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Immunology

Expression of the adenovirus *E1A* oncogene during cell transformation is sufficient to induce susceptibility to lysis by host inflammatory cells

(neoplasia/natural killer cells/macrophages/cellular immunity)

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ABSTRACT **Mammalian cells transformed by nononcogenic human adenoviruses exhibit high susceptibility to destruction by host mononuclear inflammatory cells. We have analyzed the viral gene regulation of the susceptibility of transformed cells to lysis by natural killer cells and activated macrophages. Comparisons of target cell lines transformed by overlapping segments of the adenovirus *E1*-transforming gene region revealed that isolated expression of a single oncogene, *E1A*, was sufficient to cause increased cytolytic susceptibility in the absence of detectable transformed cell-surface expression of viral transplantation antigens and irrespective of histocompatibility antigen identity between killer cells and target cells. These results suggest that oncogene functions that are not linked to the expression of previously recognized cell-surface target structures may actively induce neoplastic cell elimination by components of the host immune surveillance system.**

Progress has been made in identification and definition of functional activities of viral and cellular oncogenes that transform normal cells to neoplastic cells. However, an understanding of factors that determine survival of oncogene-transformed cells when they are confronted with host immune responses is less complete. Our previous studies suggest that protein products of DNA tumor virus oncogenes mediate expression of different levels of susceptibility of virus-transformed cells to destruction by immunologically nonspecific host inflammatory cells such as natural killer (NK) cells and activated macrophages (1). These inflammatory cells may represent the earliest host immune defenses against neoplastic cells (2). Therefore, the capacity of virus-transformed cells to establish incipient malignancies might depend on the level of transformed cell susceptibility to destruction by such host cellular immune defenses.

Based on our data and that reported by others, it can be concluded that expression of one or more early adenovirus (Ad) genes governs the cytolytic susceptibility of virus-transformed rodent cells to immunologically nonspecific (e.g., NK cells and activated macrophages) (3–5) and immunologically specific (e.g., cytotoxic T lymphocytes) host mononuclear cells (6, 7). Neoplastic transformation of normal cells by Ad requires interaction between products encoded by two early viral genes, *E1A* and *E1B*, that constitute the *E1*-transforming region (Fig. 1) (8, 9). Two hypotheses concerning the relationships between Ad early gene expression and transformed cell susceptibility to lysis by host mononuclear inflammatory cells derive from this information. Coexpression of *E1A* and *E1B* gene products may be required for induction of increased cytolytic susceptibility of Ad-transformed cells. Alternatively, *E1A* gene products might induce the cytolytic susceptible transformed cell phenotype independent of *E1B*-encoded proteins. In this study, we present data supporting the second hypothesis and demonstrating that the isolated expression of a single oncogene may determine transformed cell susceptibility to lysis by NK cells and activated macrophages.

