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Presence of Herpes Simplex Virus-Related Antigens in Transformed L Cells

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Antiserum prepared against herpes simplex virus type 1 (HSV-1)-infected L cells, i.e., lytic antiserum, was shown by an indirect immunofluorescence test to stain 90 to 95% of HSV-transformed L or HeLa cells. Immunofluorescence in these cells was always most intense in the perinuclear cytoplasmic region. Similar results were obtained with antiserum prepared against HSV-transformed L cells. These data indicate that HSV-transformed cells (both L and HeLa) express HSV-related antigens. Antiserum prepared against HSV-1-transformed L cells, i.e., transformed-cell antiserum, was found to agglutinate purified HSV type 1 virions but failed to neutralize infectivity. This suggests that HSV-1 structural antigens are expressed in HSV-1-transformed L cells. Immunodiffusion studies showed that at least two HSV-related antigens could be demonstrated with antigens from HSV-1-transformed L cells and transformed-cell antiserum. These two antigens were shown to be present in all clonal lines of HSV-1-transformed cells examined, six L cell lines and one HeLa cell line. Therefore, we conclude that transformation of cells by HSV-1, which is known to be associated with acquisition of viral thymidine kinase, must also be associated with the presence of these two antigens. We performed experiments showing that there are species of HSV-related antibody in HSV-transformed cell antiserum that could not be absorbed out with antigens from HSV-infected L cells. Antibodies present in lytic antiserum were completely removed by antigen preparations from cells lytically infected with HSV-1. Also, lytic antiserum failed to block HSV-related staining of transformed L cells in a direct immunofluorescence test. These results are compatible with one of two notions: either (i) certain genes are expressed during transformation that are not expressed during lytic infection, or (ii) these genes are expressed to a much more reduced extent during lytic infection than in transformed cells.

Mammalian cells grown in tissue culture can be transformed by herpes simplex virus (HSV). For example, Munyon et al. (16) have shown that L cells lacking in thymidine kinase (TK) (Ltk^- cells) can be stably transformed to TK^+ phenotype by infecting these cells with UV-irradiated HSV. Davidson et al. (5) confirmed these results and studied the reversion of the TK^+ cells to a TK^- phenotype. Duff and Rapp (7, 8) reported that hamster embryo fibroblasts could acquire a malignant potential due to infection by HSV type 1 or type 2 when irradiated with UV light. A small percentage of these transformed cells (approximately 5%) showed HSV-specific antigens when stained by an indirect immunofluorescence test. Garfinkle and McAuslan (11) demonstrated that TK^- XC cells can be converted to TK^+ phenotype by infecting them with viable (nonirradiated) HSV. Transformed XC cells were also shown to express HSV antigens.

We now report experiments that were carried out to determine whether L and HeLa cells that acquire the ability to make TK due to infection with UV-irradiated HSV (6, 16) also express other HSV-related antigens. Our results indicate that at least two HSV-related antigens were expressed in all HSV-transformed cell lines (both L and HeLa) examined. In addition, we show that transformed cells also expressed an HSV structural antigen.

The term "HSV-related antigens" is used in this text to indicate those antigens that are expressed either in cells lytically infected with HSV or in cells that have been transformed by UV-irradiated HSV and are not detectable in cells not infected by HSV.

MATERIALS AND METHODS

Virus. HSV-1 (strain KOS) was obtained from E. Kraiselburd. Virus stocks were prepared as described previously (16) except that virus was grown in mono-

layers of BHK cells and plaque assayed in African green monkey kidney (CV_1) cells.

Cell lines and media. Eagle medium (9) containing nonessential amino acids and glycine and supplemented with 5% calf serum (EM5C) was used as the basic cell culture medium. Human embryonic lung (HEL) cells, BHK, HEp2 and CV_1 cells were grown in EM5C medium with no additional supplements. Ltk^- and HeLa cells both lacking in TK activity were grown in EM5C supplemented with 20 μg of 5-bromodeoxyuridine per ml. All transformed cell lines (both L and HeLa cells) were grown in EM5C supplemented with methotrexate (6×10^{-7} M), thymidine (1.6×10^{-5} M), adenosine (5.0×10^{-5} M), and guanosine (10^{-5} M).

Cell lines were subcultured by trypsinization every 72 to 96 h. Cell cultures were grown at 37 C in a humidified atmosphere containing 10% CO_2 and 90% air. Ltk^- cells and clone 139 cells were tested and found negative for mycoplasma contamination by Leonard Hayflick.

Antigen preparation for immunization of rabbits. (i) Transformed cell antigens. Five-day-old monolayers of cells were washed three times with phosphate-buffered saline, and cells were scraped and pelleted at $1,000 \times g$. The cell pellet, resuspended in three times its volume in phosphate-buffered saline, was sonicated at full power for 10 s in a Bronson sonifier (model 875, Danburg, Conn.). The sonic fluid was then thoroughly mixed with Freund adjuvant and used for immunization.

(ii) Lytic-cell antigens. HEL monolayers were infected with an input of 10 PFU/cell and the infection was allowed to proceed for 18 h at 37 C. The cells were collected by low-speed centrifugation and the pellets were treated as described above.

Antiserum preparation. New Zealand white rabbits weighing about 6 lb (about 2.72 kg) were used for immunization. For the first injection, equal volumes of an antigen preparation and Freund complete adjuvant were used. For subsequent injections, Freund incomplete adjuvant was used. A properly emulsified antigen preparation was injected subcutaneously once a week for 5 weeks. At the end of this time, the animals were rested for 2 weeks and then given another booster injection of antigens without adjuvant. Rabbits were bled 1 week after the last injection. Three rabbits were used for each class of antigens, and for each injection sonicate from 8×10^7 cells (25 mg of protein) was used. All animals were bled before immunization.

Antiserum was prepared against HSV-1-transformed L cells (clones 139 and 122), Ltk^- cells, and HEL cells lytically infected for 18 h with HSV-1. The antiserum prepared against transformed cells will be referred to as "transformed-cell antiserum" and that prepared against lytically infected cells as "lytic antiserum."

Absorption of immune serum. Transformed-cell antiserum and lytic antiserum were absorbed with antigens from Ltk^- and HEL cells, respectively. Both types of antisera were also absorbed with calf serum antigens. One milliliter of the wet cell pellet, disrupted by sonication, was mixed with an equal volume of antiserum and 0.5 ml of calf serum. The mixture was stirred for 4 h at room temperature and overnight at 4 C. The mixture was then centrifuged at $10,000 \times g$ for 20 min, and the supernatant serum was saved. This procedure was continued until transformed-cell antiserum and lytic antiserum failed to give any reaction in immunodiffusion and immunofluorescent tests against Ltk^- and HEL cells, respectively. For immunodiffusion studies, the absorbed serum was concentrated to its original volume.

