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Altered growth of a human neuroendocrine carcinoma line after transfection of a major histocompatibility complex class I gene

(H-2 antigen/gene transfer/nucleic acid hybridization/soft agar clonogenic assay)

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ABSTRACT **The major histocompatibility complex (MHC) class I molecules are known to serve as recognition elements for cytotoxic T cells in mediating the rejection of transplanted tumors. We demonstrate that MHC molecules may have nonimmune functions in modulating tumor cell growth in addition to their classical role in antitumor immunity. A human neuroendocrine carcinoma cell line, COLO 320, with low levels of endogenous class I expression was transfected with the murine *H-2L*^{d} gene. Eleven independent stable clones were established, four containing only pRSV-neo and seven also containing varying copy numbers of the transfected *L*^{d} gene. The ability of the different clones to grow as colonies in soft agar correlated strongly with the relative amounts of L^d antigen expression ($r = 0.89$; $P < 0.001$). There was a weaker correlation between increased clonogenic ability and higher levels of L^d mRNA ($r = 0.67$; $P < 0.05$). There was no correlation between clonogenic ability and relative expression of amplified c-*myc* gene or of integrated pRSV-neo. Furthermore, in nude mice, L^d antigen expression was associated with increased formation of metastatic lung colonies 6 weeks after intravenous injection of 10^5 cells. These observations are consistent with the concept that MHC class I antigens may have a role in modulating the growth potential of certain tumor cells independent of their involvement in immune responses.**

The major histocompatibility complex (MHC) is a cluster of genes that encodes several classes of antigens of fundamental importance in immune responses (1, 2). Class I molecules of the MHC comprise the classical transplantation antigens, which were the first cell surface targets identified in antitumor immunity (3). To analyze the role of MHC class I antigens in tumor growth and metastasis, two general approaches have been taken: (*i*) mapping MHC class I antigen expression of different tumors; and (*ii*) introducing class I antigens by gene transfer into tumor cells with decreased or absent class I antigen expression (3–15). Either of these approaches may lead to comparative analyses of tumorigenicity or metastatic potential in the different cell lines. For instance, loss of surface expression of HLA antigens in colorectal carcinoma appears to be correlated with the degree of dedifferentiation (4, 5). Other studies demonstrate low expression of HLA class I antigens in mucinous colorectal carcinomas, in which the poor prognosis appears to be associated with a weak immune response to the tumor cells (6, 7). Other undifferentiated carcinomas also have markedly decreased class I antigen expression associated with a poor prognosis, including small cell lung carcinoma (8, 9), neuroblastoma (10), and germ cell tumors (11).

Experimental transfection of class I genes into class I-negative malignant cell lines leads to *de novo* class I antigen expression associated with reduced tumorigenicity and abrogation of metastatic properties (12–15). Most of these experiments have been carried out in immunologically competent animals. Thus, it has been concluded that class I antigen expression leads to effective recognition of the tumor cells by the host immune system.

However, there is no obligatory association between reduced HLA expression and increased malignancy, and the opposite may be observed. In several instances class I genes may be activated in transformed cells (13, 16). Also, although normal hepatocytes have low to undetectable levels of class I antigen (17), hepatocellular carcinomas have been shown to have enhanced expression of these molecules (18, 19). In other instances, allogeneic class I expression is insufficient for tumor rejection (20). These apparently contradictory observations raise questions about the role of MHC class I antigens in tumor rejection.

We have tested the hypothesis that MHC class I antigens may have a direct effect on growth of tumor cells by using the soft agar clonogenic assay and genetically immunodeficient mice. Recently, similar experiments have shown decreased clonogenic growth of a human colonic adenocarcinoma (21). Our observations here demonstrate that increased MHC class I gene expression may also lead to increased growth.

METHODS

Cell Lines and Stable Transfectants. The COLO 320 cell line (22) (American Type Culture Collection) was grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) (GIBCO) and transfected by the calcium phosphate precipitation method (23). Transfected DNAs were the pL^{d}4 plasmid (24) containing the entire *H-2L*^{d} gene including 6 kilobases (kb) of 5′ flanking sequence with the native promoter and the pRSV-neo plasmid (25) containing the neomycin-resistance gene with the long terminal repeat of the Rous sarcoma virus (RSV). Cells were transfected with pRSV-neo alone or pL^{d}4 together with pRSV-neo at a 10:1 molar ratio, and stable transfectants were selected in G418 (1 mg/ml). Three cycles of G418-resistant clones were picked at successive monthly intervals and expanded in selective medium.

