HL-A Antigens of Human Tumor-Derived Cell Lines and Viral Transformed Fibroblasts in Culture[1] (37341)

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Histocompatibility antigens are demonstrable using cytotoxicity techniques on normal and neoplastic cells (1–5), including cells in culture (6, 7). Malignant transformation *in vivo* frequently results in decreased H-2 reactivity of mouse cells (3–5, 8) and HL-A reactivity of human cells (9). The present study utilized a microcytotoxicity assay to compare HL-A reactivity of normal human diploid cells, human tumor cell lines and cells transformed *in vitro* by oncogenic viruses.

Materials and Methods. Skin punch biopsies (4 mm) were obtained from individuals of varying racial backgrounds in Eastern and Middle Western USA. Biopsies were minced and explanted to 30 ml tissue culture flasks (Falcon Plastics). Cells were grown in Dulbecco's modification of Eagle's medium containing 10% calf serum (Colorado Serum Co.). Fibroblasts were used within the first 5–10 passages after explanting. Diploid skin fibroblasts were transformed by the small plaque mutant of simian virus 40 (SV40) (10). The viral transformed clones from five such cultures were isolated (11). A diploid fibroblast strain (M. C.) transformed by the Kirsten strain of murine sarcoma virus (12) was also used in these studies. Established human cell lines included HeLa (human cervix carcinoma), KB (oral carcinoma), HEp-2 (laryngeal carcinoma); all were obtained from the American Type Culture Collection, Rockville, MD. Additional human tumor lines included Levine-3 (13) (breast carcinoma obtained from L. Old, Sloan Kettering, New York) and XC-114 (rhabdosarcoma line supplied by R. McAllister, University of Southern California (14)) A bronchogenic carcinoma line (9812) and a fibrosarcoma line (8387) (15) were obtained from A. Freeman, Microbiological Associates, Bethesda, MD. A sarcoma cell line (SA-4) (16) was provided by D. Morton, UCLA. HeLa, HEp-2, and KB cell lines were initially established prior to 1955; the remaining cell lines were established after 1969.

Leukocyte typing sera for histocompatibility antigens of the HL-A series (HL-A 1, 2, 3, 9, 10 of the first locus and HL-A 5, 7, 8, 12, 13 of the second locus) were obtained locally and from the Transplantation Immunology Br., NIH. Standard monospecific sera were used and two or more typing sera were tested for each HL-A specificity.

A modification of the lymphocyte microcytotoxicity assay using a trypan blue exclusion end point (17) was used. Cultures were trypsinized and inoculated at 500–1000 cells/well to microtest plates (Falcon Plastics). After 1–3 days at 37° in a CO_2 humidified environment, a monolayer formed which was ready for cytotoxicity testing. After removal of medium from the cells of the microtest plates, 2 μl of leukocyte typing sera were added to the wells in triplicate. After incubation for 45 min at 37° in presence of 10% CO_2, 5μl of noncytotoxic rabbit serum (Grand Island Biological) diluted 1:2 were added to each well, and incubation was carried out for 3 hr at room temperature. The plates were then flicked to remove serum and complement; trypan blue (0.25% in phosphate-buffered saline) was added to each well for a period of 5 min at room temperature, and the cells were washed once with phosphate-buffered saline. Cytotoxicity was scored according to the degree of rounding up of

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TABLE I. HL-A Antigens of Adult Diploid Fibroblasts.[a]

HL-A locus		%
First		
HL-A 1	73/217	33
HL-A 2	96/217	44
HL-A 3	49/217	22
HL-A 9	34/217	16
HL-A 10	15/217	7
Second		
HL-A 5	10/217	5
HL-A 7	61/217	28
HL-A 8	7/217	3
HL-A 12	6/217	3
HL-A 13	29/217	13

[a] Percentage of strains with positive reactions in cytotoxicity assay.

cells and trypan blue exclusion; the degree of cytotoxicity was reported as 0 (No killing) to 4+ (90–100% killing). Results are reported as the average of the degree of killing in three wells.

Results and Discussion. Two hundred and seventeen adult diploid fibroblast strains were tested for HL-A reactivity. Ninety-five percent (207/217) reacted with antisera of one or more specificities. The frequency of positive reactions for each specificity is found in Table I. All of the tested specificities gave positive reactions with frequencies that ranged from 3% for HL-A 8 and HL-A 12 to 44% for HL-A 2.