MATERIALS AND METHODS

Cells and Cell Lines. Ad2HE1 is an Ad2 virus-transformed LSH Syrian hamster embryo cell (HEC) line that is susceptible to lysis by NK cells and activated macrophages (1). SV40HE1 is a simian virus 40 (SV40)-transformed LSH HEC line that is resistant to lysis by both effector cells (1). Embryo cells were prepared as described (10). HT514b is a Syrian HEC line transformed by nononcogenic Ad5 (11). 972-3 and 983-2 are baby hamster kidney (BHK) cell lines transformed by Ad5 *Xho* I C fragment (Fig. 1) (12). K562 is a NK-susceptible human erythroleukemia cell line. A549 is a human lung carcinoma cell line that is NK resistant. 293 is a human embryonic kidney cell line transformed by sheared Ad5 DNA; 293 expresses only the *E1* region of Ad5 (13). 945-C1 and 954-21 are BHK cell lines transformed by *Hin*dIII-digested Ad5 DNA. 954-5 and 954-6 are BHK cell lines transformed by Ad5 *Hin*dIII G fragment (Fig. 1) (12). A2T2C4 is an Ad2 virus-transformed Hooded Lister rat embryo cell (REC) line (14). 309-10-1 is an Ad5 *dl*309 (wild type) virus-transformed Fisher baby rat kidney (BRK) cell line. 338-500-1 is a Fisher BRK cell line transformed by Ad5 *dl*338, which is a mutant derived from Ad5 *dl*309 with an out-of-frame deletion from 2804 to 3329 bp (see Fig. 1) (15). 1019-C1 and 1019-C3 are BHK cell lines transformed by DNA from the Ad5 host range mutant *hr*50, which has a point mutation between 6.1 and 8.0 m.u. and is defective for synthesis of the *E1B* 58-kDa protein (16). MT1A is a Fisher BRK cell line immortalized by DNA from the Ad5 *E1A*-containing plasmid pMTE1A (Fig. 1) (17). 13S is a Fisher BRK cell line immortalized by DNA from the recombinant Ad2/Ad5 *E1A* plasmid pMT13S, which contains cloned

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Abbreviations: *E1*, *E1A*, and *E1B*, early region 1 of the adenovirus genome and the *A* and *B* genes that constitute this region; NK, natural killer; HEC, hamster embryo cell; REC, rat embryo cell; Ad, adenovirus(es); BHK, baby hamster kidney; BRK, baby rat kidney; m.u., map unit representing 1% of the viral genome; SV40, simian virus 40; bp, base pair(s); BCG, bacillus Calmette–Guérin.

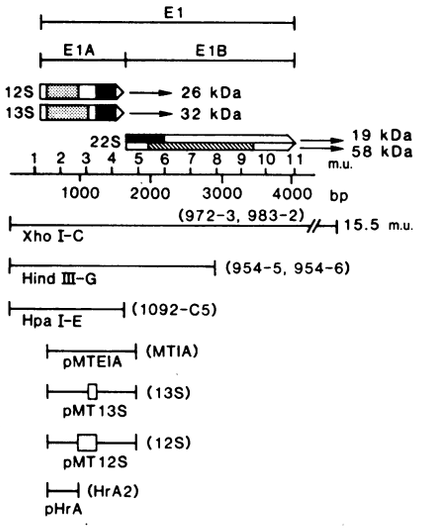

FIG. 1. Schematic diagram showing the overlapping segments (restriction enzyme fragments *Xho* I C, *Hin*dIII G, and *Hpa* I E and DNA segments from plasmids pMTE1A, pMT13S, pMT12S, and pHrA) of the Ad2 and Ad5 *E1*-transforming region contained in the transformed cell lines tested (names in parentheses). The *E1* region, represented here, maps between 1.3 and 11.2 map units (m.u.) on the left-hand end of the Ad2 and Ad5 genome. The *E1A* (1.3–4.6 m.u.) and *E1B* (4.6–11.2 m.u.) genes of the *E1*-transforming region are indicated. The mRNAs (horizontal bar/arrows labeled 12S, 13S, and 22S) transcribed from these genes in transformed cells, the predicted molecular masses of the major *E1A* protein products (12S mRNA, 26-kDa protein; 13S mRNA, 32-kDa protein), and the observed molecular masses of the two proteins encoded by the *E1B* 22S mRNA (19-kDa and 58-kDa proteins) are related to the positions [designated by m.u. and base pairs (bp)] on the r strand (transcribed from left to right) of the left-hand end of the viral genome from which they are encoded. Shaded areas in mRNA bar/arrows indicate reading frames. Open spaces between shaded areas in *E1A* mRNAs indicate intervening sequences (introns) removed during RNA processing. Open boxes in plasmid *E1A* DNA segments (pMT13S and pMT12S) indicate intron deletions represented in cloned cDNAs.