Antigen preparation for agar-gel diffusion tests. The details of antigen preparation for agar-gel diffusion tests are essentially the same as described for rabbit immunization except that the cells were sonicated for 45 s at 4 C. The sonicate was centrifuged for 10 min at $10,000 \times g$ and the supernatant (5.5 mg of protein/ml) was used as an antigen preparation.

Immunofluorescent staining. (i) Indirect staining. Cells were grown as monolayers on glass slides in Lux (Lux Scientific Corp., Thousand Oaks, Calif.) four-well tissue culture trays. Cells were fixed in acetone and stained according to the procedure of Weller and Coons (22). For immunofluorescent staining of lytically infected cells, monolayers were infected with HSV-1 for 4 h before use. The fluorescein conjugate consisted of goat anti-rabbit gamma globulin (Cappel Laboratories, Inc., Downingview, Pa.) absorbed three times with mouse liver powder.

(ii) Direct staining. The gamma globulin fraction from clone 139 antiserum was obtained by percolating the serum through a DEAE-Sephadex A-50 column according to the procedure of Baumstock et al. (3). The gamma globulin fractions were pooled and brought back to their original sample volume by pressure dialysis. It was then conjugated with fluorescein isothiocyanate (FITC) according to the procedure of McKinney et al. (14). Unbound FITC was removed from the conjugate first by extensive dialysis against phosphate-buffered saline and finally by passage through a Sephadex G-25 column. Before use, conjugated serum was absorbed twice with mouse liver powder (3 h, 4 C) and three times with Ltk^- cells and calf serum antigens. Slides were mounted in glycerol and examined in a Leitz Ortholux fluorescent microscope equipped with a vertical Ploem-type illuminator at oil immersion ($\times$100).

Immunodiffusion tests. The immunodiffusion tests were performed in 1% (wt/vol) Ionagar in Veronal buffer, pH 8.4, containing 0.015 M sodium azide (17). Antiserum was placed in the central well (6 mm in diameter) separated by gel from a hexagonal pattern of six peripheral wells, each 4 mm in diameter. The various antigen preparations were added to the peripheral wells. The precipitation reaction was allowed to develop for several days in a moist chamber at 37 C.

Test for neutralizing antibody. Two dilutions of HSV-1 containing approximately 1,500 and 150 PFU, respectively, in 0.3 ml were each mixed with an equal volume of a 1:2 dilution of the antiserum. This mixture was incubated at 37 C for 30 min and then cooled in ice. Aliquots (0.1 ml) were assayed for plaque-forming activity on CV_1 cells; the inoculum was removed after 1 h and the dishes were overlaid

with EM5C containing 2% methylcellulose (4,000 centipoise).

Virus purification. Infected CV_1 cells were harvested 34 to 42 h postinfection, and virus was purified on a Dextran T-70 gradient according to the procedure of Spear and Roizman (19). A purified virus preparation, when examined by electron microscopy, was seen to be essentially free from any cellular contaminants.

Virus-antibody immune complex. Transformed-cell antiserum was heated to 60 C for 30 min to inactivate the complement activity. The heated serum was then centrifuged at 15,000 × *g* for 30 min. For immune complex formation, 0.2 ml of purified HSV-1 (absorbance at 260 nm = 3.0) was mixed with 0.2 ml of physiological saline and 0.4 ml of antiserum. The mixture was held for 1 h at 37 C and overnight at 4 C as described by Almeida and Waterson (1).

Selection of TK⁻ revertants from HSV-1-transformed L cells. HSV-1-transformed L cells (clone 139) containing HSV-specific TK activity were grown for 5 weeks in EM5C medium containing thymidine (1.6×10^{-6} M), adenosine (5.0×10^{-6} M), and guanosine (10^{-6} M). At the end of this time, 2×10^6 cells were seeded in plastic petri dishes (60-mm diameter) in EM5C medium containing adenosine and guanosine. After 1 h, when cells had attached, the medium was replaced with EM5C containing adenosine, guanosine, and 4 μCi of [^{3}H]thymidine per ml (20 Ci/mmol). Dishes were incubated at 37 C until colonies appeared. A colony (R-139) was picked and cultured in EM5C containing 20 μg of 5-bromodeoxyuridine per ml. After 10 passages in this medium, cells were checked for TK activity and their ability to incorporate [^{3}H]-thymidine into an acid-insoluble product. These tests confirmed that R-139 was TK negative.

Protein determination. Protein was estimated by the method of Lowry et al. (13), with bovine serum albumin as calibrating standard.

RESULTS

Detection of HSV-related antigens in HSV-transformed cells by immunofluorescence. A lytic antiserum, after absorption with Ltk⁻ cells and calf serum antigens, was exposed to HSV-1-transformed L cells; 90 to 95% showed bright fluorescence. The staining was particularly intense in the perinuclear cytoplasmic region and less so throughout the remainder of the cytoplasm (Fig. 1a). Transformed-cell antiserum, exhaustively absorbed with LtK⁻ cells and calf serum antigens, also stained HSV-transformed L cells. The location of staining was similar to that observed with lytic antiserum. At times, the staining was localized on one side of the nucleus (Fig. 1b). No nuclear staining was observed with transformed cells and lytic or transformed-cell antiserum. Controls consisted of Ltk⁻ cells stained with either lytic or transformed-cell antiserum and transformed cells stained with preimmune serum. These controls were negative.

Similar experiments were carried out with HSV-1-transformed HeLa cells. Lytic antiserum stained 90 to 95% of the transformed HeLa cells. Staining was localized in the cytoplasmic region as in the case of transformed L cells with the exception that part of the fluorescence was in the form of granules in the cytoplasm surrounding the nucleus (Fig. 1c). When transformed HeLa cells were stained with transformed-L-cell antiserum, the entire perinuclear region fluoresced uniformly and stain was not localized in any particular region as in case of transformed L cells (Fig. 1d). Controls, identical to those described above, were uniformly negative.

