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The Level of Expression of Adenovirus Type 2 Transforming Genes Governs Sensitivity to Nonspecific Immune Cytolysis and Other Phenotypic Properties of Adenovirus 2-Simian Virus 40-Transformed Cell Hybrids

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Syrian hamster embryo cells transformed by adenovirus type 2 (Ad2) or simian virus 40 (SV40) differ markedly in morphology, tumorigenicity, and susceptibility to in vitro lysis by nonspecific cytotoxic cells. Hybrid cells formed by fusing Ad2- and SV40-transformed Syrian hamster embryo cells may express only SV40 T antigens or both SV40 and Ad2 T antigens. Hybrids that express only SV40 T antigens are indistinguishable from the nonhybrid SV40-transformed phenotype, wheras hybrid cells that express T antigens from both viruses closely resemble the nonhybrid parental Ad2-transformed phenotype. Because these hybrid cells have been useful in the study of neoplastic transformation, we determined the amount of viral antigens that they accumulate in an attempt to correlate the level of expression of the transforming viral genes with some of their phenotypic properties. Hybrid cells that expressed proteins from both viruses showed reduced levels of SV40 T antigens compared with those of hybrid cells that did not express Ad2 T antigens. We also found that the production of several cellular proteins that influence cytomorphology was inhibited in hybrid and nonhybrid cells that expressed Ad2 T antigens, and the repression of these cellular proteins correlated with a change in cytomorphology from fibroblastic to spherical. Finally, we showed that the susceptibility of our hybrid cells to in vitro lysis by natural killer cells and activated macrophages, two putative host-effector cells involved in defense against neoplasia, correlated closely with the level of expression of a 58,000-dalton Ad2 protein. The results reported here, together with the results of previous studies, indicate that the oncogenic potential of hybrid cells that express both Ad2 and SV40 T antigens is extremely sensitive to Ad2 expression, whereas other phenotypic properties depend on Ad2 expression in a dose-dependent manner.

Syrian hamster embryo (SHE) cells transformed by adenovirus type 2 (Ad2) or simian virus 40 (SV40) differ markedly in morphology and in sensitivity to nonspecific immune effector cells (8, 21). SHE cells transformed by Ad2 are usually small and round and very sensitive to in vitro lysis by natural killer (NK) cells and activated macrophages (MP), two putative nonspecific host-effector cells involved in defense against neoplasia. In contrast, SV40-transformed cells, which resemble untransformed SHE cells, are more fibroblastic and are relatively insensitive to in vitro lysis by NK cells and MP (7, 8, 22). Thus, by these criteria, transformation of SHE cells by SV40 induces fewer phenotypic changes than does transformation by Ad2. Although SV40-transformed cells appear to be less modified by transformation than Ad2-transformed cells, they are more oncogenic (9, 20). Ad2-transformed SHE cells readily induce tumors in immunoincompetent newborn hamsters but rarely in immunocompetent adult syngeneic (LSH strain) and never in allogeneic (CB strain) hamsters. SV40-transformed SHE cells are highly oncogenic in adult syngeneic and allogeneic hamsters as well as in newborn hamsters. Thus, although both Ad2- and SV40-transformed cells have been immortalized and are capable of in vivo propagation in immunoincompetent hamsters, the latter are either not recognized as transformed cells by immune surveillance cells in immunocompetent hamsters or they are able to resist the cytotoxic action of these cells. Moreover, because SV40-transformed cells are oncogenic in allogeneic hamsters, it is possible that expression of histocompatibility antigens might be impaired in these cells (2, 20, 24, 31).

Hybrid cells formed by fusing Ad2- and SV40-transformed SHE cells were of two types when characterized for expression of viral antigens by immunofluorescent staining (27). Some hybrids (S^+A^-) expressed only SV40 T antigens; these cells were indistinguishable from nonhybrid SV40-transformed cells (S^+) morphologically, in resistance to NK cells and MP (7), and in their capacity to induce tumors in syngeneic and adult allogeneic hamsters. Other hybrids (S^+A^+) expressed Ad2 T antigens and SV40 T antigens; these cells closely resembled the nonhybrid parental Ad2-transformed cells (A^+) in morphology, sensitivity to nonspecific cytotoxic cells, and tumorigenicity. Only one S^+A^+ hybrid, $S1^+A1^+2.4$, induced tumors in immunocompetent syngeneic hamsters; none induced tumors in allogeneic hamsters (27). These results imply that Ad2 T antigens function to govern the tumorigenic phenotypes of these hybrid cells.

Although cells of the Ad2-transformed phenotype were always more sensitive to cytolysis than were cells of the SV40-transformed phenotype, they were not all lysed to the same extent. Because recent evidence indicates that the phenotypic properties of transformed cells may depend on the quantitative level of expression of oncogenes within those cells (10, 17), it seemed reasonable to determine whether the cytolytic susceptibilities and morphological

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characteristics of our hybrid cells are related to differences in the level of expression of viral antigens. Accordingly, we made a quantitative analysis of the Ad2 and SV40 T antigens expressed in these cells and of the amounts of fibronectin and actin that they accumulate. Our results show a direct correlation between the level of the Ad2 58,000-dalton (58K) T antigen, used as an indicator of Ad2 expression, and the cytolytic susceptibility of transformed hybrid cells.

