Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 8543–8547, November 1988
Genetics

Partial suppression of anchorage-independent growth and tumorigenicity in immunodeficient mice by transfection of the H-2 class I gene *H-2L^d* into a human colon cancer cell line (HCT)

(transformation/tumor growth control/mice)

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Contributed by Kurt J. Isselbacher, December 21, 1987

ABSTRACT **Many human tumors, particularly those of epithelial origin, appear to express greatly reduced levels of major histocompatibility complex class I antigens on their surface. It has been previously reported that the class I gene *H-2L^d*, introduced into adenovirus type 12-transformed mouse cells, induces reversal of oncogenesis in immunocompetent BALB/c mice. We have tested the hypothesis that the *H-2L^d* gene, when transfected into HCT colon cancer cells, may alter their transformed phenotype. Two *H-2L^d* transfectants, HCT-Ii and HCT-If, were found to exhibit a markedly reduced-to-virtually suppressed ability to form colonies in soft agar in comparison to a transfectant (HCTh) carrying only the neomycin-resistance gene. We also compared the tumorigenicity of HCTh vs. HCT-If cells in two different strains of immunodeficient mice: nude (T$^-$) and triple-deficient mutants (T$^-$, NK$^-$, B$^-$). At 28 days postinjection of 10^7 and 10^6 cells, the size and growth rate of HCT-If tumors were greatly reduced compared to HCTh cells. Therefore, as assayed in immunodeficient animals, expression of the class I *H-2L^d* gene in HCT cells appears to correlate with partial suppression of the tumorigenic phenotype, suggesting that the expression of a transfected class I gene may by itself alter the phenotype of the recipient cell and that such phenotypic changes may be independent of the immune system.**

Mouse *H-2* class I genes constitute a highly polymorphic gene family belonging to the major histocompatibility complex. They encode trans-membrane glycoproteins, which are expressed by virtually all cell types and are responsible for rejection of tissues transplanted in noncongenic hosts. This phenomenon has suggested an immunologic function for this family of gene products (1), which are also known as transplantation antigens. *H-2* class I glycoproteins contain five portions: two extracellular variable (polymorphic) domains (α_1 and α_2) at the NH_2 terminus, followed by a constant extracellular domain (α_3), a trans-membrane domain that anchors the antigen to the cell surface, and an intracytoplasmic domain at the carboxyl terminus. The three extracellular domains bind noncovalently to the β_2 microglobulin. It is assumed that H-2 class I antigens direct the recognition of neoplastic cells by cytotoxic T lymphocytes; H-2 molecules would provide the context (major histocompatibility complex restriction) for recognition of tumor-specific antigens, present on the neoplastic-cell surface. Therefore, class I molecules appear to play an essential role in the presentation of tumor-specific antigens to the host's immune system (1).

Certain DNA tumor viruses can efficiently transform rodent cultures, but these phenotypic transformants are not necessarily tumorigenic. Rodent cells transformed by human adenovirus types 2 and 5 (Ad2 and Ad5) are nontumorigenic, whereas transformants obtained with Ad12 are highly tumorigenic in syngeneic immunocompetent hosts (2). This different behavior is associated with a reduction in the expression of class I antigens in Ad12- vs. Ad5-*E1a* transformants (3). This observation, made in rat cells, has been confirmed in Ad12-transformed mouse cells, which have been shown to be tumorigenic in syngeneic hosts (4). Moreover, by DNA-mediated gene transfer, a cloned class I gene (*H-2L^d*) derived from BALB/c mice has been introduced into Ad12 transformants, and the transfectants have been tested for tumorigenicity. The results of these experiments showed that, regardless of the cell dose, transfectants expressing high levels of *H-2L^d* are much less tumorigenic in BALB/c mice than in the parental cells (4). Although reversal of oncogenesis is seen only below a certain threshold of tumor cell dose, this observation indicates that class I gene expression can mediate reversion of the tumorigenic phenotype.

In the present study we provide evidence indicating that HCT human colon cancer cells expressing a transfected *H-2L^d* gene show partial suppression of (*i*) the transformed phenotype as assessed by a strikingly decreased ability to form colonies in soft agar and (*ii*) the tumorigenic potential when injected into immunodeficient mice (nude as well as triple-deficient mutants).