Various cell lines (Ltk⁻, HeLa, and HEp2) lytically infected for 4 h with HSV-1 gave an intense cytoplasmic fluorescence but no nuclear fluorescence when stained with transformed-cell antiserum (Fig. 2a-c). Transformed-cell antiserum used on HeLa and HEp2 cells was absorbed with these cell lines, respectively, before use. Nuclear fluorescence was seen only when lytically infected cells were stained with lytic antiserum (Fig. 2d).

HSV-related antigens detected in a TK⁻ revertant of HSV-transformed L cells. A revertant cell line lacking in TK activity was selected from clone 139 of HSV-1-transformed L cells. The details of the selection procedure are described in Materials and Methods. This cell line (R-139) was examined for the presence of HSV-related antigens by using lytic antiserum in the indirect immunofluorescence test. The majority of cells (85 to 90%) showed perinuclear cytoplasmic fluorescence (Fig. 3). Similar results were obtained when transformed-cell antiserum was used. We compared the intensity of staining of R-139 and clone 139. R-139 cells were found to be less intensely stained than clone 139 cells. These results suggest that revertant cells that have lost their TK activity still continue to express other HSV-related antigens.

Immunodiffusion studies of HSV-related antigens in HSV-transformed cells. Two distinct lines of immunoprecipitation were seen when transformed-cell antigens prepared from clone 139 of HSV-1-transformed L cells were reacted with antiserum prepared against this same cell line (Fig. 4). The lines were visible within 24 to 48 h. The same antiserum failed to react with antigens prepared from Ltk⁻ cells or with calf serum, indicating that antibodies specific for L-cell proteins and calf serum proteins had been removed by the absorption procedure. Preimmune serum, in each test, failed to give any reaction against the test antigens.

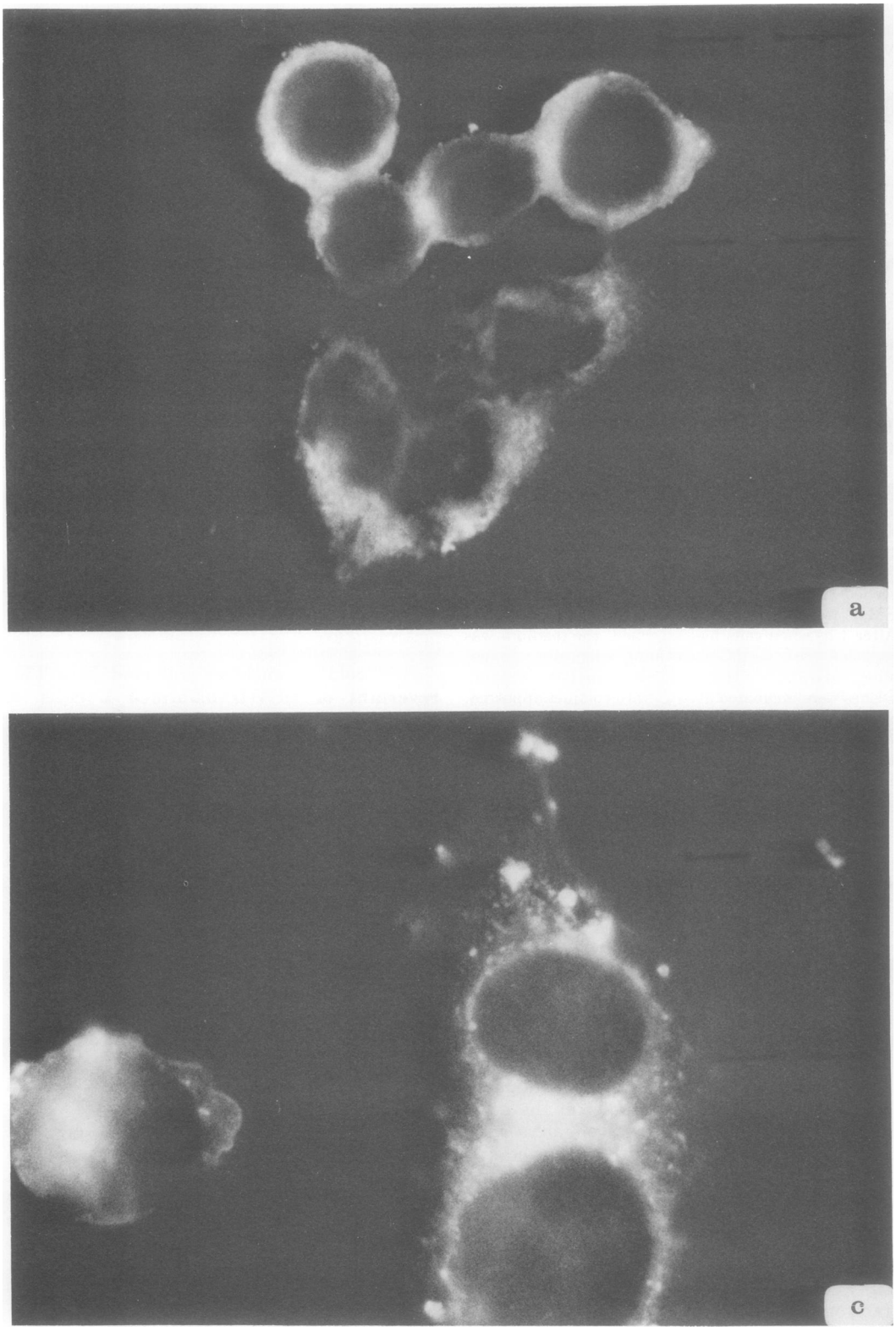


FIG. 1. *Indirect immunofluorescence test: (a) and (c) are HSV-1-transformed L and HeLa cells, respectively, stained with lytic antiserum. (b) and (d) are HSV-1-transformed L and HeLa cells, respectively, stained with transformed-cell antiserum. In each case fluorescence is localized in the perinuclear cytoplasmic region and no nuclear fluorescence is seen.*