MATERIALS AND METHODS

Cell lines. Untransformed LSH SHE cells were prepared by mincing and trypsinizing whole 14-day-old embryos. Transformed cells were established by infecting SHE cells with UV-inactivated SV40 (strain 777) or Ad2 (prototype Ad2); each line was established from a single focus of transformed cells grown on plastic dishes under agar (9). Cell lines were carried in Dulbecco modified Eagle medium containing 10% fetal bovine serum. Mutant cell lines were established which lacked either thymidine kinase (TK^-) or hypoxanthine-guanine phosphoribosyltransferase ($HPRT^-$) by selecting resistant cells in 5-bromodeoxyuridine or 6-thioguanine (100 μg/ml). Hybrid cell lines were established by fusing SV40HE1(TK^-) (S1) cells with Ad2HE1($HPRT^-$) (A1), Ad2HE3($HPRT^-$) (A3), or Ad2HE6($HPRT^-$) (A6) cells by using 50% polyethylene glycol and selecting wild-type hybrids in hypoxanthine-aminopterin-thymidine medium (27, 29). The hybrids were characterized for viral antigens by immunofluorescent staining. Hybrid designations indicate the parental transformed cells and whether viral antigens were detected (+) or not (−); the numbers designate clonal lines.

Preparation of radioactive cell proteins. Cells were radiolabeled by culturing them with [^{35}S]methionine (70 to 100 μCi/ml) for 4 h in methionine-free modified Eagle medium containing 1% fetal bovine serum. Cells were harvested by scraping, washed in cold phosphate-buffered saline, suspended in 1 ml of lysis buffer (20 mM Tris [pH 9], 0.7 M NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10% glycerol, 1% Nonidet P-40, 0.2 U of aprotinin [Sigma Chemical Co., St. Louis, Mo.] per ml), and sonicated in a Branson Sonifier (Branson Sonic Power Co., Danbury, Conn.). The lysate was clarified by centrifugation at 25,000 × *g* for 10 min at 4°C and stored at −20°C. The protein concentration of each lysate was measured with Coomassie brilliant blue (4; Bio-Rad Laboratories, Richmond, Calif.).

Immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Virus-specific proteins were immunoprecipitated with sera from hamsters bearing tumors induced by Ad2-transformed cells (23) or by ascites fluid obtained from hamsters immunized by intraperitoneal injection of a 10% extract (suspended 1:1 in Freund adjuvant) of SV40-induced hamster tumors. Fibronectin and actin were immunoprecipitated by antibody preparations obtained from Bethesda Research Laboratories, Inc. (BRL; Gaithersburg, Md.) or Miles Laboratories, Inc. (Elkhart, Ind.), respectively. The immunoprecipitates were adsorbed to inactivated *Staphylococcus aureus* (BRL), washed, and eluted by heating for 5 min in boiling water with extraction buffer (75 mM Tris [pH 6.8], 2% sodium dodecyl sulfate, 0.7 M 2-mercaptoethanol, 0.01% bromphenol blue, 50% glycerol, 0.2 U of aprotinin per ml) (30). Electrophoresis was carried out on polyacrylamide gels (7.5 to 11%) as previously described (19). Gels were fixed, saturated with Autoradiography Enhancer (New England Nuclear Corp., Boston, Mass.), dried, and exposed to X-ray film (XRP-5; Eastman Kodak Co., Rochester, N.Y.) at −76°C. The amounts of immunoprecipitated antigens were determined by densitometric analysis or by excising the bands and counting in a scintillation counter; both methods gave good agreement. The molecular weights of proteins were estimated by comparing their mobilities with marker proteins obtained from BRL. The anti-SV40 ascites fluid precipitated both 94K and 18K SV40 T antigens from all hybrid (S^+A^+ and S^+A^-) and nonhybrid (S^+) cells. Detection of Ad2 antigens varied with different sera; however, all sera tested precipitated substantial amounts of the Ad2 E1B 58K protein from all S^+A^+ and A^+ cells. None of our Ad2 antisera precipitated any proteins from S^+A^- or S^+ cells. Accordingly, we used the amount of immunoprecipitated Ad2 58K protein as an indicator of Ad2 gene expression in our transformed hybrid and nonhybrid cells.

Cytolysis assays. Monolayers of MP were prepared from peritoneal exudates from *Mycobacterium bovis* BCG-infected golden Syrian hamsters, and NK cell assays were performed with suspensions of spleen cells from normal hamsters under optimal conditions as described previously (7, 8). Target cells were radiolabeled with [^{3}H]thymidine or [^{3}H]proline (TK^- cells only), and cytolysis was measured by release of radiolabel during a 48-h incubation period at 37°C. Data are presented as the percentage of radiolabel released from target cells due to effector cell lysis, calculated as described previously (8).

