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Recognition of Adenovirus E1A Gene Products on Immortalized Cell Surfaces by Cytotoxic T Lymphocytes

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The experiments described in this report were designed to examine whether target cells transfected with the adenovirus E1A gene and exhibiting increased susceptibility to lysis by natural killer cells and activated macrophages (J. L. Cook, T. A. Walker, A. M. Lewis, Jr., H. E. Ruley, F. L. Graham, and S. H. Pilder, Proc. Natl. Acad. Sci. USA 83:6965–6969, 1986) also express E1A proteins on their surfaces. MT1A, 12S, and 13S are strain Fischer baby rat kidney (BRK) cell lines immortalized by transfection with plasmids containing only the E1A gene of nononcogenic adenovirus. All of these cell lines were effective in stimulating the generation of cytotoxic T lymphocytes (CTL) in vitro, provided that the cultures were supplemented with an exogenous source of lymphokine and that the responding lymphocytes were from syngeneic Fischer rats previously immunized with a cell line containing the intact E1A gene. HrA2, a Fischer BRK cell line immortalized by transfection with a plasmid containing only exon 1 of the E1A gene, did not generate, nor was it lysed by, E1A-specific CTL. The cytolytic activity of E1A-specific CTL was blocked by antiserum from Fischer rats immunized with purified E1A proteins synthesized in *Escherichia coli*, supporting the conclusion that an epitope on E1A proteins encoded by the intact E1A gene constitutes part of the CTL target structure on adenovirus-transformed cells. These data suggest that in addition to their functions within host cells, E1A gene products are important immunogenic determinants on the surfaces of adenovirus-transformed cells.

Virally infected and transformed cells seldom escape recognition by the immune system. Such cells may be recognized by several different potentially cytolytic host cell populations including immunologically specific cytotoxic T lymphocytes (CTL) and immunologically nonspecific natural killer (NK) cells. The fact that susceptibility of target cells to lysis by CTL can be associated with the expression of early (encoded prior to viral DNA replication) viral gene products on cell membranes and does not require viral structural protein synthesis in target cells was first demonstrated with human adenovirus-infected and transformed cells (18, 22, 25). The viral protein components of CTL target structures in these studies appeared to map to adenovirus early transcriptional regions 1 (E1 [25]) and 3 (E3 [18]). The adenovirus E1 region comprises the E1A and E1B genes and encodes nonstructural viral proteins that are involved in viral transcription, replication, and transformation of mammalian cells (15, 20, 23). Although E1A proteins have not been reported to be associated with cell membranes, the E1B 15-kilodalton protein and a 19-kilodalton protein encoded by the E3 gene have been associated with membranes in adenovirus-infected and transformed cells, and the E3 19-kilodalton protein has been shown to be associated with the cellular class I major histocompatibility antigen (18, 22). The results of these studies have suggested that these two viral proteins are candidates for the primary antigenic determinants that are recognized by CTL on adenovirus-transformed cells. However, final conclusions about the importance of the adenovirus E1B and E3 proteins in CTL recognition of virus-infected and transformed cells have remained tentative, since none of the reported studies has involved the use of CTL sensitized only to the products of a single adenovirus gene.

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Recent experiments provide evidence that adenovirus E1A gene product expression is sufficient for induction of susceptibility of transformed cells to lysis by NK cells and activated macrophages (cytolytic susceptibility) and that E1B and E3 proteins are not required for this cytolytic phenotype (6). Expression of the E1A gene in transformed cells has also been shown to be important in determining the viral specificity of tumor-specific transplantation antigens on virus-transformed cells (26). Although cytolytic susceptibility and tumor-specific transplantation antigen expression are associated with adenovirus E1A gene expression and presumably involve cell surface membrane changes on transformed cells, there is no reported evidence that E1A gene products are associated with cell membranes. Because CTL function requires binding of the T-cell receptor to cell surface antigen, one would predict that CTL could not be activated to cells immortalized by the E1A gene alone if, in fact, E1A proteins are not expressed on the cell surface. To test this hypothesis, we studied the capacity of cell lines immortalized by transfection with plasmids containing the E1A gene to induce CTL. We found that E1A-immortalized cells could induce CTL and that this cytolytic activity could be blocked by antiserum raised against purified E1A proteins. These results suggest that epitopes of E1A gene products that are localized primarily to the host cell nucleus are also expressed on the cell surface.