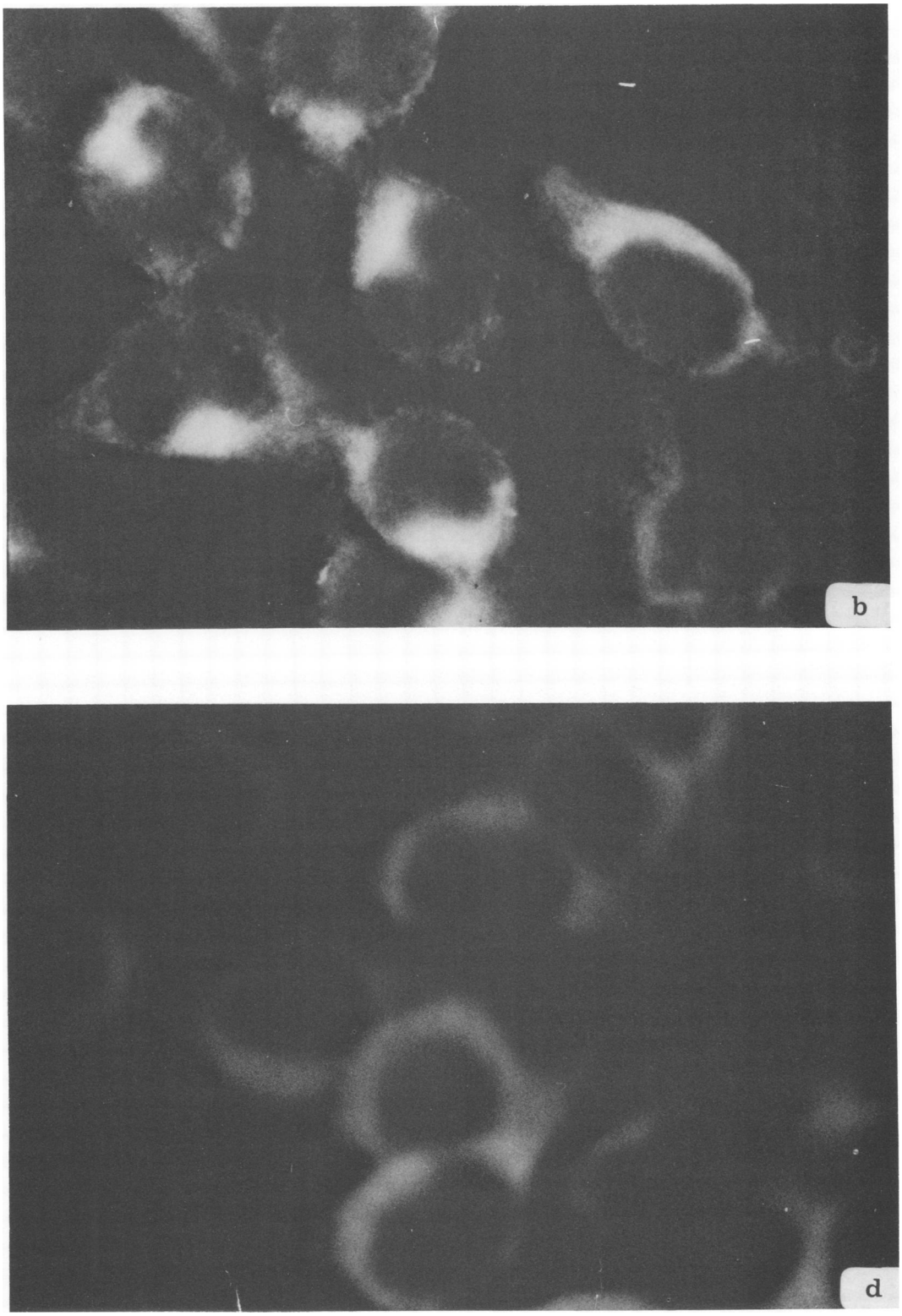
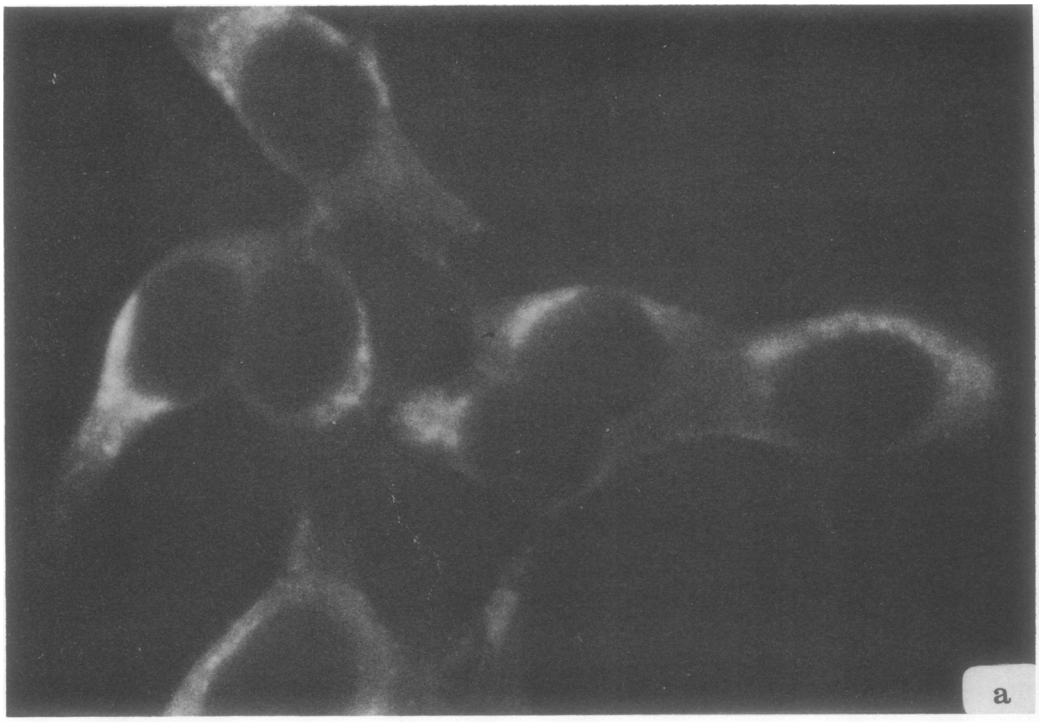