Southern and Northern Blot Analyses. Genomic DNA was prepared from cultured cells, digested with *Eco*RI and *Hin*dIII, and Southern blot analyses were carried out as described (26). ^{32}P-labeled cDNA probes were prepared by random oligonucleotide priming (27) of the purified 1.9-kb *Xba* I fragment of pL^{d}4, which is highly *L*^{d}-specific, including the 5′ three exons of *H-2L*^{d}.

Total cellular RNA was prepared by the guanidinium isothiocyanate/cesium chloride gradient method. Northern

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Abbreviations: CAT, chloramphenicol acetyltransferase; MHC, major histocompatibility complex; RSV, Rous sarcoma virus; mAb, monoclonal antibody.

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blots were performed by the formaldehyde gel method (28) and hybridized in parallel to one of four ^{32}P-labeled cDNA probes: (*i*) the *Xba* I fragment of L^d, detailed above; (*ii*) the 1-kb *Eco*RI insert of pRSV-neo; (*iii*) the 1.5-kb insert of the c-*myc* cDNA, where c-*myc* is known to be amplified in the genome of the COLO 320 HSR subline (29); and (*iv*) the 600-base-pair (bp) *Pst* I fragment of mouse β-actin cDNA. All blots were scanned with a Joyce-Loebl Chromoscan 3, and relative densitometric units normalized for actin were probed on parallel identical blots.

Transient expression of the plasmid pL^d-CAT [with the L^d promoter 5′ to the bacterial chloramphenicol acetyltransferase (CAT) as a reporter gene, donated by G. Jay, National Cancer Institute, Bethesda, MD] was carried out using calcium phosphate for transfection and harvesting cell lysates 48 and 72 hr later for thin-layer chromatography as described (30).

Immunofluorescence and Radioimmunoassay Analyses of Cell-Surface Class I Gene Expression. Two different monoclonal antibodies (mAbs) recognizing human MHC class I heavy-chain public determinants together with β_2-microglobulin were used. BB7.7, from the American Type Culture Collection; and W6/32, donated by Jordan Pober (Brigham and Women's Hospital, Boston). Two distinct L^d-specific mAbs were also used, recognizing separate domains of the L^d molecule (31): 28.14.8, specific for the α_3 domain, donated by J. Forman (University of Texas Southwest Medical Center, Dallas); and 30.5.7, specific for α_1 and α_2 domains, donated by G. Jay. Cells were treated with a 1:50 dilution of mAbs or control ascites at 4°C for 16 hr, followed by the standard protocol for the avidin–biotin complex/phycoerythrin or avidin–biotin complex/immunoperoxidase techniques (Vector Laboratories). Flow cytometric analyses were carried out on cultured cells removed from tissue culture plastic with a rubber policeman, as described (21).

For more sensitive quantitation of L^d antigen expression, a cellular radioimmunoassay (RIA) was performed. Cells grown in 96-well plates to confluence were incubated for 1 hr on ice with a 1:500 dilution of W6/32, L^d-specific mAbs (see above), or mAbs for an irrelevant surface antigen (UDC10) present only in normal kidney (donated by J. Pober), with four to eight wells per group per experiment. After a phosphate-buffered saline (PBS) wash, cells were incubated for 1 hr on ice with 1 μCi of ^{125}I-labeled Fab fragment per well of sheep anti-mouse IgG (1 Ci = 37 GBq) (DuPont/NEN). Cells were washed with PBS before solubilization in 10% SDS. All counts were normalized by expressing the percentage increase over background cpm. Total L^d antigen expression was taken as the sum of results obtained with mAbs 28.14.8 and 30.5.7.

Soft Agar Colony-Forming Assay. Single cell suspensions were prepared at confluency and viable cells were counted using trypan blue dye exclusion as an indicator. Viable cells (10^3) were mixed with 10 ml of 0.3% Bacto-agar (Difco) at 42°C in DMEM/10% FBS and were layered on a feeder layer of 0.5% Bacto-agar in DMEM/10% FBS (21, 32). The total number of colonies per plate was scored blindly by two independent observers at 3 wk using a phase-contrast microscope.

Metastatic Potential Assays in Immunodeficient Animals. Nude mice (*nu/nu* on an outbred NIH background) were bred and maintained in the pathogen-free colony at the Massachusetts General Hospital. Single cell suspensions were prepared with 10^5 cells in 0.1 ml of saline injected into each mouse via the tail vein (33). At 6 wk, mice were sacrificed and lung colonies were quantitated by gross examination under a dissecting microscope after fixation of the lungs in Bouin's fixative. Hematoxylin and eosin-stained sections were also prepared for light microscopy and the number of tumor foci were counted for a minimum of four complete cross-sections of each lung.