RESULTS

In vitro lysis by NK cells and activated-MP. Cook et al. (8) demonstrated that in an in vitro assay, Ad2-transformed SHE cells are much more sensitive to cytolysis by nonspecific cytotoxic cells than are untransformed SHE cells or SHE cells transformed by SV40 (Table 1). Similarly, hybrid cells formed from SV40HE1 and Ad2HE1 which expressed Ad2 T antigens and exhibited an Ad2 phenotype were readily lysed by NK cells and MP, whereas hybrids which had not retained the Ad2 genome and expressed only SV40 T antigens were relatively resistant (7; Table 1). We subsequently showed that hybrid cells formed from SV40HE1 and two other lines of Ad2-transformed SHE cells, Ad2HE3, and Ad2HE6, were also relatively more sensitive to in vitro lysis if they expressed Ad2 T antigens. However, the $S1^+A3^+$ and $S1^+A6^+$ hybrids were more resistant than $S1^+A1^+$ hybrids to in vitro lysis (Table 1).

Immunoprecipitation of viral antigens. We also noted that $S1^+A3^+$ and $S1^+A6^+$ hybrid cells, although very similar to each other, were both morphologically different from $S1^+A1^+$ hybrids (Fig. 1). Thus, these two properties, cytomorphology and sensitivity to nonspecific immune effector cells, appeared to be correlated; moreover, it seemed possible that both properties might be related to the relative amounts of Ad2 and SV40 genetic expression in our hybrid cells. To examine this possibility, we carried out a quantitative analysis of the viral antigens expressed in these cells.

Ad2 and SV40 tumor antigens were immunoprecipitated from whole-cell lysates and analyzed by electrophoresis on polyacrylamide gels as described in Materials and Methods. Typical electropherograms of radiolabeled viral antigens and fibronectin immunoprecipitated from hybrid cell lysates are shown in Fig. 2. It should be noted that normal hamster serum precipitates little protein from either lysate (Fig. 2A and B, lane a). Figures 2C and D show electropherograms comparing Ad2 and SV40 T antigens immunoprecipitated from equal amounts of protein extracted from $S1^+A1^-$ and $S1^+A1^+$ hybrids and the nonhybrid parental cells from which

they were derived. There was considerable variability in the amount of Ad2 and SV40 T antigens expressed in the different cell lines. The relative amounts of the Ad2 58K protein and the SV40 94K and 18K proteins immunoprecipitated from 14 hybrid cell lines and 4 nonhybrid parental cell lines are shown in Table 2. Hybrid cells derived from Ad2HE1 generally accumulated more Ad2 58K protein than did hybrids derived from Ad2HE3 or Ad2HE6, presumably reflecting the larger amount of viral expression in the parental cells (Table 2). Johansson et al. (18) also showed that Ad2HE1 accumulated substantially more Ad2 58K protein than did Ad2HE3, although both cell lines contained about equal amounts of the B fragment (*Bam*HI) of Ad2 DNA which encodes the Ad2 E1 region. All hybrid cells accumulated reduced amounts of viral antigens when compared with their parental nonhybrid cells. This may have been due to a dilution effect because our data were all normalized to the amount of cellular protein in each lysate; the hybrid cells have more chromosomes and may have more protein for equivalent numbers of viral genomes compared with nonhybrid cells (27).

In general, the cells which expressed the highest level of Ad2 58K protein accumulated the smallest amounts of SV40 T antigens. Thus, SV40 expression in the $S1^+A1^+$ hybrids, which accumulated high levels of Ad2 58K protein, is highly reduced, whereas SV40 expression in $S1^+A3^+$ hybrids, which accumulated relatively small amounts of Ad2 58K protein, is suppressed to a much lesser extent or not at all. The amount of suppression of SV40 expression in the $S1^+A6^+$ hybrids, which had intermediate amounts of Ad2 58K protein, appeared to be between that of the other two types of hybrids. This apparent dose-dependent suppression of SV40 antigens by Ad2 expression did not result from instability of the SV40 proteins in the hybrid cells because measurements of the decay of SV40 94K protein in $S1^+A1^-8.2$ and $S1^+A1^+11.4$ showed that it disappeared at approximately the same rate in both cell lines, with a half-life of 5 to 10 h (data not shown). Similarly, the Ad2 58K protein decayed at about the same rate in both hybrids, although the half-life was somewhat longer.

There appears to be a good correlation between suppression of expression of SV40 T antigens in our hybrid cells and the level of expression of the Ad2 58K protein in the same cells (Table 2). Moreover, there was also a direct correlation between expression of Ad2 58K protein and the increased sensitivity of these cells to lysis by NK cells and MP (Table 1). This correlation is illustrated graphically by plotting the amount of Ad2 58K protein expressed in our hybrid and nonhybrid cells (abscissa) and the sensitivity of the same cells to NK cells (Fig. 3A) or MP (Fig. 3B). A statistical analysis (Student's t test) of these data indicated that both correlations are valid ($P < 0.0005$). However, nonhybrid Ad2HE3 appeared to be considerably more sensitive than $S1^+A3^+$ hybrids, although it accumulated no more Ad2 58K protein.