FIG. 1. *b and d.*



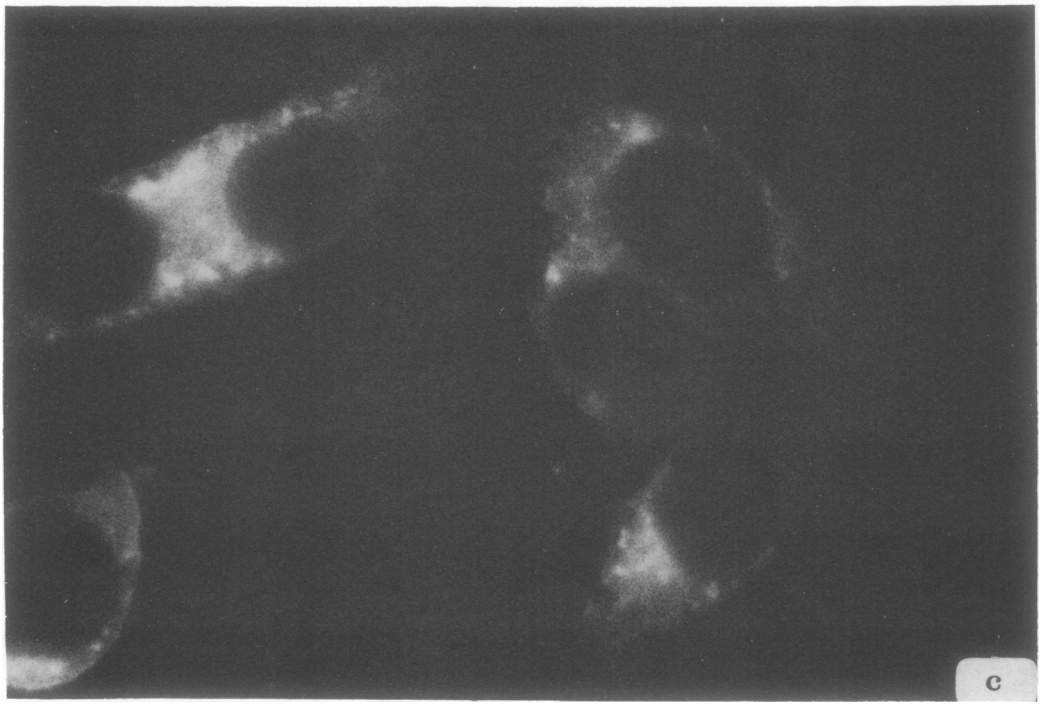


FIG. 2. *Indirect immunofluorescence test.* (a), (b), *and* (c) *are* Ltk^-, *HeLa, and HEp2 cells lytically infected for 3 h with HSV-1 before staining with transformed-cell antiserum. No nuclear fluorescence is seen in any of the cell lines.* (d) *is* Lkt^- *cells lytically infected for 3 h with HSV-1 before staining with lytic antiserum. Both nuclear as well as perinuclear cytoplasmic fluorescence is seen.*