Statistical Analyses. SEM was calculated for pooled data by the paired Student's *t* test.

RESULTS

Expression of Transfected H-2L^d in COLO 320 Carcinoma Cells. The COLO 320 colon carcinoma cell line, with amine precursor uptake and decarboxylation properties, including synthesis and secretion of serotonin, epinephrine, and corticotropin (22), was of potential interest as a model for understanding the role of MHC gene expression in neuroendocrine cell biology. Many different neuroendocrine tumors are deficient in class I antigen expression (8, 9), similar to nonneoplastic endocrine cells and neurons (17). The murine L^d gene was selected for gene transfer because both the gene and its protein product have been well characterized, and monoclonal antibodies are available that could be used to specifically detect L^d antigen at the human host cell surface (31) and thus distinguish between expression of the transfected gene and endogenous class I genes. Because of the high degree of conservation between human and murine class I genes, which is similar to homologies between different MHC class I genes within a species, the use of a murine gene in human cells was considered a valid model. The pL^{d}4 plasmid with the native L^d promoter region has been shown to be expressed at high levels (equivalent to pRSV-L^d) even in cells that cannot efficiently transcribe their own endogenous class I genes (12). Eleven G418-resistant clones were isolated for Southern blot analyses. As shown in Fig. 1*A*, *Eco*RI/*Hin*dIII cuts on either side of the 12-kb L^{d}4 gene insert, demonstrated by the arrow as a major band migrating slightly ahead of the 23-kb band of the λ *Hin*dIII marker fragment (indicated by the upper bar). Additional smaller bands in B31 and H11 were not present on all Southern blots and are believed to represent shearing of genomic DNA during manipulation and/or recognition of additional sites (star activity) by *Eco*RI. Thus, four clones contain only

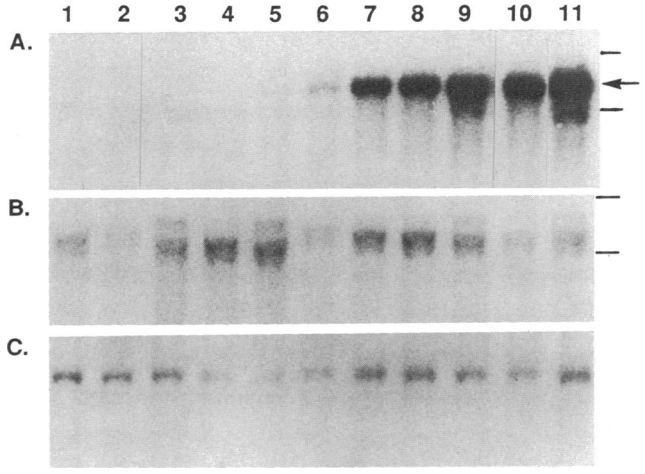


FIG. 1. Southern blot analysis of DNA from COLO 320-derived clones after transfection with pSVneo with or without pL^{d}4 containing the entire *H-2L^d* gene including the native promoter. Southern blots were incubated with cDNA probes. (*A*) The 2-kb *Xba* I fragment of L^d, corresponding to the L^d-specific outermost N-terminal regions, α_1 and α_2. (*B*) The 1.5-kb *Bam*HI fragment of human c-myc cDNA. (*C*) The 600-kb *Pst* I fragment of the murine β-actin cDNA. Lanes contain ≈15 μg of genomic DNA from the clones indicated: 1, I11; 2, F11; 3, F21; 4, B21; 5, B32; 6, K11; 7, J11; 8, J12; 9, B31; 10, B11; 11, H11. Arrow points to the major L^d (12 kb) band. Bars indicate the position of λ *Hin*dIII marker fragments: 23,000 bases (upper), and 9600 bases (lower).

pRSV-neo (I11, F11, F21, and B21), two clones have barely detectable L^d (B32 and K11), and five have integrated high copy numbers of L^d gene. Relative amounts of the c-*myc* oncogene (Fig. 1*B*) were also quantitated [since c-*myc* is known to be amplified in the parental COLO 320 HSR cell line (28)] and normalized to human placental genomic DNA on a longer exposure (unpublished data), which is taken as the single c-*myc* copy number. The presence of multiple c-*myc* bands (28) is believed to correspond to (*i*) the largest as the 12-kb *Eco*RI gene fragment; (*ii*) the second major band as the 10-kb *Eco*RI/*Hin*dIII fragment; and (*iii*) additional minor bands due to shearing and/or star activity by *Eco*RI. Another identical blot was probed with β-actin (Fig. 1*C*) to normalize all of the previous data precisely for the amount of genomic DNA actually loaded per lane.