cDNA derived from Ad2 *E1A* 13S mRNA (Fig. 1) (17). 12S is a Fisher BRK cell line immortalized by DNA from the recombinant Ad2/Ad5 *E1A* plasmid pMT12S, which contains cloned cDNA derived from Ad2 *E1A* 12S mRNA (Fig. 1) (17). Since the Ad2/Ad5 recombinant cDNAs contained in pMT13S and pMT12S were derived from processed mRNA, they lack sequences present in the respective *E1A* introns, as indicated by open boxes in the gene segments in Fig. 1. 1092-C5 is a Fisher BRK cell line immortalized by purified *Hpa* I E fragment of Ad5 DNA (see Fig. 1). HrA2 is a Fisher BRK cell line immortalized by DNA from the pHrA plasmid, which contains the *E1A* region from the Ad5 host range mutant virus *hr*440 (16). HrA2 contains Ad-specific sequences as evidenced by a single hybridizing band detected by Southern blot analysis (H.E.R., unpublished data). The *hr*440 mutation results in synthesis of a truncated polypeptide containing 140 amino acids derived from the amino-terminal (left-hand) sequences common to both *E1A* proteins (represented in Fig. 1 by the stippled area designating the first exon of the 12S mRNA) (18). All cell lines were tested for mycoplasma and were negative.

Immunoprecipitation and NaDodSO$_4$/Polyacrylamide Gel Electrophoresis. Virus-specific proteins were immunoprecipitated with specific polyclonal antisera and monoclonal antibodies. Anti-*E1B* 19-kDa fusion protein antibody was obtained from A. Berk. The monoclonal antibody 2A6, directed against the *E1B* 58-kDa protein, was obtained from A. Levine. The polyclonal antibody E1A-C1, directed against the carboxyl-terminal peptides shared by the major polypeptide products of the *E1A* gene, was obtained from P. Branton. Preparation of [^{35}S]methionine-labeled cells for analysis of expression of Ad *E1B* 19-kDa and 58-kDa proteins was performed as described (19). These same techniques were used to prepare lysates of cells labeled with $H_3{}^{32}PO_4$ [200 μCi/ml (1 Ci = 37 GBq) for 4 hr] for analysis of expression of *E1A* phosphoproteins. Immunoprecipitated proteins were resolved by electrophoresis on polyacrylamide gels and visualized by fluorography.

Cytolysis Assays. NK cell assays were performed with spleen cells from adult (2- to 5-month-old) golden Syrian hamsters and Sprague–Dawley rats or Ficoll/Hypaque-separated human blood mononuclear cells, and macrophage monolayers were prepared from peritoneal exudates obtained from adult hamsters previously infected intraperitoneally with Pasteur strain bacilli Calmette–Guérin (BCG) (3). Target cells were labeled with [^{3}H]thymidine, and cytolysis was measured by release of radiolabel during a 48-hr incubation period at 37°C. The average spontaneous release of radiolabel from the target cells tested ranged from 6.5% for SV40HE1 to 33.5% for 1092-C5. Data are presented as the percentage of total radiolabel released from target cells due to effector cell-induced lysis (3).

RESULTS

Cytolytic Susceptibility of Transformed Cells Is Regulated by Genes Within the *E1* Region of the Ad Genome. Previous studies of NK cell susceptibility of mammalian cells transformed by nononcogenic Ad have utilized, as targets, cells transformed by intact virions or large segments of viral DNA (20–22). Such cells contain diverse segments of viral DNA and may express proteins from multiple regions of the early viral genome. Results of these studies have suggested that the function of the *E1*-transforming region alone or of early gene regions other than *E1* might influence transformed cell susceptibility to NK cell-mediated lysis. Therefore, we asked whether, in the hamster system, transformation by the isolated *E1* region of a nononcogenic Ad, Ad5, and expression of the gene products encoded by this region were sufficient to induce increased susceptibility to destruction by NK cells and activated macrophages (Fig. 2*A*). Two hamster cell lines (972-3 and 983-2) transformed by purified Ad5 *E1* fragment were tested for cytolytic susceptibility and compared to cytolytic susceptible cells transformed by intact Ad2