Immunoprecipitation of fibronectin and actin. Because hybrid cells of the Ad2-transformed phenotype appeared to suppress SV40 expression, it seemed reasonable to consider the possibility that the marked differences in the morphological phenotypes of Ad2- and SV40-transformed cells (Fig. 1) resulted from suppression of expression of proteins which influence cellular morphology (1, 33, 36) and whether this suppression too might be related to the level of Ad2 expression. Accordingly, we immunoprecipitated fibronectin and actin from the same lysates used in our analysis of viral antigens (Fig. 2A and B; Table 3). Hybrid and nonhybrid cells of both transformed phenotypes accumulated much less fibronectin and actin than did the untransformed primary

TABLE 1. Sensitivity of hybrid and nonhybrid cell lines to in vitro lysis by NK cells and activated MP

Nonhybrid parental cell lines and hybrids	% Cell lysis induced by[a]:	
	NK cells	Activated MP
Untransformed SHE	2.7 ± 3.3	9.5 ± 1.0
SV40HE1	6.9 ± 1.0	23.8 ± 2.6
$S1^+A1^-8.2$	2.3 ± 0.9	27.3 ± 3.8
$S1^+A1^-9.4$	9.6 ± 1.6	26.9 ± 4.6
$S1^+A1^-10.3$	12.3 ± 1.8	28.1 ± 4.3
$S1^+A3^-9.2$	10.3 ± 1.2	26.3 ± 2.7
$S1^+A6^-7.1$	8.5 ± 2.2	30.0 ± 6.4
Ad2HE1	59.2 ± 5.9	88.0 ± 3.8
$S1^+A1^+2.4$	51.5 ± 4.7	82.2 ± 5.0
$S1^+A1^+11.4$	44.5 ± 1.8	90.4 ± 3.1
Ad2HE3	52.8 ± 2.5	88.1 ± 6.0
$S1^+A3^+1.1$	26.5 ± 2.5	64.7 ± 8.7
$S1^+A3^+3.3$	26.1 ± 3.5	61.2 ± 10.5
$S1^+A3^+4.2$	35.0 ± 3.5	56.3 ± 4.3
$S1^+A3^+6.3$	23.3 ± 4.5	46.8 ± 2.1
Ad2HE6	41.3 ± 3.7	75.2 ± 5.0
$S1^+A6^+5.1$	28.9 ± 4.9	67.0 ± 4.5
$S1^+A6^+12.1$	34.2 ± 4.2	73.4 ± 5.6
$S1^+A6^+12.2$	29.7 ± 4.8	68.8 ± 8.3

[a] The numbers indicate the percentage of cell lysis induced by effector cells compared with lysis by 0.5% sodium dodecyl sulfate. Average values for multiple (four to eight) determinations ± standard deviations are indicated.

TABLE 2. Quantitative amounts of viral antigens immunoprecipitated from hybrid and nonhybrid cell lines

Nonhybrid parental cell lines and hybrids	cpm of Ad2 58K/μg[a]	cpm of T antigen/μg[a]	
		SV40 94K	SV40 18K
SV40HE1	0	186.0 ± 62.4	81.5 ± 3.0
$S1^+A1^-8.2$	0	121.0 ± 17.1	8.7 ± 2.7
$S1^+A1^-9.4$	0	136.7 ± 13.8	13.1 ± 1.8
$S1^+A1^-10.3$	0	113.3 ± 14.7	20.2 ± 8.0
$S1^+A3^-9.2$	0	164.9 ± 21.5	40.6 ± 17.0
$S1^+A6^-7.1$	0	187.7 ± 46.5	21.0 ± 11.8
Ad2HE1	170.0 ± 9.0	0	0
$S1^+A1^+2.4$	121.2 ± 27.5	33.5 ± 11.98	6.9 ± 1.1
$S1^+A1^+11.4$	108.6 ± 32.0	23.0 ± 7.8	4.8 ± 0.6
Ad2HE3	54.0 ± 12.7	0	0
$S1^+A3^+1.1$	41.0 ± 7.4	117.7 ± 18.1	20.4 ± 5.0
$S1^+A3^+3.3$	48.6 ± 6.4	112.9 ± 1.5	18.5 ± 8.1
$S1^+A3^+4.2$	54.2 ± 10.4	81.7 ± 6.6	11.7 ± 2.0
$S1^+A3^+6.3$	31.7 ± 10.4	70.8 ± 17.3	14.5 ± 3.0
Ad2HE6	131.9 ± 4.0	0	0
$S1^+A6^+5.1$	82.6 ± 8.5	29.8 ± 10.8	4.7 ± 2.8
$S1^+A6^+12.1$	57.9 ± 17.1	73.7 ± 10.0	8.2 ± 3.2
$S1^+A6^+12.2$	100.5 ± 18.8	163.5 ± 22.6	9.8 ± 2.0

[a] The numbers indicate the counts per minute of specific proteins in each band per microgram of input lysate protein; average values for four determinations are shown ± standard deviations.