To determine whether the transfected genes were being transcribed, Northern blot analyses were performed (Fig. 2). Only those clones containing transfected L^d expressed an RNA species (Fig. 2*A*) migrating at the same position as L^d in normal mouse tissues bearing the *H-2L*^{d} gene (lane 7). The relative amounts of L^d mRNA, normalized for actin, did not correlate significantly with the number of L^d gene copies present, consistent with earlier observations that the chromosomal site of integration of transfected genes can have a significant bearing on the degree of resulting expression (34). Both the transfected and endogenous class I genes in COLO 320 were up-regulated by α and γ interferons (data not shown), indicating that the L^d promoter is probably retained in its native configuration 5′ to the integrated L^d gene. All transfected clones (as well as the parental COLO 320) expressed c-*myc* mRNA at high levels (Fig. 2*B*). Expression of pRSV-neo by all transfected clones (Fig. 2*C*) is not correlated with the degree of L^d gene expression, indicating independent transcriptional control. Northern blots were scanned by densitometry to determine the relative quantities of L^d, c-*myc*, *neo*, and actin in each lane. These results are summarized in Table 1.

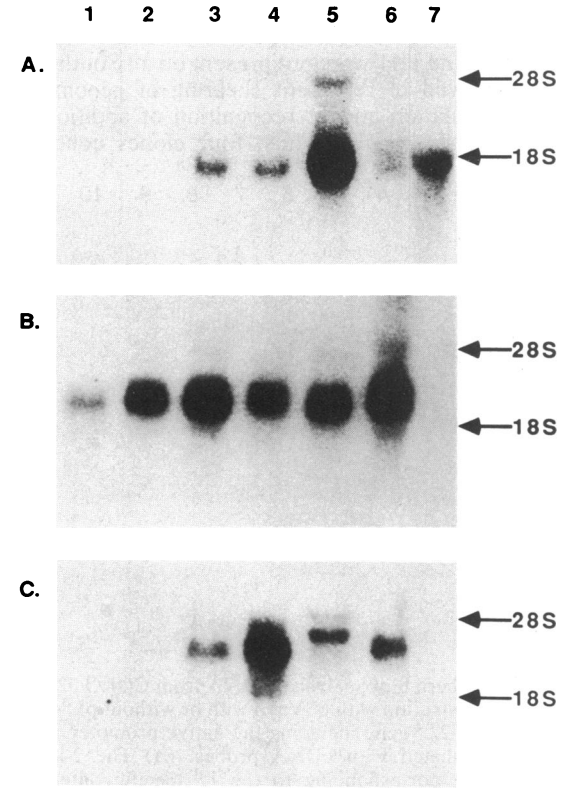


FIG. 2. Northern blot analyses of total cellular RNA incubated with cDNA probes. (*A*) L^d. (*B*) c-myc. (*C*) pSVneo. Lanes contain 15 μg of total RNA from COLO 320 (HSR) parental cell line (lane 1), B21 (lane 2), J12 (lane 3), B31 (lane 4), H11 (lane 5), B11 (lane 6), mammary tissue from the C.B-20 mouse (H-2^d), which carries the *H-2L*^{d} gene (lane 7).

To clarify whether the different clones were capable of expressing the foreign L^d promoter more or less to the same extent, a transient transfection assay was carried out with the pL^d-CAT construct. All clones expressed pL^d-CAT to a similar extent (4–11% of baseline CAT activity).

Surface expression of the L^d heavy chain was detectable by immunofluorescent light microscopy only in the H11 clone and by flow cytometry in J11, J12, and H11 (unpublished data). However, with the more sensitive technique of radioimmunoassay, L^d antigen could be detected in all clones carrying the L^d gene (Table 1), with the highest levels in J11, J12, and H11, which formed the highest number of colonies in soft agar.

Endogenous human class I antigen was not detectable in any of the clones by immunofluorescent light microscopy. However, by flow cytometry and radioimmunoassay, similar low levels of endogenous class I antigen expression were detected in all of the clones.