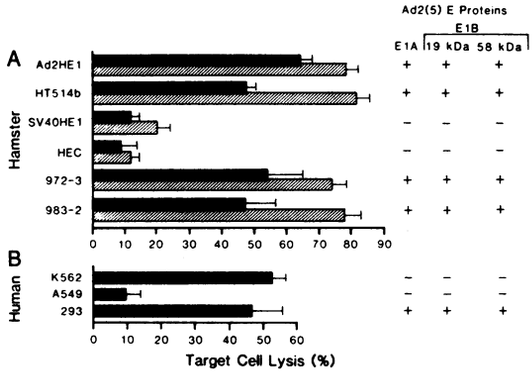

FIG. 2. Cytolytic susceptibilities of transformed cells expressing only the Ad5 *E1* gene region. Bars represent the results (mean ± SEM) of three to five experiments. Solid bars indicate NK cell-induced lysis at optimal 100:1 lymphocyte-to-target cell ratios. Hatched bars indicate BCG-activated hamster macrophage-induced lysis at approximately a 33:1 macrophage-to-target cell ratio. Ad2 or Ad5 *E1A*- and *E1B*-specific proteins were detected by immunoprecipitation with specific antibodies and electrophoretic analysis on NaDodSO$_4$/polyacrylamide gels. 972-3, 983-2, and 293 were highly susceptible to lysis compared to cytolytic resistant control cells ($P <$ 0.001).

(Ad2HE1) and Ad5 (HT514b) virions and to cytolytic resistant, nontransformed HECs and SV40-transformed HECs (SV40HE1). 972-3 and 983-2 were highly susceptible to NK cell- and macrophage-mediated lysis. Thus, functions of Ad gene regions other than *E1* are not required for induction of the cytolytic susceptible phenotype in transformed hamster cells. Studies using the Ad5 *E1*-expressing human cell line, 293, as the target cell in human NK cell assays (Fig. 2*B*) showed that the increased cytolytic susceptibility associated with the *E1* region is also exhibited by transformed human cells and is detected by human killer cells.

***E1B* Protein Function Is Not Required for Expression of Cytolytic Susceptibility in Transformed Cells.** Two products are translated from different reading frames in the *E1B* 22S mRNA in transformed cells (Fig. 1)—a polypeptide of 176 amino acids with an estimated molecular mass of 19 kDa and a second polypeptide of 496 amino acids with an estimated molecular mass of 58 kDa (reviewed in ref. 9). The *E1B* 58-kDa product is present in the transformed cell nucleus and the cytoplasm, is not associated with cellular membranes, and is required for transformation of mammalian cells by virus but not by purified viral DNA (16, 23, 24). The *E1B* 19-kDa product is associated with transformed cell membranes and, along with a different 19-kDa protein encoded by Ad early region 3 (located between 76.6 and 86.0 m.u.), may be involved in expression of virus-specific cell-surface transplantation antigen (23, 25–29).