MATERIALS AND METHODS

Animals. Inbred specific-pathogen-free rats were obtained from the following sources. Strain Fischer rats were purchased from Taconic Laboratory Animals and Services, Germantown, N.Y., and strain DA rats were purchased from Trudeau Institute, Saranac Lake, N.Y. Fischer and DA rats are major histocompatibility complex (MHC) incompatible. Fischer types as $RT1^{lvl}$, whereas DA is $RT1^{a}$.

Target cell lines. MT1A is a cell line immortalized by transfection of DNA from the recombinant adenovirus type 2-adenovirus type 5 (Ad2-Ad5) E1A-containing plasmid pMTE1A (31) into baby rat kidney (BRK) cells from Fischer inbred rats. 12S is a Fischer BRK cell line immortalized by transfection with DNA from the recombinant Ad2-Ad5 plasmid pMT12S containing cloned cDNA derived from Ad2 E1A 12S mRNA (31). 13S is a Fischer BRK cell line immortalized by transfection with DNA from the recombinant Ad2-Ad5 plasmid pMT13S containing cloned cDNA derived from Ad2 E1A 13S mRNA (31). HrA2 is a Fischer BRK cell line immortalized by DNA from the pHrA plasmid containing the E1A region from the Ad5 host range mutant virus, *hr*440 (28, 31). The *hr*440 mutation results in synthesis of a truncated polypeptide containing 140 amino acids derived from the amino-terminal (left-hand, exon 1) sequences common to both E1A 12S and 13S mRNA-encoded proteins. MT1A, 12S, 13S, and HrA2 were provided by H. E. Ruley. These four cell lines were derived by transfection as stated above of BRK cells from Fischer inbred rats (Taconic) (H. E. Ruley, personal communication). A2T2C4 is an Ad2 virus-transformed Hooded Lister strain rat embryo fibroblast cell line (8). F-4 is an Ad2 virus-transformed AS strain rat embryo fibroblast cell line (12). A2T2C4 and F-4 were provided by P. Gallimore. All of these cell lines were screened for mycoplasma by the Mycotect assay (Bethesda Research Laboratories, Inc., Bethesda, Md.) and were negative.

Generation of CTL. Thoracic duct lymphocytes (TDL) from DA or Fischer donors were collected from surgically implanted thoracic duct cannulas as described previously (2). Fischer rats were immunized intraperitoneally with 5×10^6 transformed cells at least 3 weeks prior to cannulation. A total of 10^7 responder TDL were cultured with either 25×10^6 lymph node cells or 10^6 transformed cells in a final volume of 25 ml. The lymph node cells received 2,000 rads and the transformed cells received 5,000 rads of gamma irradiation prior to culture. The culture medium was Iscove modified Dulbecco medium supplemented with 10 mM glutamine, 5×10^{-5} M 2-mercaptoethanol, penicillin, streptomycin, and 10% fetal bovine serum. The medium was further supplemented with an enriched source of interleukin-2 produced by passage of supernatants from concanavalin A-activated lymph node cells through a Sephadex G-100 column as described by Grönvik and Andersson (9). The interleukin-2 was added to the medium at a final concentration of 10% by volume. Cells and medium were cultured in upright 25-cm^2 tissue culture flasks (no. 25100; Corning Glass Works, Corning, N.Y.) for 6 days at 37°C in a humidified atmosphere containing 5% CO_2.

CTL assay. TDL cultured for 6 days as described above were washed and suspended in RPMI 1640 medium containing 20% fetal bovine serum. Targets, either transformed cells or mitogen-activated lymph node cells, were prepared and labeled with ^{51}Cr as described previously (2). CTL (effector cells) were added to target cells at the indicated effector-to-target-cell ratios in V-bottom microdilution plates. The final volume did not exceed 0.125 ml. After 4 h of incubation, the plates were centrifuged at $500 \times g$ for 3 min, after which time half the supernatant from each well was harvested and counted in a gamma counter. The percent specific (CTL-induced) release of radiolabel from target cells was calculated as described previously (2).