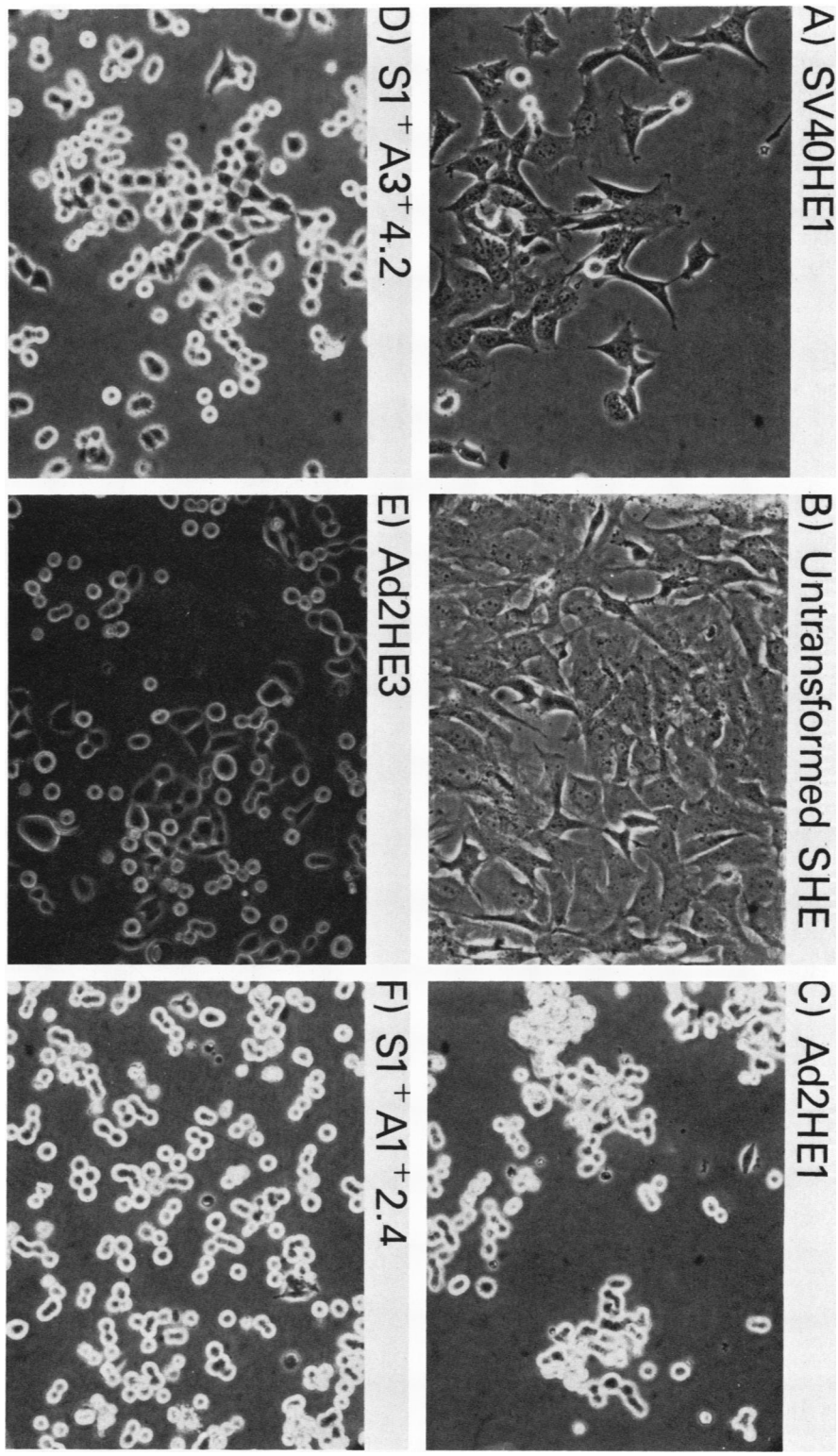

FIG. 1. Selected hybrid and nonhybrid cell lines. Magnification, ×200.

cells from which they were derived (Table 3). $S1^+A1^+$ hybrids, which accumulated the highest levels of Ad2 58K protein and low levels of fibronectin and actin, were mainly small round cells, whereas $S1^+A3^+$ and $S1^+A6^+$ hybrids, which accumulated less Ad2 58K protein and more fibronectin and actin, are more cuboidal (Fig. 1). Cells of the S^+A^- phenotype, which express no Ad2 antigens, had a fibroblastic shape; they usually accumulated substantial amounts of fibronectin and actin. There was no obvious reason why $S1^+A1^-10.3$ cells should accumulate much less fibronectin than the other S^+A^- hybrids (Table 3), since their shape was not notably different. However, in general it would appear that transformation by Ad2 leads to suppression of these cellular proteins and that this suppression correlates well with changes in the cell shape from fibroblastic to spherical.