Interest in the *E1B* 58-kDa product as a mediator of *E1* region-induced cytolytic susceptibility in transformed cells was derived from the correlation observed between expression of this protein and the cytolytic susceptibility of somatic cell hybrids formed between Ad2 (cytolytic susceptible) and SV40 (cytolytic resistant) transformed hamster cells (19). To evaluate the requirement for expression of the *E1B* 58-kDa product for induction of cytolytic susceptibility, two types of transformed rodent cells were tested in NK cell and macrophage assays (Fig. 3). First, hamster cell lines transformed by Ad5 DNA digested with the restriction enzyme *Hin*dIII (945-C1 and 954-21) or by the *Hin*dIII G fragment (Fig. 1; 954-5 and 954-6) were tested (Fig. 3*A*). One *Hin*dIII cleavage site is in the *E1B* 58-kDa coding region (Fig. 1). Of the *Hin*dIII digest-transformed lines, one (954-21) expressed *E1B* 58-kDa protein, and one (945-C1) did not. As expected, neither of the lines transformed by purified *Hin*dIII G fragment (lines 954-5 and 954-6) expressed the 58-kDa protein. All four *Hin*dIII fragment-transformed cell lines were highly susceptible to lysis by NK cells and activated macrophages, and there was no correlation between the presence of detectable *E1B* 58-kDa protein and cytolytic susceptibility. In the second series of experiments (Fig. 3*B*), rat cell lines transformed by wild-type Ad5 (309-10-1) or by an Ad5 deletion mutant, *dl*338 (338-500-1), which lacks sequences required for expression of *E1B* 58-kDa protein, were compared for susceptibility to lysis by rat NK cells. As observed in the previous study (Fig. 3*A*), there was no correlation between the presence of *E1B* 58-kDa protein and cytolytic susceptibility, as wild-type and mutant virus-transformed cells were as susceptible to NK cell-mediated lysis as was the positive control, Ad2 virus-transformed cell line A2T2C4.

Since the *E1B* 19-kDa protein is expressed on the cell surface, its possible role as a mediator of increased cytolytic susceptibility was also of interest. To evaluate the importance of the *E1B* 19-kDa protein in the cytolytic susceptible transformed cell phenotype, two cell lines transformed by DNA from the host range Ad5 mutant *hr*50 (1019-C1 and 1019-C3) were tested for NK susceptibility (Fig. 3*C*). As reported (16), neither cell line contained detectable *E1B* 58-kDa protein, and only 1019-C1 contained detectable *E1B* 19-kDa protein. Both cell lines were highly susceptible to hamster NK cell-mediated lysis. The 1019-C3 cell line (lacking *E1B* 19-kDa protein) was also highly susceptible (92.4% ± 7.6% specific lysis) to lysis by activated macrophages. These results confirm the lack of a correlation between the presence of the *E1B* 58-kDa protein and cytolytic susceptibility observed in the studies shown in Fig. 3 *A* and *B* and also suggest that the expression of the *E1B* 19-kDa protein is not required.

Expression of Either the 13S or 12S mRNA Species of the Ad5 *E1A* Gene in Transformed Cells Is Sufficient for Induction of the Cytolytic Susceptible Phenotype. The above data raise the question as to whether the function of only the *E1A* gene of the *E1*-transforming region is required for expression of the cytolytic susceptible transformed cell phenotype. The *E1A* gene of nononcogenic Ad encodes two mRNA species in transformed cells that can be translated into at least two major polypeptide products (Fig. 1) (reviewed in ref. 9). However, unlike *E1B* 22S mRNA translation, which produces proteins encoded by two different reading frames, the two *E1A* mRNAs are translated in the same reading frames and differ only by the sizes of the introns deleted during mRNA processing. Thus, *E1A* 13S mRNA theoretically encodes a product of 289 amino acids with a predicted molecular mass of 32 kDa, and the *E1A* 12S mRNA theoretically encodes a product of 243 amino acids with a predicted molecular mass of 26 kDa. These two major polypeptides have identical amino (left-hand) and carboxyl (right-hand) termini and differ only by an internal 46 amino acids present in the larger protein. Ad gene fragments containing only the *E1A* region were used to partially transform primary BRK cells to an immortalized phenotype (MTIA, 1092-C5; Fig. 1). In addition, immortalized BRK cell lines were established by transfection of cloned cDNAs derived from the 13S or 12S mRNAs (pMT13S and pMT12S, respectively; Fig. 1). These *E1A* immortalized cell lines contained *E1A*-specific phospho-