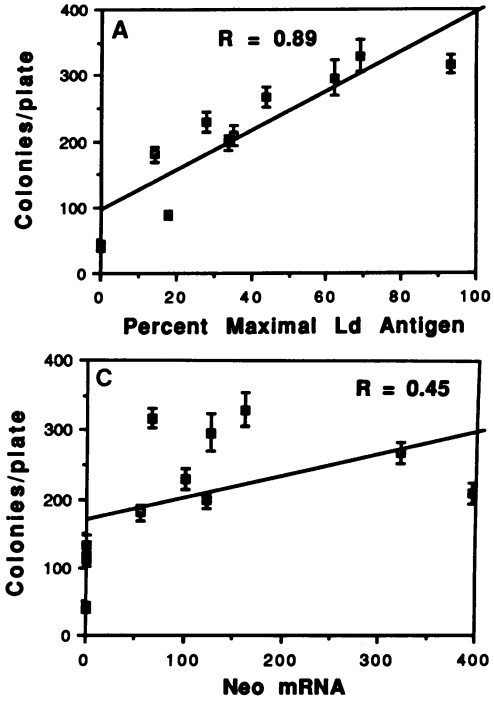
Morphological analyses of the clones revealed no differences by either light microscopy or electron microscopy. All had occasional desmosomes, rare neurosecretory granules, and no evidence of lumen formation. All clones stained equally well by the ABC immunoperoxidase technique for neuron-specific enolase and corticotropin (ACTH) (rabbit antibodies from Dako) as well as by using our own rabbit antibodies to the prohormone form of gastrin-releasing peptide (35).

Anchorage-Independent Growth of L^d Transfectants in Soft Agar. We assayed the clonogenic potential of all 11 clones in soft agar at 3 wk (32). As shown in Table 1, clones lacking the L^d gene yielded from 41 to 180 colonies per plate, with the highest number occurring in B21. Two clones with low levels of L^d mRNA and antigen (B32 and K11) did not form significantly more colonies than B21, the highest L^d-negative clone. However, the four clones with the highest copy numbers of L^d gene together with moderate to high levels of L^d mRNA and antigen (J11, J12, B31, and H11) demonstrated an increased number of colonies per plate (267–329; all with $P < 0.001$) compared to the highest colony-forming L^d-

Table 1. Summary of COLO 320-derived transfected clones

Clone	Northern blots			RIA level, % increase over background (L^d antigen)	Soft agar colonies per plate
	L^d	c-myc	Neo		
HSR	0	644	0	0 ± 0	88 ± 7 (5)
I11	ND	ND	ND	−9 ± 9	41 ± 8 (11)
F11	ND	ND	ND	ND	133 ± 14 (11)
F21	ND	ND	ND	18 ± 9	112 ± 10 (11)
B21	0	371	54	14 ± 5	180 ± 11 (18)
B32	0	1085	122	34 ± 11*	198 ± 12 (11)
K11	64	1013	397	35 ± 12*	209 ± 16 (6)
J11	154	1085	126	69 ± 10*	296 ± 27 (17)†
J12	54	412	161	62 ± 14*	329 ± 24 (18)†
B31	42	362	322	44 ± 4*	267 ± 15 (17)†
B11	21	549	100	28 ± 7*	229 ± 15 (17)†
H11	260	489	65	93 ± 5†	317 ± 15 (16)†

Relative amounts of L^d, c-myc, and neo mRNA were determined by densitometry of Northern blots normalized for actin mRNA. Cell-surface L^d antigen was determined by RIA. Results of four experiments are pooled and are expressed as percentage increase over background ± SE. Numbers in parentheses refer to the number of plates per group. Results represent pooled data from five experiments ± SE. ND, not determined.
*$P < 0.05$.
†$P < 0.001$.



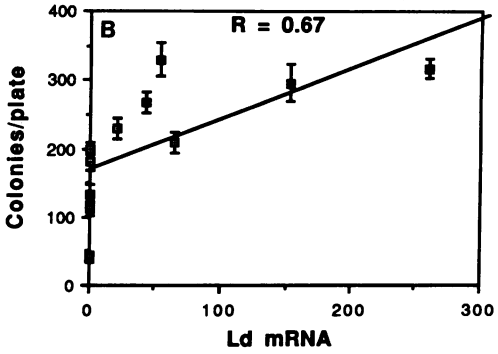


FIG. 3. Correlation of anchorage-independent growth of COLO 320-derived clones and cell-surface L^d antigen (determined by RIA) (*A*), relative L^d mRNA levels (*B*), and relative pRSVneo mRNA levels (all taken from Table 1) (*C*). Mean numbers of colonies per plate are given ± 1 SE. Correlation coefficients are indicated. (*A*, $P < 0.001$; *B*, $P < 0.05$; *C*, $P > 0.10$.)

negative control, B21. One clone, B11, with low surface L^d, had 229 colonies per plate, which was also significantly above B21, although this is in the region of overlap between L^d-positive and L^d-negative clones.