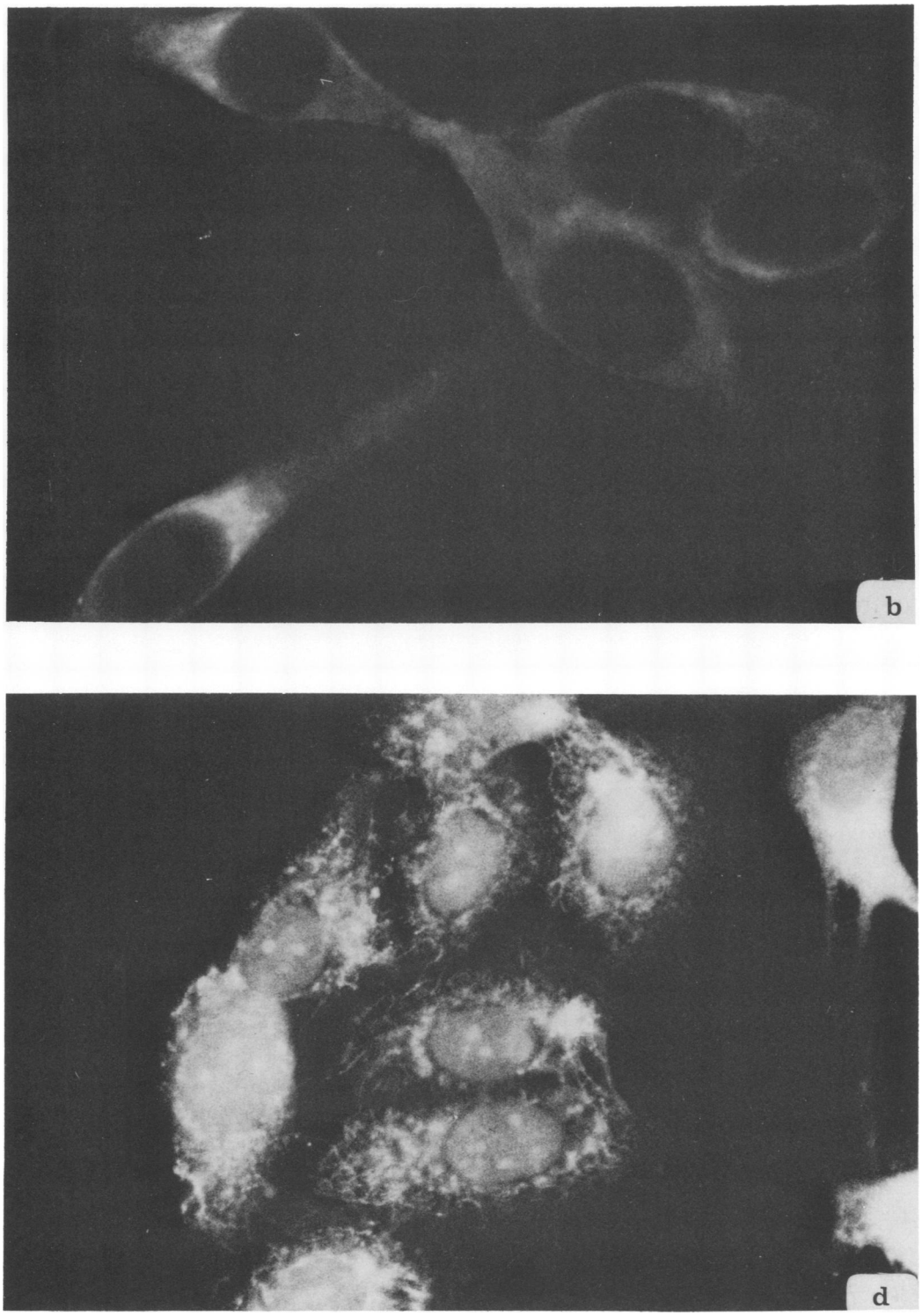


FIG. 2. *b and d.*

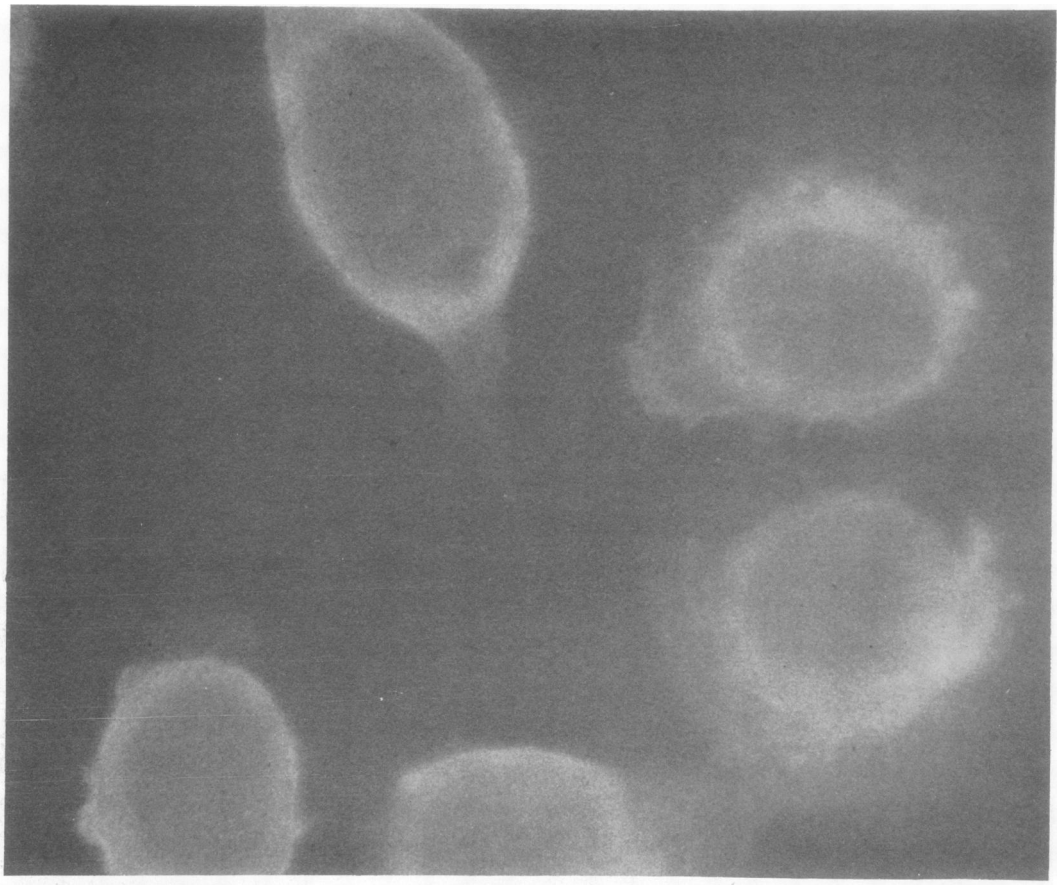


FIG. 3. *Indirect immunofluorescence test on a revertant cell line (R-139) obtained from HSV-1-transformed L cells using lytic antiserum. Note the reduced but specific perinuclear cytoplasmic fluorescence of these revertant cells.*

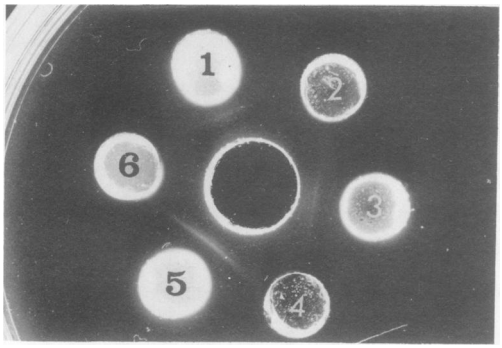


FIG. 4. *Agar-gel diffusion analysis of antigens from HSV-1-transformed L cells (clone 139). Central well: Transformed-cell antiserum prepared against clone 139 cells and adsorbed with Ltk⁻ cells and calf serum antigens. Peripheral wells: 1, 3, and 5 contain various preparations of clone 139 antigens; 2 and 4 contain Ltk⁻ cell antigens; and 6 contains calf serum antigens. Protein concentration for each antigen preparation was approximately 5.5 mg/ml.*

If the immunodiffusion plates were retained for 7 to 10 days, a third line was observed close to the antigen well in two out of five experiments. These three immunoprecipitin lines will be referred by their respective numbers, I, II, and III (Fig. 4). Line III was closer to the antigen well and line I was closer to the antiserum well.

Two immunoprecipitin lines were seen when lytic antigens were tested against transformed-cell antiserum, and these two lines were identical to lines I and II of transformed-cell antigens.

We could not see any reaction when various dilutions of antigens from transformed (L or HeLa) cells were checked against various dilutions of lytic antiserum. To determine whether this failure to detect a reaction was due to a deficiency in our lytic antiserum, anti-HSV rabbit antiserum was obtained from Gary Cohen. This also failed to give any precipitation reaction with antigens from HSV-transformed cells.

We examined a total of six different clones of HSV-1-transformed L cells in immunodiffusion tests. Antigens from each of these clones produced two HSV-related lines (I and II), using transformed-cell antiserum. All these clones expressed the same two HSV-related antigens since they showed lines of identity with clone 139 antigens (Fig. 5).

Antigens from HeLa cells transformed by HSV-1, when examined against transformed-L-cell antiserum, gave two immunoprecipitin lines, also corresponding to lines I and II of clone 139 antigens (Fig. 6). The antigen preparations used in various immunodiffusion tests were usually made fresh each time. Under no circumstances was any antigen preparation stored at Revco temperature (−70 C) for any longer than 48 h.

Detection of antibody to virion structural antigen(s) in transformed-cell antiserum. Transformed-cell antisera prepared against HSV-1-transformed L cells (clones 139 and 122) did not give any significant neutralization of HSV-1 infectivity in plaque reduction assays (Table 1). Lytic antiserum, on the other hand, gave complete neutralization of virus infectivity. This indicates that our transformed-cell

FIG. 5. *Agar-gel diffusion analysis of antigens from various clones of HSV-1-transformed L cells. Central well: Transformed-cell antiserum prepared against clone 139 cells and absorbed with Ltk⁻ cells and calf serum antigens. Peripheral wells: 1, antigens from clone 120; 2, clone 122; 3, clone 160; 4, calf serum; 5, clone 190; and 6, Ltk⁻ cells. Protein concentration for each antigen preparation was approximately 5.5 mg/ml.*