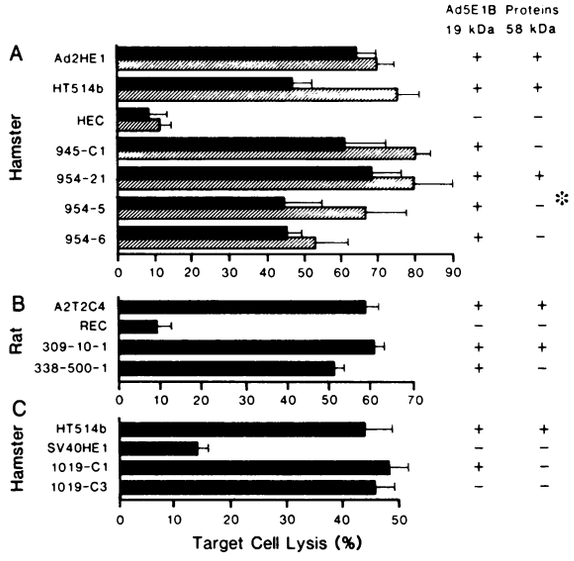

FIG. 3. Lack of correlation between cytolytic susceptibility and transformed cell expression of Ad5 *E1B*-specific proteins. Bars represent the results (mean ± SEM) of three to five experiments. Solid bars indicate NK cell-induced lysis: hamster spleen cells at an optimal 100:1 spleen cell-to-target cell ratio (*A* and *C*); rat spleen cells at an optimal 200:1 spleen cell-to-target cell ratio (*B*). Hatched bars indicate BCG-activated hamster macrophage-induced lysis. Ad5 *E1B*-specific proteins were detected by immunoprecipitation with specific antibodies (see Fig. 2 legend) and electrophoretic analysis on $NaDodSO_4$/polyacrylamide gels. 945-C1, 954-21, 954-5, 954-6, 338-500-1, 1019-C1, and 1019-C3 were all highly susceptible to lysis compared to cytolytic resistant control cells ($P < 0.001$). *, 954-5 cell lysates did not contain detectable *E1B* 58-kDa protein; however, hamsters bearing tumors induced by this cell line had detectable *E1B* 58-kDa-specific antibody in their sera (17).

proteins detectable by immunoprecipitation (Fig. 4; 1092-C5 not shown); none contained detectable *E1B* proteins (not shown). Using these *E1A*-transformed rat cells as targets in rat NK cell assays, two questions were asked: (*i*) whether the function of the *E1A* region alone in immortalized cells is sufficient to induce cytolytic susceptibility and (*ii*) whether there is a difference in the capacity of the 13S and 12S coding regions of the *E1A* gene to induce cytolytic susceptibility during transformation (Fig. 5*A*). Cell lines transformed by the whole *E1A* gene (MT1A and 1092-C5), 13S cDNA (13S), or 12S cDNA (12S) were as sensitive to lysis by rat NK cells as the Ad2 virus-transformed, positive control cell line A2T2C4. Thus, the *E1A* transcription unit contains all of the information required for induction of cytolytic susceptibility during cell transformation, and the internal 46 amino acid difference between the putative major polypeptides encoded by the 13S and 12S mRNAs has no effect on the ability of the gene products to induce cytolytic susceptibility during transformation.

The importance of intact *E1A* function in induction of cytolytic susceptibility was suggested by the results of experiments in which the HrA2 line was used as a source of target cells in rat NK cell assays (Fig. 5*A*). HrA2 was transformed by transfection with pHrA plasmid DNA (Figs. 1 and 5*A*), containing a mutated *E1A* gene that encodes a truncated amino-terminal *E1A* product of 140 amino acids from the first exon of the 13S mRNA (17). The fact that this cell line exhibits a cytolytic resistant phenotype similar to that observed with nontransformed rat embryo cells suggests two conclusions. (*i*) Function of a product encoded by both exons of the *E1A* messages is required for induction of cytolytic susceptibility, either as a result of the presence of a functional domain encoded by the second *E1A* exon or of a functional change in the *E1A* gene product resulting from a conformational change induced by the presence of the additional polypeptide segment encoded by the second exon. (*ii*) Induction of the immortalized state following DNA fragment transfection does not guarantee increased cellular cytolytic susceptibility.