The effect of virus transformation on the expression of HL-A reactivity in human cells was examined. As shown in Table II with 5 different human fibroblast strains, there were no differences in HL-A reactivity between the SV40 transformants and the uninfected parent cell strains. Quantitative differences in the degree of killing were minimal. No difference was noted in the reactivity between clones originating from a given parent strain, including one instance (M. A.) where near diploid and polyploid clones were isolated from the same fibroblast culture (11). A murine sarcoma virus-transformed human cell strain (M. C.) also showed unchanged HL-A reactivities when compared with the untransformed parent cells.

The HL-A phenotypes of eight established human tumor-derived lines are recorded in Table III. HL-A reactivity could be detected

TABLE II. HL-A Reactivity of Virus-Transformed Human Fibroblasts.

	Locus									
	First					Second				
	1	2	3	9	10	5	7	8	12	13
	Strength of cytotoxic reaction (0–4+) SV40-transformed diploid strains									
Control (F.L.)	0	4+	0	0	0	0	0	0	0	0
SV40 Clone 1	0	4+	0	0	0	0	0	0	0	0
SV40 Clone 3A	0	4+	0	0	0	0	0	0	0	0
Control (A.L.)	0	4+	0	4+	0	0	0	0	0	0
SV40 Clone	0	4+	0	4+	0	0	0	0	0	0
Control (37)	4+	2+	0	0	0	0	3+	0	0	0
SV40 Clone	4+	1+	0	0	0	0	3+	0	0	0
Control (J.A.)	0	3+	0	0	0	0	0	0	0	0
SV40 Clone	0	4+	0	0	0	0	0	0	0	0
Control (M.A.)	0	0	4+	0	0	0	4+	0	0	0
SV40 Near diploid	0	0	4+	0	0	0	4+	0	0	0
SV40 Polyploid	0	0	4+	0	0	0	4+	0	0	0
	MSV-transformed diploid strain									
Control (M.C.)	0	4+	0	0	4+	0	0	0	0	0
MSV-transformed	0	4+	0	0	4+	0	0	0	0	0

TABLE III. HL-A Reactivity of Cell Lines Derived from Human Tumors.

	Locus									
	First					Second				
	1	2	3	9	10	5	7	8	12	13
	Strength of cytotoxic reaction (0–4+)									
HeLa	0	0	0	0	0	0	0	0	0	0
KB	0	0	0	0	0	0	0	0	0	0
HEp-2	0	0	0	0	0	0	0	0	0	0
SA-4	0	0	0	0	0	0	0	0	0	0
8387	4+	4+	0	0	0	0	2+	1+	0	0
9812	0	0	2+	0	0	0	2+	1+	0	0
XC-114	2+	0	0	0	0	0	0	0	0	0
Levine-3	0	0	2+	0	0	0	0	0	0	0

in four cultured human tumor cell lines (Levine-3, XC-114, 9812, 8387). In four tumor-derived lines (HeLa, KB, HEp-2, SA-4), however, no HL-A reactivity could be detected. Cytotoxic reactions were generally weaker than those of adult fibroblast or *in vitro* transformed strains. Neuraminidase treatment of one of the nonreactive tumor-derived cell lines (HeLa) resulted in no change in HL-A reactivity.

In all fibroblast strains tested, HL-A antigenic reactivity was unchanged following *in vitro* transformation with a DNA virus, SV40, or an RNA virus, murine sarcoma virus (Kirsten). By contrast, our results with tumor-derived cell lines extend previous observations that cells obtained following transformation *in vivo* frequently have decreased HL-A reactivity when tested directly (9) or after passage in culture (7). Decreased frequency and strength of HL-A reactivity of tumor-derived cell lines is possibly due to cross-contamination of cell lines (18), immunoselection *in vivo* (3, 4), and/or antigenic loss after prolonged passage in tissue culture, as was reported to occur in human diploid fibroblasts (19). Three of four cell lines in which we demonstrated no HL-A reactivity have been in continuous culture more than 18 yr.

Summary. Histocompatibility (HL-A) antigens were demonstrated on 95% of 217 cultured adult human fibroblast strains using a microcytoxicity method. Four of eight lines established from human tumors showed no HL-A reactivity. By contrast, no loss of HL-A reactivity was found in fibroblast strains transformed *in vitro* by simian virus 40 (SV40) or murine sarcoma virus (Kirsten strain).

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