Analyses of these data indicate a strong linear correlation between clonogenic ability and the relative levels of L^d antigen ($r = 0.89$; $P < 0.001$) (Fig. 3*A*). There is a weaker linear correlation between clonogenic ability and relative amounts of L^d mRNA ($r = 0.67$; $P < 0.05$) (Fig. 3*B*). In contrast, there was no correlation between clonogenic growth and relative amounts of c-*myc* or RSV-neo mRNA (Fig. 3*C*).

Metastases in Immunodeficient Mice. For *in vivo* analyses, several clones were selected based on the number of L^d gene copies—namely, F21 (no L^d), B32 (low L^d), and J12 (high L^d). Results of *in vivo* metastatic or clonogenic ability were correlated directly with the *in vitro* data (Fig. 4). The number of colonies per lung in nude mice correlated closely with the number of colonies formed by these clones in soft agar ($r = 1.00$ in this experiment; $r = 0.98$ overall). Thus, clones with increased L^d antigen formed more metastases *in vivo*, such that the clonogenic ability in soft agar appeared to correlate with tumor formation in nude mice. The presence of malignant cells in the lung foci was confirmed histologically and the number of positive foci per 10 low-power fields correlated directly with the soft agar cloning efficiency of these clones (unpublished data).

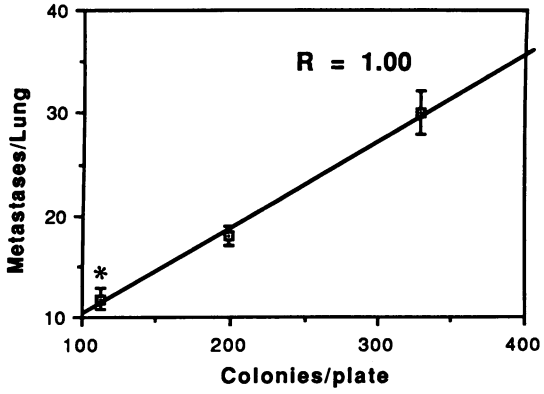


FIG. 4. Correlation of lung colony-forming ability in nude mice and the number of colonies formed in soft agar. Cell lines tested in this experiment are F21 (no L^d), B32 (low L^d), and J12 (high L^d). Thus, F21 was the only L^d-negative cell line tested. The number of lung specimens examined was 12, 12, and 10, respectively.

DISCUSSION

We here demonstrate that MHC class I gene expression can result in a positive modulation of malignant growth potential of a neuroendocrine cell line, which supports the concept that these antigens may have a nonimmunological role (21). The principal evidence supporting this contention was the strong correlation between soft agar clonogenic potential and levels of L^d antigen (Fig. 3*A*; $r = 0.89$; $P < 0.001$). There was a weaker correlation between growth and L^d mRNA levels (Fig. 3*B*; $r = 0.67$; $P < 0.05$). Thus, five distinct clones with more than two or three copies of the murine *H-2L*^{d} gene demonstrated increased clonogenic ability, which was highly significant ($P < 0.001$) as compared to the negative control with the highest clonogenic ability, B21 (Table 1 and Fig. 3). This increased clonogenic ability could be a primary occurrence, with increased L^d gene expression occurring secondarily, perhaps due to cross-binding of growth-potentiating trans-acting factors. However, transient expression of L^d-CAT was not significantly different between L^d-negative controls and high L^d clones with high L^d gene copy number. This suggests that there is no intrinsic difference in the capacity of the distinct clones to initiate transcription at the L^d promoter.

Another possible explanation is that chromosomal perturbations induced by the calcium phosphate precipitation transfection method (36) or spontaneously occurring in the parental cell line (22) may have given rise to clones with greater or lesser clonogenic potential. However, it seems reasonable to suggest that four L^d-negative controls and three low L^d clones with 41–229 colonies (mean) per plate and four moderately to strongly L^d-positive clones with 267–329 colonies (mean) per plate are unlikely to result from random chromosomal aberrations, especially in view of the high statistical significance.

Finally, there was no correlation between growth and c-*myc* gene copy number or relative abundance of c-myc mRNA. Similarly, there was no correlation between clonogenic ability and pRSV-neo gene expression (Table 1 and Fig. 3*C*), indicating that the results were not simply due to integration of foreign DNA.