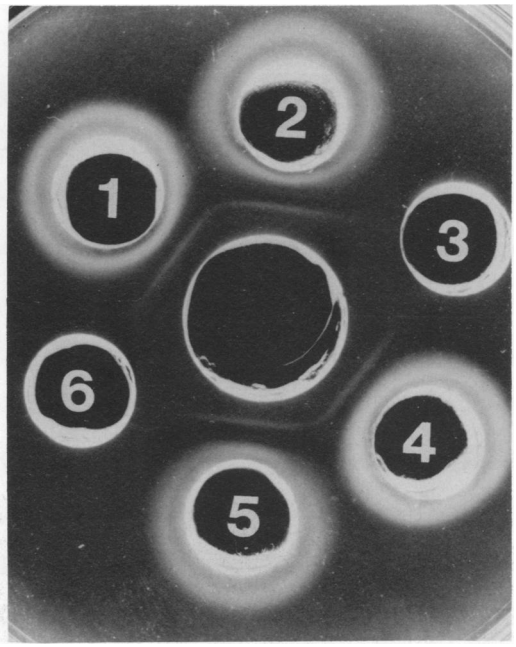


FIG. 6. *Agar-gel diffusion analysis of antigens from HSV-1-transformed L and HeLa cells. Central well: Transformed cell antiserum prepared against clone 139 cells and absorbed with Ltk⁻, HeLa cells, and calf serum antigens. Peripheral wells: 1 and 4, antigens from clone 139 cells; 2 and 5, antigens from transformed HeLa cells; 3, HeLa cell antigens; 6, Ltk⁻ cell antigens. Protein concentration for each antigen preparation was approximately 5.5 mg/ml.*

TABLE 1. *Neutralization of HSV-1 infectivity by rabbit antisera*

Antiserum[a]		Avg no. of plaques/dish[b]	
Immunogen[c]	Immune state	A[d]	B[e]
HEL cells infected with HSV-1 for 18 h	Preimmune	39	TMTC[f]
	Postimmune	0	0
HSV-1-transformed L cells, clone 139	Preimmune	39	TMTC
	Postimmune	43	TMTC
HSV-1-transformed L cells, clone 122	Preimmune	48	TMTC
	Postimmune	55	TMTC

[a] All antisera were used at a final dilution of 1:4.

[b] Virus-antiserum mixtures were incubated for 30 min at 37 C.

[c] Prepared as described in Materials and Methods.

[d] Represents the residual infectivity of virus-antiserum mixtures. A virus-calf serum control gave an average of 34 plaques/dish.

[e] Represents data from virus-antiserum mixtures that contained a 10 times greater number of PFU than column A.

[f] Too many to count.

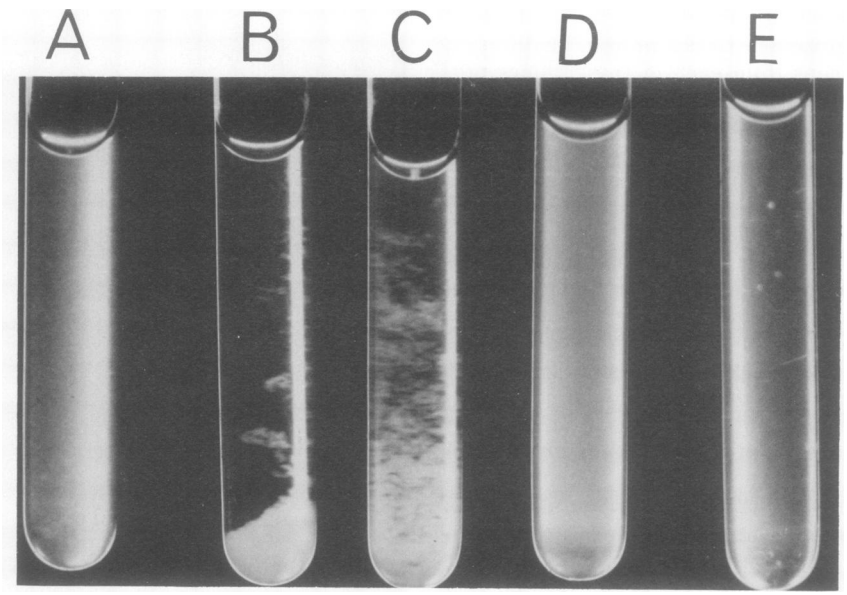


FIG. 7. *Virus-antibody agglutination test. Purified HSV-1 was mixed with (A) preimmune rabbit serum, (B) transformed-cell antiserum, (C) lytic antiserum, (D) antiserum prepared against Ltk⁻ cells, and (E) control-purified HSV-1. Virus-antibody mixture was held for 1 h at 37 C and overnight at 4 C.*

antisera did not contain any antibody that would neutralize virus infectivity. Controls consisted of calf serum and preimmune serum; neither of these sera gave any neutralization of virus infectivity.

Purified HSV-1 virus grown in CV_1 cells, when mixed with transformed-cell antiserum prepared against clone 139, resulted in virion agglutination (Fig. 7). Electron microscopic observation of this agglutinated material showed aggregates of virions in a protein matrix. As a positive control we mixed purified virions with lytic antiserum, and this also resulted in virion agglutination. Other controls consisted of purified virus mixed with either the preimmune serum or antiserum prepared against Ltk⁻ cells. Neither of these two controls agglutinated virions. These data indicate that antiserum prepared against HSV-1-transformed L cells contain an antibody(s) to an HSV-1 structural protein(s).

Do transformed cells express a protein(s) that is not expressed during lytic infection? If transformed cells contain a protein(s) that is not expressed during lytic infection, it should be possible to detect such a protein(s) by (i) exhaustively absorbing transformed cell antiserum with lytic antigens and then using this absorbed serum to stain transformed cells in the indirect immunofluorescence test, or by (ii) attempting to block fluorescence of transformed cells by using lytic antiserum as a blocking agent and subsequent staining with fluorescein-conjugated transformed-cell antiserum.

A batch of antiserum prepared against HSV-1-transformed L cells (clone 139), which had been made free of Ltk⁻ and calf serum antibodies by the absorption procedure described earlier, was divided into six aliquots. Antigens from disrupted Ltk⁻ cells, which had been lytically infected with HSV-1 at a multiplicity of 20 PFU per cell for 18 h, were used to absorb antibodies to HSV-1-related proteins from transformed-cell antiserum. Aliquot 1 was absorbed once, aliquot 2 was absorbed twice, and aliquots 3, 4, 5, and 6 were absorbed 3, 4, 5, and 6 times, respectively. At the end of each absorption step, antiserum was centrifuged (10,000 $\times$ *g* for 10 min) and the supernatant was saved for the next absorption step. Each aliquot was centrifuged (15,000 $\times$ *g* for 30 min) at the end of its absorption and the supernatant was concentrated to its original volume.

