell-mediated lysis ($P < 0.001$). Differences between the killing of the *ras*-transfected target cells and parental cells were not significant ($P > 0.05$).

spectrum of cell morphologies and the inherent resistance of parental NIH 3T3 cells to NK cell cytotoxicity allowed examination of the independent significance of oncogene-induced morphological transformation as related to NK cell susceptibility. An NK-susceptible, E1A oncogene-transfected NIH 3T3 cell line, PE1A (1) (Fig. 4b), was used as a cytotoxicity-susceptible control for these assays. The results (Fig. 5) showed that the degree of *ras*-induced morphological transformation was not correlated with the level of target cell susceptibility to cytotoxicity. These data indicate that oncogene-induced morphological transformation alone is not sufficient to explain differences in oncogene-transfected target cell cytotoxic susceptibilities and suggest that oncogene product activities that are dissociable from morphological transformation are responsible for regulation of target cell cytotoxic phenotypes.

DISCUSSION

Transformation experiments using single oncogenes have provided evidence that normal primary cells may be rendered immortal as a result of expression of certain single oncogenes but may not exhibit some of the traits of cells that are completely transformed by combinations of oncogenes (19, 28). These observations are the basis for the concept that immortalization may be the first step in the transformation process and must be complemented by a second event for completion of transformation. This conceptual two-step transformation process is compatible with observations from studies of chemical-induced carcinogenesis and with epidemiological data on tumor progression *in vivo* (8, 33). What, then, is the role of the host in this process of cell transformation? Is the host merely a provider of the cells for oncogene-induced transformation and of a permissive environment in which such oncogene-transformed cells can proliferate to form a progressive malignancy? Or is the host an active participant in determining which oncogene-ex-

pressing cells will, and which will not, survive and progress? On the basis of observations that oncogene-transformed cells may be tumorigenic in immunodeficient hosts (e.g., nude mice) but not immunocompetent hosts (e.g., adult, syngeneic animals), it appears likely that the host cellular immune response is, in fact, an active determinant of transformed cell regression or progression. The outcome of the interactions between transformed cells and the cellular immune response is likely to depend on a variety of factors, among which may be the growth rate of the transformed cell population, the quality and quantity of the host cellular immune response elicited by transformed cells, and the susceptibility of the transformed cell population to the destructive effects of the host effector cells that constitute the cellular immune response. It is the latter that is addressed in these studies.

In this report, evidence is presented showing that oncogene expression at the first step of cell transformation, cell immortalization, may result in significant differences in the survival of immortalized cells when confronted with host NK cells and activated macrophages, putative components of the early-appearing, antineoplastic, cellular immune response. The Ad2/5 E1A oncogene renders the cells it immortalizes highly susceptible to lysis by NK cell and macrophage lytic mechanisms (cytotoxicity-susceptible phenotype), whereas *myc* and *ras* oncogenes do not change the inherent cytotoxic resistance of host cells during immortalization. Therefore, E1A, *myc*, and *ras* share immortalizing activity but do not share the ability to induce susceptibility to cytotoxicity. Genetic analysis of functional domains of the E1A gene that are conserved among adenovirus serotypes has shown that the domains required for cellular immortalization and for collaboration with other oncogenes during transformation are located in the E1A first exon (18, 22, 25, 32). The cytotoxic resistance of the HrA2 cell line (Fig. 1 and 2), immortalized by transfection with the functional equivalent of the E1A first exon, suggests that expression of this minimal essential Ad2/5 E1A transforming region is insufficient for induction of susceptibility to cytotoxicity and that the E1A second exon may be required for induction of this phenotype. Whether the E1A second exon contains a functional domain whose expression is required for induction of susceptibility to cytotoxicity or whether the absence of the E1A polypeptide encoded by this exon indirectly alters the function of an E1A first-exon domain remains to be determined. However, it appears that *myc* and *ras* lack the functional equivalent of this postulated E1A domain. Furthermore, the ability of all three of these oncogenes to immortalize primary cells suggests that the E1A function(s) that induces susceptibility to cytotoxicity may be dissociable from those required for immortalization.

These results and related data from other reports (12, 20, 36) also suggest general conclusions about the determinants of oncogene-induced susceptibility to cytotoxicity. An overview of these data indicates that the identity of the oncogene, the host cell in which it is expressed, the level of oncogene expression in that cell, and the state of activation of the host killer cell being tested are all important in determining expression of the cytotoxicity-susceptible transformed cell phenotype. The observations that E1A-expressing cells of hamster, rat, mouse, and human origin are all susceptible to NK cell-mediated lysis (5, 6; Cook et al., *in press*) suggest that there is a broad host cell range in which the E1A oncogene can induce susceptibility to cytotoxicity. In contrast, *ras* oncogene expression appears to be unable to induce susceptibility to NK cell cytotoxicity in

primary rat cells (Fig. 1 and 2), continuous REF52 rat cells (Fig. 3), or continuous NIH 3T3 mouse cells (Fig. 5; 12, 20) but able to induce susceptibility to cytolysis in continuous Rat-1 cells (15) and continuous mouse C3H10T1/2 cells (12, 36) if the level of *ras* expression is high and if the NK cell activity is augmented by prior activation. The results of cytolysis assays in which xenogeneic and allogeneic NK cells and xenogeneic, activated macrophages were used as killer cells suggest that the cytolytic phenotypes of these oncogene-immortalized cells may be determined by differential susceptibilities to cytolytic mechanisms that NK cells and macrophages from different species have in common. It has been reported that the E1A oncogene renders transfected NIH 3T3 cells susceptible to cytotoxicity by recombinant human tumor necrosis factor alpha (TNF), whereas *myc* and *ras* do not (1). Since TNF is one of the cytolytic molecules produced both by activated macrophages and by certain populations of NK cells (7, 24, 26, 27, 37), further analysis of oncogene activities in immortalized cells that may be involved in triggering TNF release and altering TNF receptor function and post-receptor-binding pathways of TNF cytolytic activity may be useful for an understanding of oncogene-related differences in susceptibilities of target cells to cytolysis.

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