MATERIALS AND METHODS

Cell Cultures and Transfection. HCT (originally designated HCT-15) are human colon adenocarcinoma cells (5) (American Type Culture Collection), that were propagated in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS). Exponentially growing HCT were transfected using the calcium phosphate-precipitation technique (6) as modified by Wigler *et al.* (7). Donor DNAs were the pL^{d}4 plasmid (8) containing the complete genomic sequence of class I *H-2L^d* gene, including its regulatory sequences, and the pRSV-neo plasmid (9) containing the neomycin-resistance gene under the transcriptional control of the long terminal repeat of the Rous sarcoma virus. The two plasmids were cotransfected, and the resistant cells were selected in DMEM plus 10% FBS containing G418 (GIBCO) at 800 μg/ml. Neomycin-resistant clones were picked 3 weeks after transfection and expanded in selective medium for DNA and RNA extraction.

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Abbreviations: TD, triple-deficient mice (T$^-$, NK$^-$, B$^-$); DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; Ad2, Ad5, and Ad12, adenoviruses type 2, type 5, and type 12, respectively.

DNA and RNA Blot Hybridization. Chromosomal DNA from neomycin-resistant clones was collected as previously described (10), digested with *Eco*RI, electrophoresed through a 1% agarose gel, and transferred onto nitrocellulose filter following alkali denaturation (11). Hybridization was performed (12) using as a probe a purified *Xba* I–*Xba* I fragment of ≈1.9 kilobases (kb) encompassing the 5′ three exons of *H-2L*^{d}. This fragment was ^{32}P-labeled by nick-translation (13) as modified by Weinstock *et al.* (14). After hybridization, filters were washed at 65°C in 0.1× SSC (1× SSC = 0.15 M sodium chloride/0.015 M sodium citrate, pH 7) and 0.1% $NaDodSO_4$ before exposing for autoradiography using x-ray films at −80°C. Total RNA was extracted with the method of Cathala *et al.* (15); poly(A)$^+$ RNA was selected through oligo(dT)-cellulose chromatography (16), size separated by denaturing gel electrophoresis (17), and blot hybridized as described above.

Growth in Soft Agar (18). HCTh, HCT-Ii, and HCT-If cells were trypsinized to single-cell suspension and mixed with 0.3% Bacto-agar (Difco) in DMEM plus 10% FBS to a final concentration of ≈10^2 cells per ml. Ten milliliters of this suspension were layered in 10-cm culture dishes containing a base of 0.5% Bacto-agar in DMEM plus 10% FBS. Agar plates were incubated in 5% CO_2/95% air at 37°C and fed once at 10 days. Colonies were scored at 3 weeks after staining with neutral red.

Tumorigenicity Assays in Immunodeficient Animals. Two types of immunodeficient mice were used: (*i*) nude (*nu/nu*) mice lacking T cells (T$^-$) and (*ii*) triple-deficient mutants (TD) lacking T cells, natural killer cells, and an effective B-cell response because of the *xid* mutation (T$^-$, NK$^-$, B$^-$) (19–22). Approximately 10^7 and 10^6 cells from HCTh and HCT-If cultures were prepared after trypsinization in 0.1-ml inoculum and injected s.c. into the right axilla of five animals for each strain. The mice were followed for 4 weeks for tumor development. Animals were kept in the pathogen-free colony at Massachusetts General Hospital (23).

RESULTS

Transfection and Expression of *H-2L*^{d} in HCT Colon Cancer Cells. HCT cells derived from colon adenocarcinoma are fast-growing, epithelial-like human cells that we have previously characterized (24). We have used these cells as recipients in transfection experiments using the calcium phosphate-DNA precipitation technique (6, 7). As donor DNA, we used the plasmid pL^{d}4, which contains the entire genomic sequence for the *H-2L*^{d} gene (25), including the promoter and regulatory sequences (8). Because no selection is available to identify and clone stable HCT transfectants carrying the *H-2L*^{d} gene integrated into the chromosomal DNA, we cotransfected pL^{d}4 with the plasmid pRSV-neo (9), containing a selectable marker for neomycin resistance. After selection with G418-containing culture medium, we isolated neomycin-resistant clones that we expanded to extract chromosomal DNA for Southern blot analysis. Fig. 1 shows the short and long exposure (*Left* and *Right*, respectively) of four different chromosomal DNAs, clearly indicating positive hybridization for clones f and i (designated HCT-If and HCT-Ii, respectively). The marker identifies the linear pL^{d}4 (≈15 kb), because we used *Eco*RI as a restriction enzyme, which cuts only once in the plasmid. This is also the major band in HCT-Ii, whereas HCT-If exhibits two additional slower-migrating bands. The additional signal evident in the long exposure is probably due to cross-hybridization between our probe and endogenous *HLA* class I sequences.