Generation of antiserum. E1A protein was expressed in *Escherichia coli* containing the expression vector pAS1-E1A410, provided by M. Rosenberg, Smith Kline & French Laboratories, Swedeland, Pa. This expression vector permits high-level expression in *E. coli* of the product of Ad2-Ad5 E1A 13S mRNA. E1A proteins were purified from *E. coli* as described by Krippl et al. (17). Control protein preparations were made by using the same purification scheme starting with *E. coli* containing the pAS1 plasmid lacking E1A sequences, also provided by M. Rosenberg. Purified protein (200 μg) was emulsified in 0.5 ml of incomplete Freund adjuvant and injected subcutaneously into adult Fischer rats. The animals were immunized three times at monthly intervals. E1A antiserum was collected via cardiac puncture 7 days after the last immunization.

RESULTS

Generation of E1A-specific cytotoxicity. MT1A, 12S, 13S, and HrA2 are cell lines produced by transfection of Fischer BRK cells with plasmids containing various components of the human adenovirus E1A gene. The E1A gene is the only adenovirus gene that is essential for cell immortalization (14). This gene encodes two mRNA species that can be translated into at least two major polypeptide products. Because the two mRNAs are translated in the same reading frames and differ only by the sizes of the introns that are deleted by mRNA processing, the larger RNA species (13S) theoretically encodes a product of 289 amino acids, whereas the smaller species (12S) theoretically encodes a 243-amino-acid protein (1, 21). These two major polypeptides have identical amino and carboxyl termini and differ only in the internal 46 amino acids present in the 13S mRNA product. MT1A was immortalized by the whole E1A gene, whereas 12S and 13S were immortalized by cDNA copies of the E1A 12S or 13S mRNA, respectively (30). Therefore, the MT1A cell line contains proteins encoded by both mRNAs, whereas the 12S and 13S cell lines contain proteins encoded by their respective mRNAs.

Of interest to this study was the observation that MT1A, 12S, and 13S are susceptible to lysis by NK cells and activated macrophages, whereas HrA2 is not (6). One interpretation of the differences in lytic susceptibility of these cell lines is that the intact E1A gene in target cells (i.e., MT1A, 12S, and 13S) is required for the cytolytic-susceptible transformed cell phenotype, whereas expression of E1A exon 1 alone in target cells (i.e., HrA2) is insufficient for induction of this phenotype. Among the questions that arise from the hypothesis that E1A gene products induce this cytolytic-susceptible transformed cell phenotype are those of whether the mechanism of action of E1A proteins involves their expression on the cell surface and whether E1A proteins mediate induction of the cytolytic-susceptible phenotype indirectly by altering the expression of certain cellular genes. To begin to examine these possibilities, it is important to determine whether E1A proteins can be detected on the surfaces of immortalized cells. If these proteins are present on the cell surface, it should theoretically be possible to generate CTL to these cell surface antigens. To test this possibility, CTL were generated by immunizing Fischer rats intraperitoneally with 5×10^6 gamma-irradiated MT1A, 12S, 13S, or HrA2 cells. Fischer is the strain of origin of all four of these cell lines. At 4 to 6 weeks after immunizations, TDL were collected from animals and cultured for 6 days with the same cell line with which the donors had been primed (see Materials and Methods). MT1A, 12S, and 13S all generated lymphocytes that lysed MT1A, 12S, and 13S (Fig. 1). In addition, all three cytotoxic populations lysed F-4, an adenovirus-transformed rat embryo fibroblast cell line from