Potential mechanisms involved in the observed growth enhancement by elevated MHC class I gene expression include (*i*) "presentation" of peptide growth factors to growth factor receptors such as the epidermal growth factor receptor and the insulin receptor, which have been demonstrated to be physically associated with class I antigens in the plasma membrane (37, 38); (*ii*) stabilization of growth factor receptor conformation to enhance binding of peptide growth factors to their receptors, which could explain the correlation between MHC haplotype and the K_a of the glucagon receptor (39); (*ii*) increased expression of class I antigens with their intracellular domain associated with cytoskeletal proteins (40) may favor a cell shape that optimizes cell division (41); (*iv*) increased MHC with its phosphorylated intracellular serine and threonine (42) could serve as a phosphate donor in tyrosine phosphorylation of growth factor receptors or otherwise facilitate the signal transduction mechanism triggering DNA synthesis and cell division.

MHC class I gene transfection has been recently demonstrated to inhibit rather than increase clonogenic growth of a different human colon carcinoma cell line, HCT-15 (21). These data are not viewed as contradictory, but rather as an indication of the complexity of growth control signaling pathways. Several other molecules have both positive and negative growth regulatory effects depending on the target cell type and/or the hormonal milieu, including transforming growth factor β (43), amphiregulin (44), and nerve growth factor (45). The relative lack of MHC class I antigens on normal hepatocytes (17) but their presence on regenerating liver and hepatocellular carcinoma cells (18, 19) suggests their possible involvement in the growth of other cell types besides the COLO 320 model described here.

That the MHC might have nonimmunological functions is not completely unexpected. It has been hypothesized that the MHC might act to stabilize organogenesis-directing signals, such as the H-Y antigen during testicular embryogenesis (46). MHC has also been demonstrated to have an effect on cell position (47), olfactory perception, and mating preference of inbred mice (48), and it is linked to genes controlling growth and development in rats and mice (49, 50). Although the mechanisms by which these diverse functions are mediated remains to be elucidated, the present data support the concept that MHC molecules may be involved in cellular signal transduction.