After three steps of absorption with lytic antigens, transformed-cell antiserum failed to stain Ltk⁻ cells lytically infected with HSV-1 (Fig. 8). However, the same antiserum caused decreased but significant fluorescence of transformed cells. Any further absorption steps did not change the degree nor quality of specific fluorescence.

In the same test, transformed-cell antiserum was absorbed with transformed-cell antigens.

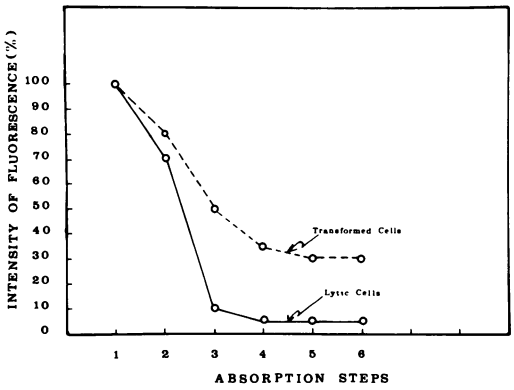


FIG. 8. *Blocking of HSV-specific fluorescence in HSV-transformed cells using transformed-cell antiserum that had been absorbed out step by step several times with lytic antigens.*

At the end of three successive absorption steps, transformed-cell antiserum failed to show any fluorescence of either the transformed cells or cells lytically infected with HSV-1. These results indicate that all the antibodies present in transformed-cell antiserum that react with transformed cells can be removed by absorption with preparations of transformed-cell antigens but not with lytic antigens.

In the direct immunofluorescence staining procedures, transformed cells were first exposed to lytic antiserum (blocking agent) and then stained with FITC-conjugated transformed-cell (clone 139) antiserum. A majority of cells (80 to 90%) showed specific fluorescence, although the intensity of stain was reduced by the blocking antiserum. Control experiments in which transformed cells were first exposed to nonconjugated transformed-cell (clone 139) antiserum as a blocking agent and then stained with FITC-conjugated transformed-cell antiserum were carried out. None of these control slides showed any specific fluorescence. We have made the same observation with FITC-conjugated antiserum made against clone 122 of HSV-1-transformed L cells. Collectively our results indicate that lytic antiserum reduces, but cannot completely block, the staining of transformed cells by FITC-conjugated transformed-cell antisera.

DISCUSSION

HSV-1-related antigens are present in Ltk$^-$ cells that have acquired the ability to make TK due to an abortive infection with UV-irradiated HSV. The location of these antigens in the perinuclear cytoplasm is similar to that reported by Duff and Rapp (7, 8) and Garfinkle and McAuslan (11) in their HSV-transformed cells. It is interesting that a nuclear DNA virus such as HSV-1 does not make a nuclear antigen in transformed cells. Preliminary attempts have failed to detect a complement-dependent nuclear antigen (18) in our transformed cells. Moreover, the strong association of the HSV-related antigenic material with the nuclear membrane is reminiscent of a lytic HSV infection.

The synthesis of virus proteins in the cytoplasm and their transport to the nucleus in herpesvirus-infected cells has been reported by Fujiwara and Kaplan (10) and Sydiskis and Roizman (20). There are two possible explanations for the exclusive cytoplasmic location of HSV antigens in transformed cells: (i) HSV antigens present in transformed cells are those that are never transported to the nucleus in lytically infected cells, and (ii) antigens are present in transformed cells that normally are transported to the nucleus in lytic infection, but this transport does not occur in transformed cells. TK activity in both lytically infected cells and in HSV-1-transformed L cells is cytoplasmic in location (S. Lin, personal communication). The virion structural protein expressed in these transformed cells would be expected to be transported to the nucleus during lytic infection if it is part of the capsid but not necessarily if it is part of the envelope.

A much higher proportion of transformed cells show HSV-related antigens when derived from procedures that we have previously reported (15, 16), which involve selecting for TK-positive cells, than in cells identified on the basis of morphological and growth character changes (7, 8). The reason for this difference is at present not understood.

Two and occasionally three HSV-related antigens can be demonstrated to be present in HSV-transformed cells by immunodiffusion. This indicates that there are at least two and possibly three HSV-related proteins expressed in HSV-transformed L cells. We believe it is significant that all of the individually isolated clones of HSV-transformed L cells which we have studied contain these two antigens. The fact that transformed HeLa cells also contain these antigens further indicates that they are virus and not cell related. In addition, the presence of these antigens indicates that certain HSV genes are expressed and that many other virus genes are not expressed in transformed cells. On the basis of these data, we postulate that these antigens may have a functional role in transformation.

Since we identify transformed cells by their ability to express TK, it is obviously possible

that one of these two antigens is TK. Work is now in progress to evaluate this possibility. Preliminary observations of the TK$^-$ revertant line of transformed L cells (clone R-139) indicate that only one antigen is detected in the immunodiffusion test. This revertant cell line was lost due to bacterial contamination. We are presently isolating other revertant cell lines to determine whether all revertants lack the same antigen in immunodiffusion tests.

If one of the antigens observed in immunodiffusion is in fact TK, we still have no idea of the identity of the second. Conceivably it may be the virion antigen expressed in transformed cells.

We have presented evidence suggesting that an antigen is present in transformed L cells which is not present in their parental Ltk$^-$ cells and is also not detectable in HSV-1 lytically infected Ltk$^-$ cells. It is generally assumed that all virus genes would be expressed during lytic virus multiplication. For example, the fact that the T antigen induced by papovaviruses in transformed cells is also made during lytic infection supports this assumption (4, 12). Our data suggests that this assumption may not be true in the case of HSV-1. Our observations may also be explained by assuming that this antigen is expressed in much greater amounts in transformed cells than in lytically infected cells.

We have reported that, under two different experimental conditions, transformed-cell antiserum agglutinated highly purified and concentrated preparations of HSV-1 but did not reduce the infectivity at low concentration of unpurified HSV-1. The failure of the antiserum to reduce HSV-1 infectivity by agglutination is probably due to the low concentration of virus particles and the high concentration of antiserum used in infectivity neutralization tests.

Several instances have been described in the literature in which antibody combines with, but does not neutralize, virus infectivity (2, 21).

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