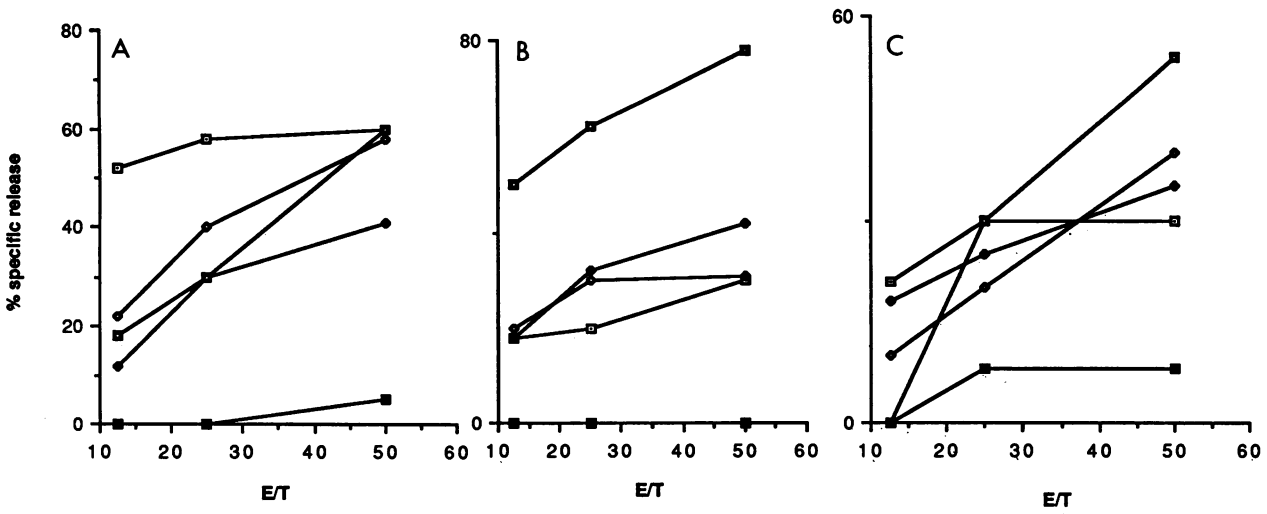

FIG. 1. E1A-specific cytotoxic lymphocyte induction by and lysis of cell lines immortalized by an intact E1A gene. TDL from Fischer rats immunized with MT1A (A), 13S (B), or 12S (C) cells and cultured in vitro with the immunizing cell line for 6 days were tested for specific cytotoxicity in a 4-h chromium release assay against F-4, MT1A, 12S, 13S, and HrA2 as described in Materials and Methods. On the abscissa, E/T refers to the effector-to-target-cell ratio. The ordinate is the percent specific release of chromium, calculated as described previously (2). Symbols: ⊡, F-4; ◆, MT1A; ◪, 12S; ◆, 13S; ■, HrA2.

strain AS. Strain AS is compatible with Fischer in the class I and II regions of the MHC. None of these cytotoxic lymphocyte populations lysed HrA2 target cells. In addition, HrA2 failed to generate E1A-specific cytolytic activity (data not shown).

Evidence that cytotoxic lymphocyte activity is T cell mediated. The profile of lysis in Fig. 1 matches that observed for NK cells (6). MT1A, 12S, and 13S are all NK susceptible, whereas HrA2 is not. F-4 is also NK susceptible (27). Therefore, it was conceivable that the cytotoxic activity observed in these assays was mediated by NK cells. If the lysis was mediated by NK cells, this cytolytic population should also lyse other NK-susceptible targets and should not be restricted to lysis of MHC-identical target cells, since MHC restriction is a property of CTL and not of NK cells (10). We tested such populations for lysis of A2T2C4, which is an NK-susceptible adenovirus-transformed rat embryo fibroblast line derived from strain HL (6). Strain HL is MHC incompatible with strain Fischer. Cytolytic populations from Fischer rats primed in vivo and restimulated in vitro with MT1A lysed MT1A but not A2T2C4 (Fig. 2). The fact that A2T2C4 is an NK-susceptible target cell line that is not lysed by these MT1A-induced cytotoxic lymphocytes indicates that the effector cells are not NK cells. Because A2T2C4 and F-4 are both Ad2- transformed rat embryo fibroblast lines, the observation that Fischer cytotoxic lymphocytes primed to MT1A lyse only F-4 and not A2T2C4 also suggests that the effector cells are MHC restricted, since AS, the strain of origin of F-4, is compatible in the class I and II loci of the MHC, whereas HL, the strain from which A2T2C4 was derived, is not. These data support the conclusion that the effector cells in these assays are CTL.