To determine whether the transfected sequences were being transcribed in HCT-If and HCT-Ii cells, we isolated poly(A)$^+$ RNA from both clones and from the neomycin-resistant HCTh, as a negative control; Fig. 2 shows the RNA

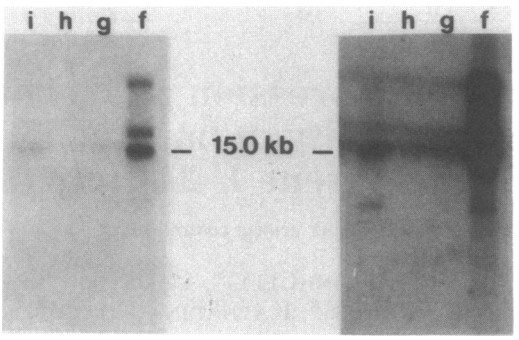

FIG. 1. Southern blot analysis of chromosomal DNAs. Chromosomal DNA was extracted from neomycin-resistant HCT clones (a–k), and ≈10-μg aliquots were loaded in each lane after *Eco*RI digestion. The nitrocellulose filter was hybridized with a ^{32}P-labeled nick-translated *Xba* I–*Xba* I fragment encompassing the 5′ three exons of *H-2L*^{d} gene. Size of the linear pL^{d}4 is indicated. *Left* and *Right* represent short and long exposure, respectively, of the same experiment.

blot hybridization of these RNAs using the *H-2L*^{d} specific probe. Although HCTh RNA exhibits no hybridization signal, both HCT-If and HCT-Ii clones appear to contain a transcript of ≈1.7 kb, which is expressed at about the same level independent of the presence or absence of G418 in the culture medium. Fig. 2 shows the short and long exposure (*Left* and *Right*, respectively) of the RNA blot hybridization of HCT-Ii, HCTh, and HCT-If clones, from which the RNA was extracted after culturing the cells in the presence (+) or absence (−) of G418. Although HCT-If cells contain ≈10-fold more *H-2L*^{d} DNA integrated into their genome than do HCT-Ii cells, the amount of H-2L^d RNA detected by RNA hybridization was about equivalent. Thus, the transcriptional state of both clones for the *H-2L*^{d} gene may be independent of copy number in confluent monolayers (which were used as our source of RNA). Alternatively, only one or a limited number of *H-2L*^{d} gene equivalents may be transcriptionally active in HCT-If. Therefore, this result indicates that HCT-Ii and If clones do express the transfected *H-2L*^{d} gene as mRNA. Moreover, HCT parental cells and seven independent G418-resistant clones (including HCTh, Ii and If) were found to be negative for surface expression of HLA and β_2-microglobulin, suggesting that the *H-2L*^{d} gene transfected in HCT cells is expressed in the absence of detectable endogenous class I antigens (S.G.-C., unpublished work).

Partial Suppression of Anchorage-Independent Growth in Semi-Solid Medium of *H-2L*^{d} Transfectants. The process of

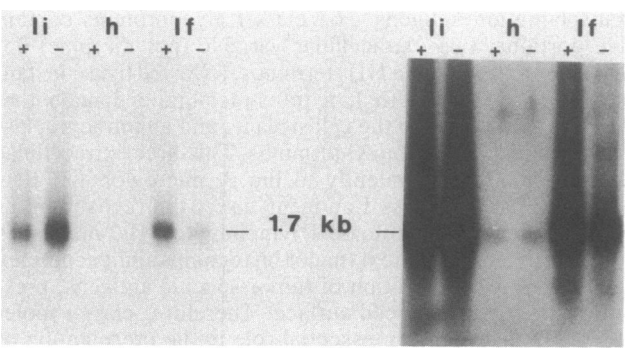

FIG. 2. RNA blot analysis of poly(A)$^+$ RNAs. Samples were obtained from HCT-Ii, HCTh, and HCT-If clones, grown in the presence (+) or absence (−) of G418. Thirteen to fifteen micrograms of RNA sample was loaded per lane. Hybridization conditions and probe used were the same as for the DNA blot. Size of the H-2L^d transcript is indicated. *Left* and *Right* represent short and long exposure, respectively, of the same experiment.