DISCUSSION

In previous studies, we showed that early Ad2 proteins function to suppress SV40 DNA synthesis in lytically infected permissive cells (28) and the SV40 tumorigenic phenotype in hybrids formed from SV40- and Ad2-transformed SHE cells (27). In a subsequent study, we demonstrated that

TABLE 3. Quantitative amounts of fibronectin and actin immunoprecipitated from hybrid and nonhybrid cell lines

Nonhybrid parental cell lines and hybrids	cpm[a]	
	Fibronectin	Actin
Untransformed SHE	1,167.6 ± 128.1	863.9 ± 129.8
SV40HE1	446.0 ± 43.4	80.6 ± 20.7
$S1^+A1^-8.2$	238.5 ± 31.4	134.4 ± 2.8
$S1^+A1^-9.4$	372.1 ± 21.2	153.7 ± 21.3
$S1^+A1^-10.3$	27.7 ± 10.9	120.3 ± 15.1
$S1^+A3^-9.2$	235.4 ± 38.6	242.6 ± 12.4
$S1^+A6^-7.1$	198.3 ± 23.9	153.0 ± 45.2
Ad2HE1	20.9 ± 1.7	2.4 ± 1.4
$S1^+A1^+2.4$	14.4 ± 4.8	6.4 ± 3.5
$S1^+A1^+11.4$	11.4 ± 4.5	5.9 ± 2.3
Ad2HE3	38.2 ± 4.1	35.9 ± 22.1
$S1^+A3^+1.1$	46.0 ± 9.2	15.0 ± 4.9
$S1^+A3^+3.3$	122.6 ± 10.1	19.0 ± 8.3
$S1^+A3^+4.2$	133.2 ± 9.5	22.0 ± 3.2
Ad2HE6	28.2 ± 8.2	35.3 ± 4.7
$S1^+A6^+5.1$	28.8 ± 0.7	9.3 ± 1.2
$S1^+A6^+12.1$	37.4 ± 4.2	14.4 ± 5.7
$S1^+A6^+12.2$	31.2 ± 3.0	20.5 ± 3.0

[a] The numbers indicate the counts per minute of specific proteins in each band per microgram of input lysate protein; average values for multiple determinations (three to five) are shown ± standard deviations.

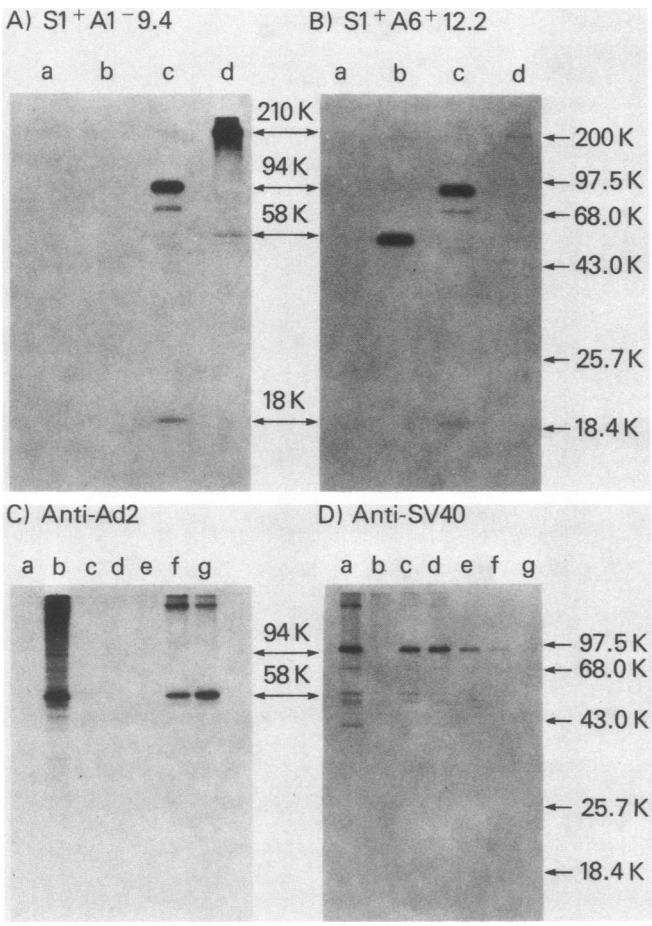

FIG. 2. Electropherograms of viral antigens immunoprecipitated from hybrid and nonhybrid cell lines. Lysates prepared from $S1^+A1^-9.4$ (A) and $S1^+A6^+12.2$ (B) and immunoprecipitated with normal hamster serum (lane a), anti-Ad2 serum (lane b), anti-SV40 ascites fluid (lane c), and antifibronectin (lane d). Viral antigens from equal amounts of lysate protein immunoprecipitated by anti-Ad2 serum (C) or anti-SV40 ascites fluid (D). Lanes: a, SV40HE1, b, Ad2HE1; c, $S1^+A1^-8.2$; d, $S1^+A1^-9.4$; e, $S1^+A1^-10.3$; f, $S1^+A1^+2.4$; and g, $S1^+A1^+11.4$. The locations of molecular weight markers are indicated. K, 10^3.

