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PRODUCTION OF GROUP- AND TYPE-SPECIFIC ANTIGENS DURING NON-PERMISSIVE INFECTION OF DOG KIDNEY CELLS WITH HERPES SIMPLEX VIRUS TYPE 2

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SUMMARY: Infection of a continuous cell line of dog kidney origin (MDCK) with herpes simplex virus type 2 (HSV-2) resulted in production of little to no new infectious virus. Serial subculture of MDCK cells inoculated with HSV-2 did not permit establishment of carrier cell cultures, as assessed by negative results of plaque assays for infectious virus and radioimmunoassay (RIA) for viral antigens. Group- and type-specific antigens were detected in lysates of non-permissive MDCK cells inoculated with HSV-2 and tested by RIA at 24 hours post-inoculation. Polypeptides produced in permissive (Vero) and non-permissive (MDCK) cell systems were labeled with [^{14}C]-amino acids and analyzed by polyacrylamide slab gel electrophoresis and autoradiography. During non-permissive infection, two polypeptides of large molecular weight, not found in uninfected MDCK cells, one of which commigrated with a major HSV-2 structural polypeptide, were synthesized and reproducibly detected.

INTRODUCTION

Non-permissive or abortive infection of various cell systems by herpes simplex virus type 1 (HSV-1) has been described. These studies showed that abortive infections occurred in dog kidney cells (1,2,3,4), chicken embryo cells (5,6) and mouse macrophages (7). In abortively-infected cells, only some sequences of the HSV-1 genome appear to be transcribed and subsequently translated, as evidenced by the accumulation of newly synthesized antigens. These polypeptide antigens could be detected by complement-fixation and fluorescent antibody techniques (6) and by polyacrylamide gel electrophoresis of cell lysates (7).

Populations of HSV-1 possess varying low percentages of virus capable of completing an infectious cycle in non-permissive cell cultures (6). From wild-type populations, variants of HSV-1 have been isolated which are capable of directing productive infection of chicken embryo cells, designated ck$^+$ (6),

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and dog kidney cells, designated dk^+ (2,3,4). In the case of abortive infection of dog kidney cells with HSV-1, dk^+ mutants could be isolated only after repeated subculture of the infected cells. Variants found in wild-type populations of HSV-1 incapable of completing the infectious cycle, designated dk^-, were isolated and found to interfere with dk^+ virus replication (4).

To our knowledge, abortive infection of cells by HSV-2 has not been described. In the present study it is shown that HSV-2 infection of Madin-Darby canine kidney (MDCK) cells is abortive and that two nascent large molecular weight viral polypeptides are reproducibly detected and it is likely that they contribute to HSV-2 group- and type-specific antigenicity detected by radioimmunoassay.

MATERIALS AND METHODS

Cell cultures used in this study included: African green monkey kidney (Vero), Madin-Darby canine kidney (MDCK) and human fetal lung (HFL). All cells were propagated in the following growth medium: basal medium Eagle's with 10% fetal calf serum, glutamine (2 mM/ml) and neomycin (20 µg/ml) (8). The 333 strain of HSV-2 was used in all experiments. Infectivity was assayed using HFL cells and a growth medium overlay method (9) employing 0.75% immune serum globulin (Armour Pharmaceutical Co., Phoenix, Arizona). Virus was purified as described by Chadha et al. (10) employing ultracentrifugation in CsCl gradients (9) rather than dextran gradients. Procedures used for solid-phase RIA have been described previously (10,11,12). Briefly, plastic coated metallic beads are coated with antigens, reacted with test serum, washed and reacted with ^{125}I-labeled antiglobulin. The viral and control cell antigens used in these experiments were prepared as previously described (10). The human sera were obtained from human beings who had been infected with HSV-1 only or HSV-2 only and have been described (14).

Confluent monolayer cultures of Vero and MDCK cells in 60 mm dishes were inoculated with 10-12 plaque-forming units (pfu) per cell. The inoculum was removed following a one hour adsorption period at 25° and the cells were then washed three times with phosphate buffered saline, pH 7.4. Fresh growth medium was added and the cells were incubated at 34° until harvested and assayed for infectivity and viral antigens. Mock-infected control cells were included and processed in the same manner as infected cells in all experiments.

Methods described by Powell and Courtney (15) were utilized for sodium docecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in slabs and autoradiographic analysis. An acrylamide concentration of 8.5% was used in the separating gel and 2.8% in the stacking gel. Cells were treated as described above except at two hours post-inoculation (p.i.), growth medium was replaced with growth medium containing 1/10 the usual concentration of amino acids and 5 µCi/ml [^{14}C]-amino acid mixture (specific activity, 45 mCi/m Atom carbon; Schwarz-Mann, Inc., Orangeburg, New York). X-ray films (Kodak, NS2T) were exposed to the dried gels for one week. The resulting x-ray film autoradiograms were scanned with a densitometer (E-C Apparatus Corp., Philadelphia, Pa.).