neoplastic transformation is accompanied by a series of phenotypic changes that are expressed during *in vitro* growth (26). In general, there is a good correlation between anchorage independence—the ability of cells to grow *in vitro* without attachment to a solid phase—and tumorigenicity (27). We, therefore, performed experiments of anchorage-independent growth to test the ability of HCTh, HCT-Ii, and HCT-If cells to form colonies in soft agar. After trypsinization of cultures grown on plastic dishes, $\approx 10^3$ cells per 10-cm plate were resuspended in DMEM containing 10% FBS and 0.3% Bacto-agar, and the suspension was layered on a base of DMEM containing 10% FBS and 0.5% Bacto-agar. The agar plates were then incubated for 3 weeks, and after staining the colonies with neutral red in phosphate-buffered saline, their size and number were scored under a dissecting microscope. Table 1 summarizes such an experiment. HCTh and HCT-Ii exhibited a colony-forming efficiency of ≈45%, although HCT-Ii cells gave rise to much smaller colonies than did HCTh (Fig. 3*a*). In separate experiments, we found no significant difference between HCTh and untransfected (neomycin-sensitive) HCT parental cells in the ability to form colonies in soft agar, as well as in colony size (data not shown). This result tends to rule out the possibility that pRSV-neo expression affects the ability of HCTh cells to grow in soft agar. Fig. 3*b* shows the comparison between HCTh and HCT-If where colonies could be identified only microscopically; the ≈6% efficiency of colony formation scored for HCT-If (Table 1) probably represents an overestimation. The star-like appearance of some HCTh colonies was due to precipitation of neutral red stain. Fig. 3 *c–e* show representative fields of HCTh, HCT-Ii, and HCT-If agar plates, respectively, photographed at higher magnification. These experiments indicate that *H-2L*^{d} gene expression is associated with partial suppression of the ability of HCT colon cancer cells to form colonies in soft agar (partial suppression of anchorage-independent growth). We have tested 13 additional clones (6 of which express the *H-2L*^{d} gene) for anchorage-independent growth and found that the *H-2L*^{d} transfectants exhibit a virtually suppressed ability to form colonies in soft agar (like HCT-If), whereas only 1 of 7 G418-resistant, *H-2L*^{d}-negative clones was unable to grow in soft agar (unpublished work).

Table 1. Growth in soft agar of HCT transfectants

Efficiency of colony formation, %		
HCTh*	HCT-Ii†	HCT-If‡
45 ± 5	47 ± 7	6 ± 2

The efficiency of colony formation was somewhat variable in different experiments, but the ratios between clones were virtually unchanged within each experiment.
*Colonies of various size and shape; many large colonies.
†Colonies of homogeneously small size.
‡Mostly microscopic colonies.

Partial Suppression of Tumorigenicity in Immunodeficient Mice. Nude mice are hairless mutants affected by an autosomal recessive mutation (*nu/nu*) associated with absence of thymus-derived mature T-lymphocytes (T^-) and thymus-dependent antibody response (19). Triple-deficient mutants, obtained through special breeding steps, are affected by two additional mutations—beige (*bg*), which is associated with a complete and selective impairment of natural killer (NK^-)