Previous reports have shown that Ad2 and Ad5 *E1A* proteins are localized to the cell nucleus and cytoplasm and are not associated with cellular membranes (23, 30). These results, along with the observations that neither the *E1B* 19-kDa protein nor the *E3* 19-kDa protein (both cell-surface proteins) is required for the induction of cytolytic susceptibility, suggest that no transformed cell-surface, virus-encoded proteins may be required for expression of the cytolytic susceptible phenotype. This is in contrast to destruction of Ad-transformed cells by immunologically specific, syngeneic, cytotoxic T lymphocytes, which requires recognition by the T-cell receptor of cell-surface, virus gene-encoded transplantation antigens associated with class I major histocompatibility antigen identical to that of the T cell (21, 31). The conclusion that the cytolytic activity observed in these studies is unrelated to identity between

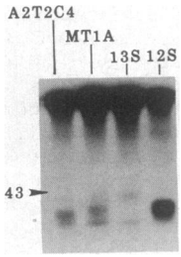

FIG. 4. *E1A* phosphoproteins immunoprecipitated from rat cell lines. The position of a 43-kDa molecular mass marker protein is indicated.

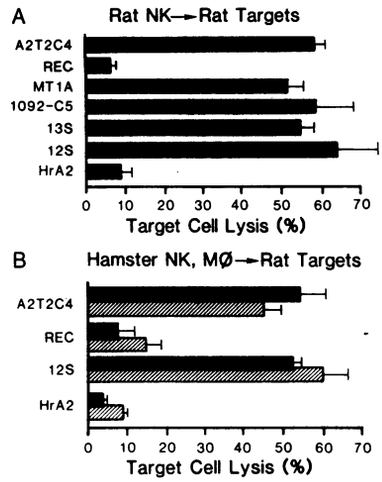

FIG. 5. Cytolytic susceptibilities of transformed rat cells expressing only Ad2(5) *E1A* proteins. Bars represent the results (mean ± SEM) of at least four experiments. Solid bars indicate NK cell-induced lysis: hamster spleen cells at an optimal 100:1 spleen cell-to-target cell ratio (*A*); rat spleen cells at an optimal 200:1 spleen cell-to-target cell ratio (*B*). Hatched bars indicate BCG-activated hamster macrophage (Mϕ)-induced lysis. MT1A, 1092-C5, 13S, and 12S were highly susceptible to lysis compared to cytolytic resistant REC control ($P < 0.001$), and HrA2 was highly resistant to lysis compared to cytolytic susceptible A2T2C4 control cells ($P < 0.001$).

class I cell-surface histocompatibility antigens expressed on target and killer cells was supported by the observations that allogeneic rat and xenogeneic hamster NK cells and hamster macrophages recognized the same cytolytic susceptibility in the 12S rat cell line and the same cytolytic resistant phenotype of HrA2 rat cells (Fig. 5*B*).

DISCUSSION

It has been proposed that NK cells and activated macrophages provide the host with a first line of defense against neoplasia, since these two classes of host mononuclear inflammatory cells exhibit spontaneous, rapidly inducible cytolytic activity toward neoplastic cells (2). Once a primary tumor nidus develops, however, a privileged tumor microenvironment may be created to which such potentially destructive host inflammatory cells may have restricted access (32–34). Thus, interactions between neoplastically transformed cells and host mononuclear inflammatory cells that occur at early stages of tumor development, and possibly before the establishment of such a protected tumor focus, may be critical in determining the outcome of an incipient malignancy in an immunologically competent host. Considering these concepts, it seems likely that progress in understanding reasons for early tumor progression or rejection will require definition of factors that regulate neoplastic cell susceptibility to the lytic mechanisms of NK cells and activated macrophages. The data presented in this report provide direct evidence that isolated expression of a single oncogene, *E1A*, can determine the cytolytic susceptible phenotype of a neoplastic cell. These data also provide an initial link between oncogene expression and the ability of NK cells and activated macrophages to selectively recognize and destroy neoplastic cells in preference to normal cells.