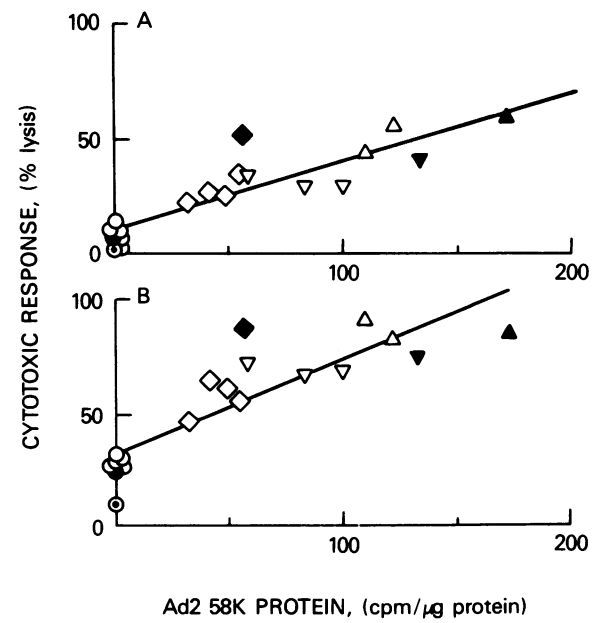

FIG. 3. Correlation of the amount of Ad2 58K protein expressed in hybrid and nonhybrid cells with their sensitivity to NK cells (A) or activated MP (B) . Symbols: ⊙, untransformed SHE cells; ●, SV40HE1; ▲, Ad2HE1; ◆, Ad2HE3; ▼, Ad2HE6; ○, $S1^+A1^-$, $S1^+A3^-$, and $S1^+A6^-$; △, $S1^+A1^+$; ◇, $S1^+A3^+$; ▽, $S1^+A6^+$. Data from Tables 1 and 2.

some of our S^+A^+ hybrids, which express both Ad2 and SV40 T antigens, are indistinguishable from the parental nonhybrid Ad2-transformed cells in their sensitivity to in vitro lysis by NK cells and activated MP (7). In contrast, hybrid cells which did not accumulate detectable Ad2 T antigens were as resistant to these nonspecific immune effector cells as were nonhybrid SV40-transformed cells. Because the interactions of these viral antigens have been useful in elucidating the mechanism involved in neoplastic transformation, we report here the results of a quantitative analysis of viral antigen expression in our hybrid cells together with a more complete analysis of the susceptibility of these cells to in vitro lysis by NK cells and MP. We also determined the amounts of cellular fibronectin and actin which accumulate in these hybrid cells and correlated this with the morphologies of the two transformed phenotypes. We show here that the sensitivity of these hybrid cells to in vitro lysis by NK cells and MP increases with increased expression of the Ad2-transforming genes (i.e., Ad2 58K protein). We also show that the amount of SV40 T antigen expression in all S^+A^+ hybrids and the levels of cellular fibronectin and actin decrease with increasing levels of Ad2 58K protein. Thus, it appears that the level of viral antigen expression (Ad2 or SV40 or both) strongly influences the transformed cell phenotype in a dose-dependent manner.

We did not show whether suppression of SV40 antigens, fibronectin, and actin by Ad2 expression is at the level of transcription or translation. However, recent evidence indicates that the AdE1A region is a strong modulator of both viral and cellular transcription (3, 16, 25, 36). Moreover, Velcich and Ziff (37) have shown that adenovirus E1a proteins repress transcription from the SV40 early promoter and that the extent of repression increases with increasing quantities of these proteins.

Our results are consistent with recent reports which indicate that the phenotype of transformed cells may depend on the level of oncogene expression. For example, the cellular equivalent of the oncogene of Harvey murine sarcoma virus is expressed during the G1 phase of the replication cycle of human WI38 cells (5, 10). However, cells transformed by murine sarcoma virus or transfected with oncogenic DNA from that virus accumulate much higher levels of p21 *ras*, and this increased expression of the oncogene is accompanied by marked changes in morphology and growth characteristics. Moreover, p21 *ras*, synthesized in *Escherichia coli*, purified, and microinjected into normal rodent cells, elicits morphological changes in a dose-dependent manner (34). Similarly, rat cells transformed by oncogenic DNA from Rous sarcoma virus may express different levels of pp60 v-*src*. These cells exhibit different phenotypic properties depending on the dose of pp60 v-*src* (17).