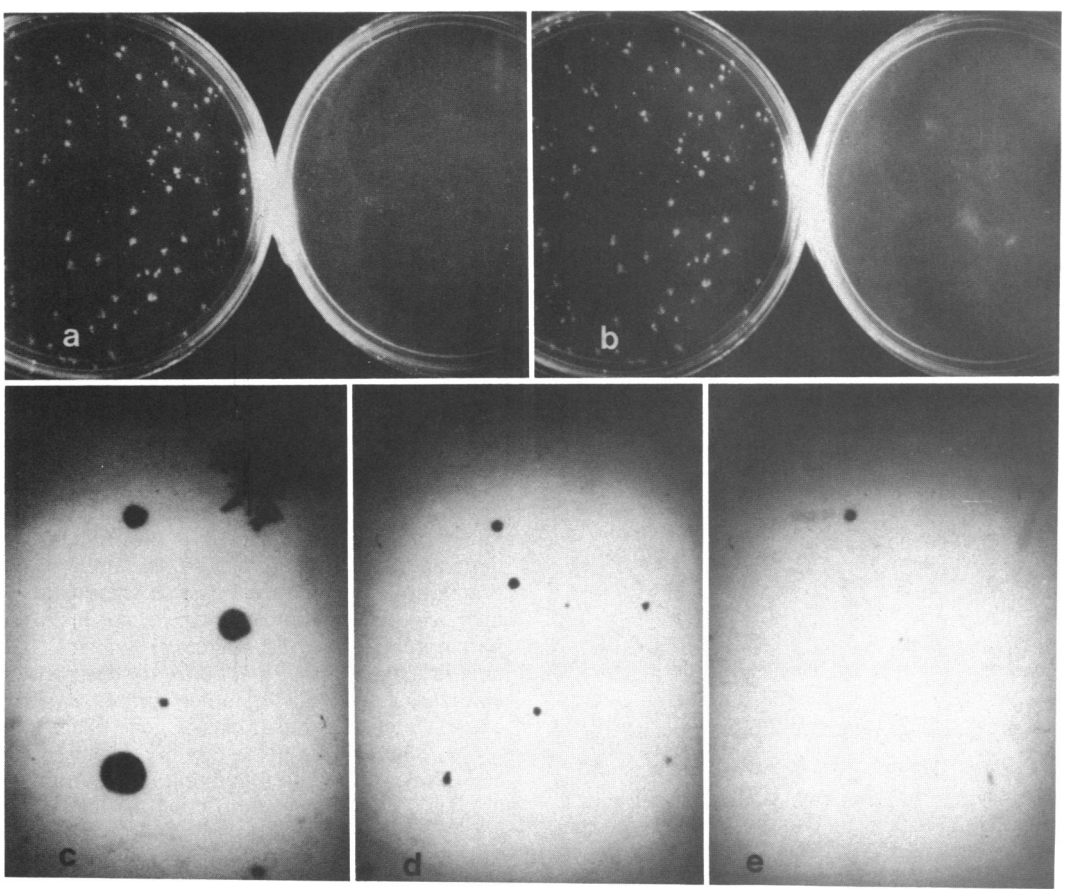

FIG. 3. Growth in semisolid medium. Single-cell suspensions from HCTh, HCT-Ii, and HCT-If were mixed in 0.3% soft agar, as described, at a concentration of 10^3 cells per 10-cm dish, incubated for 3 weeks, and stained with neutral red. (*a*) Comparison between HCTh (at left) and HCT-Ii (at right) at low magnification. (*b*) Comparison between HCTh (at left) and HCT-If (at right) at low magnification. (*c–e*) Representative fields at higher magnification of agar colonies from HCTh, HCT-Ii, and HCT-If, respectively (from left to right).

cells (20), and *xid* (21, 22), which is associated with a block in the maturation of B cells (B^-). These animals represent the host of choice for experiments aimed at assessing the tumorigenicity of cell cultures derived from spontaneous human neoplasias. Therefore, we injected 10^7 and 10^6 cells suspended in 0.1 ml of culture medium into five animals of each strain for a total of 20 animals per cell line. Fig. 4 shows the kinetics of tumor growth for HCTh and HCT-If cells up to 4 weeks after injection. Size of the tumors is expressed as the mean tumor volume of the five animals in each group. HCTh tumors grew faster and larger in triple-deficient mutants (*Upper*) than in nude mice (*Lower*). Shortly after the fourth week, most animals had to be sacrificed because of large tumor size. In the triple-deficient mutants, no detectable growth occurred after injection of 10^6 HCT-If cells. In the following weeks tumors did grow in triple-deficient mice injected with HCT-If cells, but the growth rate appeared to be much slower than with HCTh tumors. In nude mice injected with HCT-If cells, we could measure tumor growth, although the rate appeared to be much slower. Tumor volumes were calculated from their diameter on the assumption that they were essentially spherical. Fig. 5*a* shows a triple-deficient mouse at 4 weeks after injection with 10^7 HCTh cells; Fig. 5*b* shows the only triple-deficient mouse injected with 10^7 HCT-If cells that had detectable tumor growth 4 weeks after injection.