The reported activities of the *E1A* gene of nononcogenic Ad concern effects on viral and cellular transcription (reviewed in ref. 9), efficiency of transformation (35, 36), or viral replication (37). Whether the mechanisms by which these activities are expressed are also responsible for induction of the cytolytic susceptible transformed cell phenotype remain to be determined. However, certain conclusions can be

reached when comparing induction of cytolytic susceptibility to two previously reported activities associated with the Ad *E1A* gene—cell immortalization and induction of expression of other early Ad genes. (*i*) There is no clear association between the immortalized (partially transformed) or completely transformed cell phenotype, as determined by *in vitro* morphologies and growth properties of transformed cells, and the cytolytic susceptible or cytolytic resistant phenotype. For example, hamster cells transformed by Ad2, Ad12, or SV40, which share many of the commonly accepted properties of completely transformed cells, exhibit wide differences in cytolytic susceptibility in NK cell and macrophage assays (1). Furthermore, BRK cells immortalized by the first exon of *E1A* (HrA2) are highly resistant to NK cell- and macrophage-induced lysis, whereas immortalization of BRK cells by the 12S mRNA, 13S mRNA, or entire *E1A*-coding regions results in highly cytolytic susceptible transformed cells (Fig. 5). (*ii*) Susceptibility to lysis by NK cells and macrophages requires only *E1A* gene expression and does not require *E1A*-induced expression of virus-specific transplantation antigens or their association with class I major histocompatibility antigens on the cell surface, as has been suggested for cytotoxic T-lymphocyte killing of Ad-transformed rat cells (7, 38).

The observations that *E1A* gene products are found in the transformed cell nucleus and cytoplasm but may not be present on transformed cell-surface membranes suggest that *E1A* induction of cytolytic susceptibility during cell transformation may not be simply due to *E1A* products providing cell-surface target structures for NK cells and activated macrophages. There are at least two other types of mechanisms by which the *E1A* gene products of nononcogenic Ad might induce increased cytolytic susceptibility during transformation. There might be an indirect, virus-induced alteration in the target cell surface that qualitatively alters the recognition of cytolytic susceptible, compared to cytolytic resistant, cells by NK cells and macrophages, resulting in the delivery of a lethal injury to the target cell. There might also be physiological changes induced by *E1A* gene expression in transformed cells that could result in a reduced ability of the transformed cell to survive an effector cell-induced injury such as the defect in mitochondrial respiration observed in macrophage-injured cells (39). Whatever the mechanism by which *E1A* induces increased cytolytic susceptibility, the data obtained using the HrA2 cell line as a target cell (Fig. 5) suggest that the second exon of the *E1A* gene may encode a functional domain that is essential for expression of the cytolytic susceptible transformed cell phenotype. It seems likely that other viral oncogenes (e.g., Ad12 *E1* or SV40 in hamster cells) and certain nonviral oncogenes (e.g., those expressed in tumor cells such as A549 cells; Fig. 2*B*) functioning in cytolytic resistant transformed cells must lack this *E1A*-like activity or may actively express an activity associated with cytolytic resistance. Studies of mechanisms by which *E1A* induces increased cytolytic susceptibility during transformation and comparison studies between *E1A* and other viral and nonviral oncogenes regarding induction of the cytolytic susceptible and cytolytic resistant transformed cell phenotype may provide a better understanding of the genetic regulation of factors operative in transformed cells that determine the success or failure of the host in limiting the initial establishment of neoplasms.

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