The mechanism of adenovirus dominance in determining the phenotypic properties of hybrid cells is not understood, but two possibilities should be considered. The transformed hybrid cell phenotype could result from the direct action of Ad2 proteins on the expression of cellular genes, or the hybrid cell phenotype might arise from suppression of the influence of SV40 proteins (32).

The primary untransformed SHE cells from which our transformed cells were derived more closely resemble SV40-transformed cells than Ad2-transformed cells from the point of view of the phenotypic properties considered here. Thus, it is possible that transformation by SV40 leads to immortalization and in vivo propagation, whereas other phenotypic properties are modified to a lesser extent. In contrast, Ad2 transformation, although also conferring immortality and the ability to propagate in vivo, leads to much more pronounced effects on the phenotypic characteristics of the transformed cell, including a markedly increased sensitivity to lysis by nonspecific immune effector cells. This hypothesis is supported by the observation that rat and mouse cells transformed by SV40 are highly sensitive to in vitro lysis by NK cells and MP, indicating that SV40 transformation per se may lead to cytolytic susceptibility (22); only SV40-transformed hamster cells are resistant. Moreover, Franza and Garrels (14) have made an elegant analysis of two-dimensional electropherograms of radiolabeled proteins extracted from an established line of rat cells and the same cells transformed by either Ad5 or SV40. Their results showed that Ad2 transformation induces a much greater change in cellular protein expression than does transformation by SV40. These changes were effected by both repression and derepression of cellular proteins. Thus, the resistance of our S^+ and S^+A^- cells to cytolysis is most likely a property of the hamster cell which remains unaltered by SV40 transformation, implying that transformation by Ad2 renders hamster cells vulnerable to the cytolytic action of NK cells and MP.

Suppression of fibronectin and actin synthesis could contribute to the morphological differences observed between S^+A^- and S^+A^+ hybrids. Indeed, loss of fibronectin has been associated with loss of the fibroblastic morphology by some transformed cells (1). However, previous microscopic studies have suggested that loss of actin microfilaments is due to a disruption of the actin cables rather than to a suppression of actin synthesis (15). In contrast, our data indicate that immunoprecipitable actin is significantly reduced in SHE cells transformed by either SV40 or Ad2 compared with untransformed SHE cells and that the actin content of Ad2-transformed cells is 3- to 20-fold less than that of SV40-transformed cells (Table 3). This finding is supported by recent work comparing immunoprecipitable actin in untransformed REF-52 cells with that in REF-52 cells transformed by either SV40 or Ad5 (14). This study, employing methods similar to ours, demonstrated that the actin content of untransformed cells is greater than that of SV40-transformed cells and that both of these cell types contain more actin than Ad5-transformed cells. Because the earlier results regarding actin synthesis (15) were not based on immunoprecipitation, it is difficult to interpret those results quantitatively. It is likely that immunoprecipitation of actin is, in fact, revealing an important suppressive effect of Ad2.

Ad2 genetic expression also governs the tumorigenic potential of S^+A^+ hybrids (27). S^+A^+ hybrids readily induced tumors in immunoincompetent hamsters; however, they induced few tumors in syngeneic adult hamsters and none in allogeneic hamsters. In contrast, S^+A^- hybrids induced tumors in both immunoincompetent and immunocompetent hamsters. If transformed cells are to develop into tumors in immunocompetent animals, they must not only be able to propagate in vivo but they must also be able to resist the cellular immune responses of the host. Changes in fibronectin expression have been implicated in changes in tumorigenicity (6); however, recent work indicates that there is little correlation between levels of fibronectin in transformed cells and their ability to induce tumors (26). The importance of nonspecific cytotoxic cells such as NK cells and MP in tumor rejection remains to be fully elucidated (11, 13). However, there is a good correlation between the ability of transformed SHE cells to resist

cytolysis in vitro by nonspecific effector cells and the ability of the same cells to induce tumors in immunocompetent hosts (21, 22). Thus, it is reasonable to propose that the insensitivity of S^+A^- hybrids to in vitro lysis by NK cells and MP is a factor in determining the ability of these cells to resist rejection by immunocompetent hamsters. Other factors may also contribute importantly to control of tumor induction by transformed cells. For example, expression of the transforming viral genes may regulate expression of cellular histocompatibility antigens which in turn influence sensitivity of transformed cells to cytotoxic T lymphocytes (2, 31). Moreover, tumor cell secretions may influence the activity of cytotoxic cells (35) or stimulate the formation of barriers which limit access of cytotoxic cells to the tumor (12). These and other functions, which may be governed by expression of transforming viral genes, probably contribute to the tumorigenic phenotype of transformed cells. Thus, although the oncogenic potential of our hybrid cells is governed by the lowest levels of Ad2-transforming gene expression, we showed here that other phenotypic properties, including sensitivity to cytolysis by NK cells and MP, depend on Ad2 genetic expression in a dose-dependent manner.

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