Cell cultures grown in parallel with the ones used for tumorigenicity studies were characterized for their neomycin-resistant phenotype and for their karyotype to rule out major changes in their genotype. We have confirmed that both HCTh and HCT-If are G418-resistant cell lines; their respective karyotypes demonstrated that they are both male human cells with specific chromosomal markers in common, providing evidence that the two cell lines are related (data not shown).

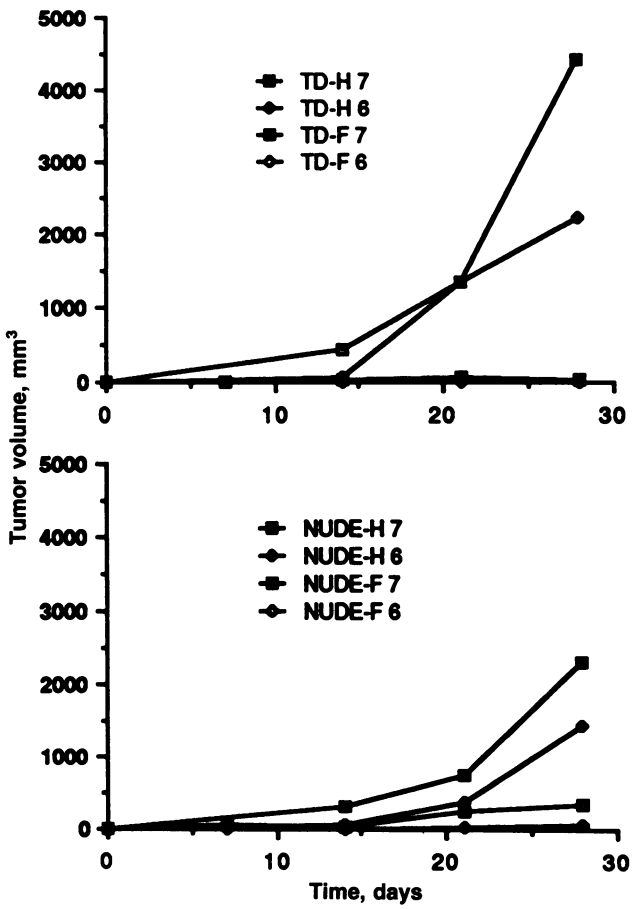

FIG. 4. Growth curve of HCTh and HCT-If tumors in immunodeficient mice. Cell suspensions (0.1-ml inoculum) were injected s.c. into the right axilla of five animals for each strain. (*Upper*) Tumor growth in triple-deficient mice (TD) injected with 10^7 HCTh cells (H7, ▣), 10^6 HCTh cells (H6, ◆), 10^7 HCT-If cells (F7, ■), 10^6 HCT-If cells (F6, ◆); (*Lower*) tumor growth in nude mice of HCTh and HCT-If cells (same symbols).