CTL responses to alloantigens. We explored two possible explanations for the failure of HrA2 to either induce or serve as targets for CTL. The first is based on the plasmid construct used to transform the BRK cells from which the HrA2 cell line was derived. The HrA2 plasmid codes for a truncated E1A protein lacking the amino acids encoded by E1A exon 2. This modification in the E1A protein could result in the absence of an essential functional E1A domain or in a conformational change in the E1A protein required for expression of CTL target structures. Alternatively, the HrA2 cell line might simply be resistant to effector cell-induced lysis in general. If the latter possibility were correct, CTL specific for Fischer strain alloantigens, the strain from which the HrA2 cell line was derived, should not lyse HrA2. The second possibility was shown to be incorrect (Fig. 3). DA CTL specific for Fischer alloantigens lysed HrA2 cells. HrA2 cells were also effective in inducing DA-anti-Fischer CTL. The effector cells generated had exactly the same specificity as DA TDL stimulated with MT1A cells. Lysis

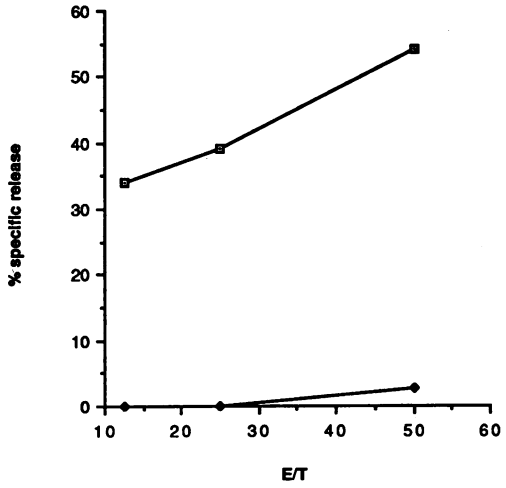

FIG. 2. Evidence that the cytotoxicity is mediated by T lymphocytes. TDL from MT1A-immune Fischer rats were stimulated in vitro with MT1A cells and tested for specific cytotoxicity on MT1A and A2T2C4 target cells. On the abscissa, E/T refers to the effector-to-target-cell ratio. The ordinate is the percent specific release of chromium, calculated as described previously (2). Symbols: ⊡, MT1A; ◆, A2T2C4.

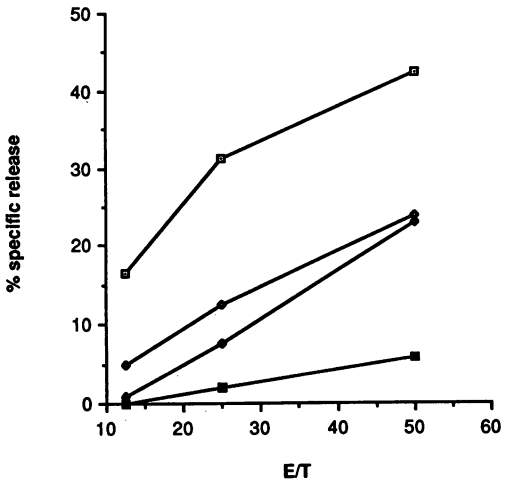

FIG. 3. Expression of rat strain-specific MHC antigens by HrA2 and MT1A. TDL from strain DA rats ($RT1^a$) were cultured with lymphoid cells from strain Fischer ($RT1^{lvl}$). These DA-anti-Fischer cytotoxic T cells were tested in a 4-h chromium release assay for their capacity to lyse MT1A, HrA2, A2T2C4, and normal Fischer T cells stimulated to blastogenesis with the T-cell mitogen concanavalin A. Symbols: ⊡, MT1A; ◆, HrA2; ■, A2T2C4; ◆, Fischer T cells stimulated with concanavalin A.

was observed with MT1A, 12S, 13S, and HrA2 target cells (Fig. 4). These experiments demonstrate that the inability of HrA2 target cells to stimulate and to be lysed by E1A-specific CTL is not due to an innate resistance to lysis or to a failure to express Fischer MHC antigens. These data are compatible with the conclusion that HrA2 cells do not express the E1A epitope(s) recognized by E1A-specific CTL.