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1. Bodmer, W. F. (1987) *J. Clin. Pathol.* **40**, 948–958.
2. Flavell, R. A., Allen, H., Burkly, L. C., Sherman, D. H., Waneck, G. L. & Widera, G. (1986) *Science* **233**, 437–443.
3. Tanaka, K., Yoshioka, T., Bieberich, C. & Jay, G. (1988) *Annu. Rev. Immunol.* **6**, 359–380.
4. Momburg, F., Moller, P., Moldenhauer, G. & Hammerling, G. J. (1986) *J. Immunogenet.* **13**, 195–199.
5. Garcia-Espejo, R., Alonso, M. C., Solana, R., Pera, C. & Pena, J. (1986) *J. Immunogenet.* **13**, 211–217.
6. Van den Ingh, H. F., Ruiter, D. J., Griffioen, G., van Muijen, G. N. P. & Ferrone, S. (1988) *Br. J. Cancer* **57**, 125–130.
7. Momburg, F., Degener, T., Bacchus, E., Moldenhauer, G., Hammerling, G. J. & Moller, P. (1986) *Int. J. Cancer* **37**, 179–184.
8. Doyle, A., Martin, W. J., Funa, K., Gazdar, A., Carney, D., Martin, S. E., Linnoila, I., Cuttitta, F., Mulshine, J., Bunn, P. & Minna, J. (1985) *J. Exp. Med.* **161**, 1135–1151.
9. Funa, K., Gazdar, A. F., Minna, J. D. & Linnoila, R. I. (1986) *Lab. Invest.* **55**, 186–193.
10. Lampson, L. A., Fisher, C. A. & Whelan, J. P. (1983) *J. Immunol.* **130**, 2471–2478.
11. Jones, E. A. & Bodmer, W. F. (1980) *Tissue Antigens* **16**, 195–202.
12. Tanaka, K., Isselbacher, K. J., Khoury, G. & Jay, G. (1985) *Science* **228**, 26–30.
13. Ljunggren, H.-G. & Karre, K. (1986) *J. Immunogenet.* **13**, 141–151.
14. Hammerling, G. J., Klar, D., Katzav, S., Segal, S., Feldman, M., Wallach, R. & Hammerling, A. (1986) *J. Immunogenet.* **13**, 153–157.
15. Wallich, R., Bulbuc, N., Hammerling, G. J., Katzav, S., Segal, S. & Feldman, M. (1985) *Nature (London)* **315**, 301–305.
16. Brickell, P. M., Latchman, D. S., Murphy, D., Willison, K. & Rigby, P. W. J. (1985) *Nature (London)* **316**, 162–163.
17. Ponder, B. A. J., Wilkinson, M. M., Wood, M. & Westwood, J. H. (1983) *J. Histochem. Cytochem.* **31**, 911–919.
18. Fukusato, T., Gerber, M. A., Thung, S. N., Ferrone, S. & Schaffner, F. (1986) *Am. J. Pathol.* **123**, 264–270.
19. Paterson, A. C., Sciot, R., Kew, M. C., Callea, F., Dusheiko, G. M. & Desmet, V. J. (1988) *Br. J. Cancer* **57**, 369–373.
20. Cole, G. A., Cole, G. A., Clements, V. K., Garcia, E. P. & Ostrand-Rosenberg, S. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8613–8617.
21. Gattoni-Celli, S., Willett, C. G., Rhoads, D. B., Simon, B., Strauss, R. M., Kirsch, K. & Isselbacher, K. J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8543–8547.
22. Quinn, L. A., Moore, G. E., Morgan, R. T. & Woods, L. K. (1979) *Cancer Res.* **39**, 4914–4924.
23. Davis, L. G., Dibner, M. D. & Battey, J. B. (1986) *Basic Methods in Molecular Biology* (Elsevier, New York), pp. 286–289.
24. Evans, G. A., Margulies, D. H., Shykind, B., Seidman, J. G. & Ozato, K. (1982) *Nature (London)* **300**, 755–757.
25. Gorman, C., Padmanabhan, R. & Howard, B. H. (1983) *Science* **221**, 551–553.
26. Davis, L. G., Dibner, M. D. & Battey, J. B. (1986) *Science* **221**, 44–78.
27. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132**, 6–13.
28. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K., eds. (1987) in *Current Protocols in Molecular Biology* (Wiley, New York), pp. 4.9.1–4.9.7.
29. Alitalo, K., Schwab, M., Lin, C. C., Varmus, H. E. & Bishop, J. M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1707–1711.
30. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2**, 1044–1051.
31. Ozato, K., Hansen, T. H. & Sachs, D. H. (1985) *Mol. Cell. Biol.* **5**, 1379–1384.
32. Carney, D. N., Gazdar, A. F. & Minna, J. D. (1980) *Cancer Res.* **40**, 1820–1823.
33. Zimmerman, R. J., Gaillard, E. T. & Goldin, A. (1987) *Cancer Res.* **47**, 2305–2310.
34. Townes, T. M., Lingrel, J. B., Chen, H. Y., Brinster, R. L. & Palmiter, R. D. (1985) *EMBO J.* **4**, 1715–1721.
35. Sunday, M. E., Kaplan, L. M., Motoyama, E., Chin, W. W. & Spindel, E. R. (1988) *Lab. Invest.* **59**, 5–24.
36. Kerbel, R. S., Waghorne, C., Man, M. S., Elliott, R. & Breitman, M. L. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1263–1267.
37. Phillips, M. L., Moule, M. L., Delovitch, T. L. & Yip, C. C. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3474–3478.
38. Ediden, M. (1988) *Immunol. Today* **9**, 218–219.
39. Lafuse, W. & Ediden, M. (1980) *Biochemistry* **19**, 49–54.
40. Pober, J. S., Guild, B. C., Strominger, J. L. & Veatch, W. R. (1981) *Biochemistry* **20**, 5625–5633.
41. Folkman, J. & Moscona, A. (1978) *Nature (London)* **273**, 345–349.
42. Pober, J. S., Guild, B. C. & Strominger, J. L. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 6002–6006.
43. Sporn, M. B. & Roberts, A. B. (1988) *Nature (London)* **332**, 217–219.
44. Shoyab, M., McDonald, V. L., Bradley, J. G. & Todaro, G. J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6528–6532.
45. Levi-Montalcini, R. (1987) *Science* **237**, 1154–1162.
46. Ohno, S. (1977) *Immunol. Rev.* **33**, 59–69.
47. Birkby, C. A., Curtis, A. S. G., McGrath, M. & Ripley, B. D. (1988) *J. Cell Sci.* **89**, 167–174.
48. Yamaguchi, M., Yamazaki, K., Beauchamp, G. K., Bard, J., Thomas, L. & Boyse, E. A. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 5817–5820.
49. Artzt, K. (1984) *Cell* **39**, 565–572.
50. Gill, T. J. & Kunz, H. W. (1979) *Am. J. Pathol.* **96**, 185–206.