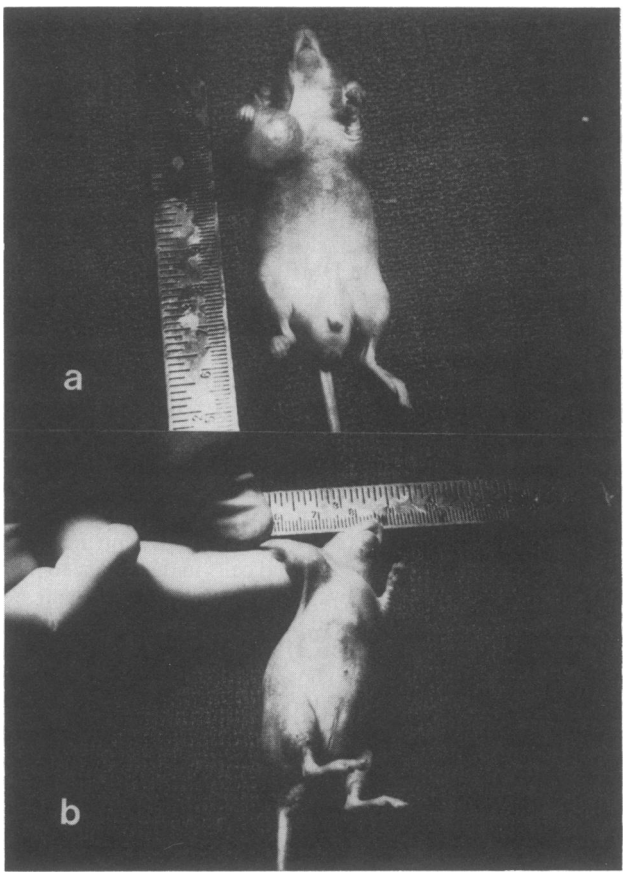

FIG. 5. Tumor growth at 4 weeks in triple-deficient (T^-, NH^-, B^-) mice. (*a*) Triple-deficient mouse 28 days after injection into the right axilla with 10^7 HCTh cells. (*b*) Triple-deficient mouse 28 days after injection with 10^7 HCT-If cells; this is the only animal in five animals that showed detectable tumor growth 4 weeks postinjection.

DISCUSSION

Our results indicate that *H-2L*^{d} expression correlates with partial suppression of anchorage-independent growth in HCT class I transfectants and decreased tumorigenicity after injection of HCT-If cells in two different strains of immunodeficient mice (nude and triple-deficient mutants). It is premature to extend the possible significance of these findings to other class I genes because of their extensive polymorphism, and although expression of class I antigens is down-regulated in several tumor tissues particularly those of epithelial origin (1, 28), other tumors have been found to express higher levels of class I gene products (1, 29–31).

Consistent with our results is the observation of Kulesh and Greene (32), who found that interferon (a potent inducer of class I gene expression) could restore contact inhibition and "shape-dependent" proliferation in malignant cells (33). In studies that parallel those reported here, D. Ingber and J. Folkman (personal communication) have observed that HCT-If cells plated on plastic substrates coated with increasing concentrations of fibronectin exhibit a higher degree of spreading than that exhibited by HCTh (measured by the surface area covered by the cells). We have also found that

HCT-If cells express less glucose transporter-specific RNA by a factor of 10 than do HCTh (R.M.S., unpublished work), consistent with previous reports of increased glucose transporter mRNA in transformed vs. normal cells (34, 35). Taken together, the present findings support the concept that expression of *H-2L*^{d} in HCT cells correlates with partial suppression of the tumorigenic phenotype.

It has been reported that the transfection procedure by itself may induce substantial phenotypic changes in independent transfectants (36). Interestingly, in that study most of the clones exhibiting a less transformed phenotype expressed higher levels of MHC class I genes. We believe those observations are more likely the result of tumor heterogeneity which, to some degree, we have also observed with other HCT transfectants. However, tumor heterogeneity does not appear to be an adequate explanation for our finding that *H-2L*^{d} expression correlated with a consistent down-regulation of the transformed phenotype in 13 additional clones tested so far, 6 of which express the transfected gene.

Our tumorigenicity studies in immunodeficient mice suggest that class I antigens, in addition to their well established role in the control of tumor growth via the immune system, may also be involved in the induction of phenotypes that can alter cell behavior independent of the immune system. These observations are also consistent with the recent report of a specific interaction between MHC-I antigens and hormone receptors, which could trigger a cellular response (37). Therefore, the phenotypic changes induced by *H-2L*^{d} are consistent with the possible functional role(s) of class I gene products as surface membrane proteins involved in organogenesis and/or cell differentiation (38, 39).

We thank Dr. Gilbert Jay for providing plasmids and monoclonal antibody 30.5.7, Dr. Herman Suit for providing access to the pathogen-free animal facility, Dr. Anthony Zeitman for his assistance and advice, Robert Sedlacek for excellent technical assistance with the immunodeficient mice, Dr. Mary Sunday for helpful discussion, and Carol Henkle for performing karyotype analysis. This work was supported by Public Health Service Research Grant CA43499-01 from the National Cancer Institute.