Blocking studies with antibody to E1A proteins. The nature of the antigens recognized by CTL in these assays was investigated. The two most likely candidates include (i) proteins encoded by the E1A gene and expressed on the cell surface and (ii) cellular antigens whose expression is induced during transformation by E1A-mediated transcriptional activation of cellular genes. To study the possibility that E1A antigens form part of the CTL target structure on E1A-immortalized cells, we performed blocking antibody studies by using E1A-specific antibodies. To generate these antibodies, we immunized Fischer rats with purified E1A proteins in incomplete Freund adjuvant (see Materials and Methods). Control animals were immunized with control protein preparations lacking E1A in incomplete Freund adjuvant. Serum samples from these immunized animals were adsorbed with lymphoid cells from normal (nonimmunized) Fischer rats. These serum samples were then tested for their capacities to block CTL specific for MT1A target cells or Fischer lymphoblasts. E1A antiserum blocked lysis of MT1A target cells by Fischer CTL specific for MT1A (Fig. 5A). Control serum did not block. CTL from strain DA rats generated against Fischer alloantigens had a marginally reduced capacity to lyse MT1A target cells in the presence of E1A antiserum (Fig. 5B), whereas the same antiserum had no effect on DA anti-Fischer CTL when the targets consisted of normal Fischer lymphoblasts (Fig. 5C). These results show that antibodies to E1A proteins can effectively block lysis of target cells immortalized by the E1A gene only if the CTL are E1A antigen specific.

DISCUSSION

The E1A gene of adenoviruses is located in the extreme left-hand end of the viral genome (reviewed in reference 23). In transformed cells and at early times after infection, two families of E1A products are detected that are translated from two overlapping E1A mRNAs (13S mRNA and 12S mRNA) that differ only by the sizes of the introns removed during posttranscriptional processing. Expression of these E1A proteins is associated with several functions including either activation or repression of viral and cellular gene transcription (reviewed in reference 3), stimulation of viral and cellular DNA replication (24, 29), establishment of transformation (15, 20), viral specificity of transformed cell tumor-specific transplantation antigen (26), and induction of susceptibility of virus-infected (5) and virus-transformed (6) cells to the cytolytic effects of NK cells and activated macrophages. Studies of the intracellular localization of E1A proteins indicate that proteins that contain the carboxy-terminal 5 amino acids encoded by the E1A gene localize to the nucleus (19). E1A proteins have also been detected in the cytoplasm of infected and transformed cells, both in the cytosol and associated with cytoskeletal elements (4). There have been no previous reports of association of E1A proteins

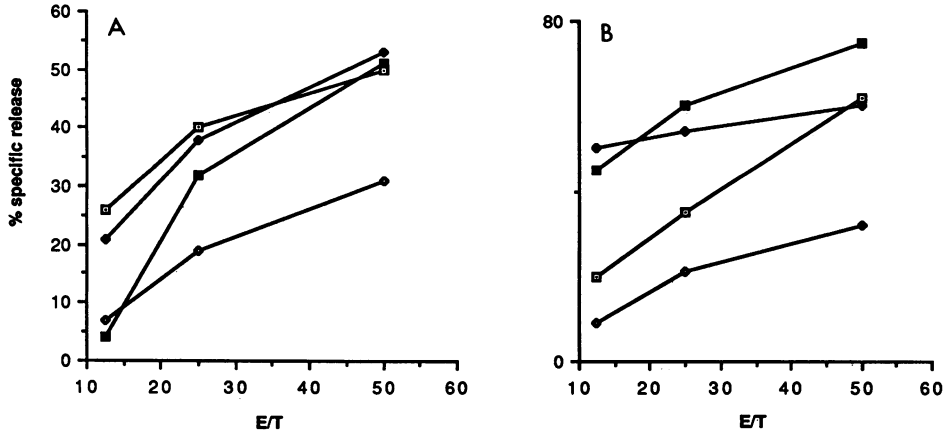

FIG. 4. HrA2 induction of and sensitivity to lysis by alloantigen-specific CTL. DA lymphocytes were cultured with HrA2 (A) or MT1A (B) as described in Materials and Methods. Both populations of effector cells were tested for specific lysis of MT1A, 12S, 13S, and HrA2 target cells. Symbols: ⊡, MT1A; ◆, 12S; ■, 13S; ◆, HrA2.

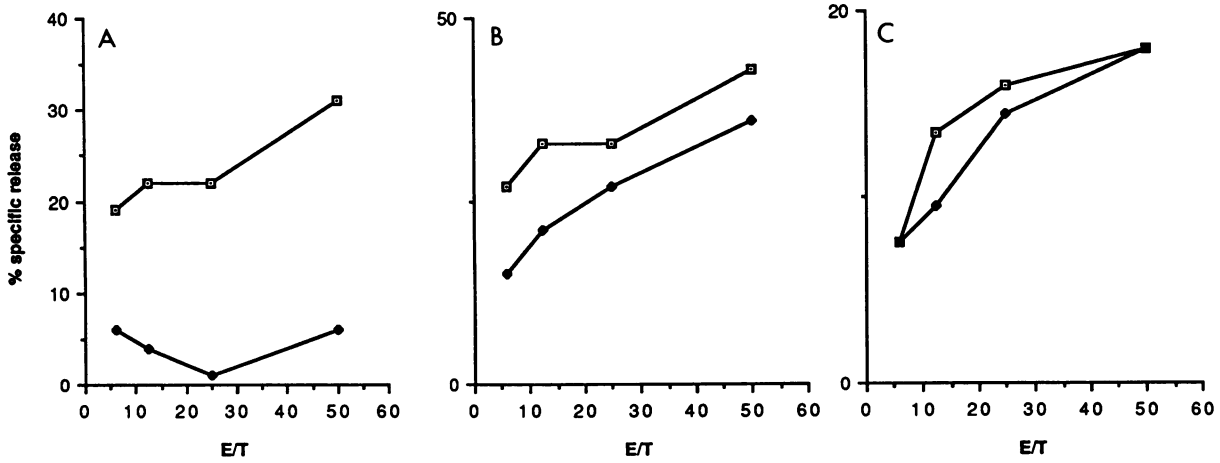

FIG. 5. Specific blocking of tumor-specific CTL with antiserum to E1A proteins. CTL populations specific for MT1A tumor antigens (panel A) and Fischer alloantigens (panels B and C) were tested for specific lysis of chromium-labeled MT1A (panels A and B) or Fischer concanavalin A lymphoblast targets (panel C). The CTL assay was performed with the addition of antiserum raised in Fischer rats against E1A proteins. Included as a control was antiserum from rats immunized with preparations lacking the E1A protein. Serum was added at a final dilution of 1:8. Symbols: ⊡, control; ◆, anti-E1A antiserum.

with cellular membranes or of E1A expression on the cell surface.

Two of the activities reported for adenovirus E1A proteins suggest that these proteins may be associated with or expressed on the surfaces of transformed cells or that E1A proteins may indirectly alter transformed cell surfaces in a way that is detectable by cells of the host cellular immune response. Sawada et al. (26) observed that E1A governs the viral specificity of tumor-specific transplantation antigen expressed on transformed cells as detected in transplantation rejection bioassays. A possible explanation for this observation is that E1A epitopes that are unique to Ad5 and Ad12 are recognized in association with host cell class I MHC antigen by viral antigen-specific CTL that are generated in the immunized hosts. In a different experimental system, we reported that expression of the E1A gene in immortalized rodent and human cells is sufficient to induce increased susceptibility of such target cells to lysis by host NK cells and activated macrophages (6). Target cell recognition and initiation of the cytolytic event in NK cell and macrophage cytolysis assays is likely to be determined by structures present on the target cell surface. It is conceivable that such postulated NK cell and macrophage target structures contain or are regulated by the cell surface expression of E1A proteins on adenovirus-transformed cells.