1. Tanaka, K., Yoshioka, T., Bieberich, C. & Jay, G. (1988) *Annu. Rev. Immunol.* **6**, 359–380.
2. Bernards, R., Schrier, P. I., Houweling, A., Bos, J. L., van der Eb, A. J., Zijlstra, M. & Melief, C. J. M. (1983) *Nature (London)* **305**, 776–779.
3. Schrier, P. I., Bernards, R., Vaessen, R. T. M. J., Houweling, A. & van der Eb, A. J. (1983) *Nature (London)* **305**, 771–775.
4. Tanaka, K., Isselbacher, K. J., Khoury, G. & Jay, G. (1985) *Science* **228**, 26–30.
5. Dexter, D. L., Barbosa, J. A. & Calabresi, P. (1979) *Cancer Res.* **39**, 1020–1025.
6. Graham, F. L. & van der Eb, A. J. (1973) *Virology* **52**, 456–467.
7. Wigler, M., Pellicer, A., Silverstein, S. & Axel R. (1978) *Cell* **14**, 725–731.
8. Evans, G. A., Margulies, D. H., Shykind, B., Seidman, J. G. & Ozato, K. (1982) *Nature (London)* **300**, 755–757.
9. Gorman, C., Padmanabhan, R. & Howard, B. H. (1983) *Science* **221**, 551–553.
10. Gattoni-Celli, S., Kirschmeier, P., Weinstein, I. B., Escobedo, J. & Dina, D. (1982) *Mol. Cell. Biol.* **2**, 42–51.
11. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
12. Wahl, G. M., Stern, M. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3683–3687.
13. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
14. Weinstock, R., Sweet, R., Weiss, M., Cedar, H. & Axel, R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1299–1303.
15. Cathala, G., Savouret, J.-F., Mendez, B., West, B. L., Karin, M., Martial, J. A. & Baxter, J. D. (1983) *DNA* **2**, 329–335.
16. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408–1412.
17. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5201–5205.
18. MacPherson, I. & Montagnier, L. (1964) *Virology* **23**, 291–294.
19. Pantelouris, E. M. (1968) *Nature (London)* **217**, 370–371.
20. Roder, J. & Duwe, A. (1979) *Nature (London)* **278**, 451–453.
21. Scher, I., Sharrow, S. O. & Paul, W. E. (1976) *J. Exp. Med.* **144**, 507–518.
22. Karagogeos, D., Rosenberg, N. & Wortis, H. H. (1986) *Eur. J. Immunol.* **16**, 1125–1130.
23. Sedlacek, R. S. & Mason, K. A. (1977) *Lab. Anim. Sci.* **27**, 667–670.
24. Gattoni-Celli, S., Kirsch, K., Kalled, S. & Isselbacher, K. J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6127–6131.
25. Evans, G. A., Margulies, D. H., Camerini-Otero, R. D., Ozato, K. & Seidman, J. G. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1994–1998.
26. Hsiao, W.-L.W., Gattoni-Celli, S. & Weinstein, I. B. (1985) *Mol. Cell. Biol.* **5**, 1800–1803.
27. Shin, S., Freedman, V. H., Risser, R. & Pollack, R. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4435–4439.
28. Momburg, F., Möller, P., Moldenhauer, G. & Hammerling, G. J. (1986) *J. Immunogenet.* **13**, 195–199.
29. Ljunggren, H.-G. & Karre, K. (1986) *J. Immunogenet.* **13**, 141–151.
30. Katzav, S., Segal, S. & Feldman, M. (1984) *Int. J. Cancer* **33**, 407–415.
31. Haddada, H., Sogn, J. A., Coligan, J. E., Carbone, M., Dixon, K., Levine, A. S. & Lewis, A. M., Jr. (1988) *J. Virol.* **62**, 2755–2761.
32. Kulesh, D. A. & Greene, J. J. (1986) *Cancer Res.* **46**, 2793–2797.
33. Folkman, J. & Moscona, A. (1978) *Nature (London)* **273**, 345–349.
34. Birnbaum, M. J., Haspel, H. C. & Rosen, O. M. (1987) *Science* **235**, 1495–1498.
35. Flier, J. S., Mueckler, M. M., Usher, P. & Lodish, H. F. (1987) *Science* **235**, 1492–1495.
36. Kerbel, R. S., Waghorne, C., Man, M. S., Elliott, B. & Breitman, M. L. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1263–1267.
37. Solano, A. R., Cremaschi, G., Sanchez, M. L., Borda, E., Sterin-Borda, L. & Podesta, E. J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5087–5091.
38. Ohno, S. (1977) *Immunol. Rev.* **33**, 59–69.
39. Dausset, J. (1981) *Science* **213**, 1469–1474.