E1A proteins appear to be very heterogeneous owing to a variety of possible transcriptional start sites and to post-translational processing of the mRNA products (11). This observation, along with the likelihood that immunofluorescence assays detect only protein species present in relatively large quantities in a given cell compartment, may provide an explanation for the previous failure to detect E1A proteins associated with infected or transformed cell surfaces. In contrast, immunologically specific CTL are highly sensitive indicators of the presence of foreign proteins or peptides on the surfaces of histocompatible target cells. In the experiments described in this report, E1A-specific CTL were derived from syngeneic rats immunized with BRK cell lines immortalized by transfection with the E1A gene of nononcogenic adenovirus. The recognition and lysis of E1A-immortalized syngeneic target cells by sensitized CTL in an antigen-specific and genetically (MHC) restricted manner suggest that E1A proteins form part of the CTL target structures on these cells. The ability of E1A-specific antibody to block this cytolytic CTL-target cell interaction supports this concept.

In contrast to the results presented here, Föhring et al. (7) were unable to induce rat CTL activity against syngeneic cells immortalized by transfection with the E1A fragment (the *Acc*I E restriction fragment) of Ad12. It is possible that technical differences such as different levels of E1A expression in the target cells, the use of TDL instead of spleen cells as the source of CTL, or the addition of interleukin-2 during in vitro CTL amplification could account for the ability to detect E1A-specific CTL activity in the present study. It is also conceivable that there are differences in the expression of E1A proteins on the surfaces of cells immortalized by the E1A gene of nononcogenic adenovirus serotypes (used here) compared with highly oncogenic Ad12 (used by Föhring et al. [7]). However, the transplantation immunity data of Sawada et al. (26) do not favor this explanation. In those studies, it was observed that the E1A gene of nononcogenic Ad5 and of Ad12 regulates the viral specificity of immunity to virus-transformed cell challenge of previously sensitized, syngeneic animals. The results of those bioassays are compatible with the conclusion that E1A proteins are expressed on the surfaces of both Ad5- and Ad12-transformed cells and that the recognition and destruction of the virus-transformed cells by immune animals is mediated by Ad5 or Ad12 E1A-specific CTL.

The observation that the HrA2 cell line could not induce or serve as targets for E1A-specific CTL in these studies but that this cell line could induce alloantigen-specific CTL suggests several conclusions. The E1A epitope that is recognized by the E1A-specific CTL populations tested in these studies may be present in the second E1A exon that is missing from the pHrA plasmid used to immortalize the HrA2 cell line. An alternative explanation for the inability of HrA2 cells to serve as targets for E1A-specific CTL is that the absence of the second E1A exon renders the truncated E1A protein encoded by the pHrA sequences integrated in HrA2 cells unstable or alters the intracellular trafficking of this polypeptide such that it is not properly exhibited on the cell surface in association with class I MHC antigens.

These data, which suggest that the cell surface expression of adenovirus E1A proteins is a major factor in recognition of virus-transformed cells by specifically sensitized CTL, are compatible with the results obtained with other virus-specific CTL models. CTL specific for cells infected with influenza virus (30) or cytomegalovirus (16) have been shown to recognize viral proteins or peptides derived from these proteins, which are not glycosylated transmembrane proteins and which are expressed primarily in the cell nucleus. The cellular pathways through which these nuclear proteins travel to the target cell surface to become associated with cellular class I MHC antigens are not clearly defined. It is possible that the expression of these viral nuclear proteins such as E1A on the cell surface simply represents the end result of a metabolic pathway through which foreign proteins are processed. The key factors in determining the ability of such proteins to serve as components of CTL target structures is the foreign nature of these viral proteins and their association with cell surface class I MHC antigens.

Whether E1A proteins have other functions on the surfaces of infected or transformed cells remains to be determined. As mentioned, we have observed that E1A expression in immortalized cells is sufficient to induce increased susceptibility to lysis by NK cells and activated macrophages (6). Neither of these killer cell populations requires expression of either viral proteins or histocompatible class I MHC antigens on the surfaces of their target cells (10, 13). The results of our preliminary studies indicate that the same E1A-induced antibodies that block E1A-specific CTL killing of E1A-immortalized target cells have little or no effect on NK cell-mediated killing of the same target cells (data not shown). These results suggest that recognition of these E1A-immortalized cells by CTL and by NK cells or macrophages is mediated through different cell surface target structures and that the E1A expression on the cell surface may be unrelated to the mechanism by which E1A induces NK cell and macrophage cytolytic susceptibility during cell transformation.

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