TABLE 1. YIELDS OF HSV-2 FROM VERO AND MDCK CELLS INCUBATED AT 34°[a]

Host Cell	Lysate Harvested (hr p.i.)	Experiment 1 (pfu/cell)	Experiment 2 (pfu/cell)
Vero	2.5	ND[b]	0.42
Vero	24	72	167
MDCK	2.5	0.04	0.14
MDCK	24	0.03	0.13

[a] Cells were inoculated with a multiplicity of 10-12 pfu/cell.

[b] Not done.

RESULTS

Yields of infectious HSV-2 per cell at 2.5 and 24 hours p.i. from Vero and MDCK cells inoculated with this virus are presented in Table 1. The results from two separate experiments revealed that Vero cells produced progeny virus, whereas little to no progeny were produced in MDCK cells. Infected MDCK cells were subcultured and assayed for infectious HSV-2. These cells were also tested for viral antigens by RIA using a serum positive for antibody against HSV-2 antigens. Infectious virus (3.8×10^4 pfu/ml) and HSV-2 antigen were found in cells inoculated with HSV-2 at passage level one (Table 2). Cells trypsinized and subcultured at passage level two contained less than 10 pfu per 2.7×10^6 cells, but were positive for viral antigens. After four passages, these cells were negative for viral antigen and contained less than 10 pfu per 2.7×10^6 cells.

The presence of HSV-2 antigens in lysates of Vero and MDCK cells at 24 hours p.i. was detected by RIA using standardized serum dilutions of 1:200. The sera were from human beings who had been infected with HSV-1 only or HSV-2 only. The results of duplicate bead RIA tests, repeated on separate days, are

TABLE 2. VIRUS YIELDS AND ANTIGENS FROM SUBCULTURED HSV-2 INOCULATED MDCK CELLS[a]

ANTIGEN	pfu/ml	RIA reactivity	
		$\frac{\text{inoculated (cpm)}^{c}}{\text{uninoculated (cpm)}}$	HSV-2 antigen[d]
HSV-2 inoculated MDCK (p1)[b]	3.8×10^4	$\frac{1154}{440}$	positive
HSV-2 inoculated MDCK (p2)	<10	$\frac{1100}{440}$	positive
HSV-2 inoculated MDCK (p4)	<10	$\frac{380}{360}$	negative

[a]Virus yields were measured 24 hrs after initial inoculation or subculture.

[b]p1, p2, p4 refer to passage levels one, two, and four, respectively.

[c]cpm represent an average from duplicate bead RIA's.

[d]HSV-2 inoculated MDCK cells were considered positive for HSV-2 antigen if inoculated cells demonstrated at least twice the reaction (cpm) as that observed in uninoculated MDCK cells tested by RIA with an HSV-2 positive serum

given in Table 3. Uninfected Vero and MDCK cells gave reactivities of 0.2-0.6 x 10^3 counts per minute (cpm) with both sera. The human serum from the individual infected with HSV-1 only bound 0.5-0.8 x 10^3 cpm with HSV-2 inoculated Vero and MDCK cells, suggesting the presence of HSV-1 and HSV-2 common or group-specific antigen in these cell lysates. The human serum from the individual infected with HSV-2 only bound 2.1-2.7 x 10^3 cpm with lysates from Vero and MDCK cells inoculated with HSV-2. The difference in reactivities between the HSV-1 only and HSV-2 only sera with Vero or MDCK cells inoculated with HSV-2 was significantly different at the 95% confidence level when analyzed by the Student's t-test. This difference suggests that HSV-2 type-specific antigens are present in lysates of both Vero and MDCK cells inoculated with HSV-2.

TABLE 3. RADIOIMMUNOASSAY OF HSV-2 INOCULATED VERO AND MDCK CELL LYSATES FOR REACTIVITY WITH ANTISERA DIRECTED AGAINST HSV-1 OR HSV-2 ONLY

Serum Used in RIA Positive for:	Antigens Tested for RIA Reactivities (cpm/antigen-coated bead x 10^3)[a]			
	Uninoculated Vero	Vero Inoculated with HSV-2	Uninoculated MDCK	MDCK Inoculated with HSV-2
HSV-1 only	0.4	0.8[b]	0.4	0.8[c]
	0.3	0.8	0.2	0.5
HSV-2 only	0.6	2.1[b]	0.6	2.7[c]
	0.5	2.3	0.4	2.3

[a]Average reactivities of two RIA tests carried out on separate days.

[b,c]Significantly different at 95% confidence level using the Student's t-test.

Densitometer tracings of autoradiograms of polypeptides labeled with [^{14}C]-amino acids and separated by SDS-PAGE from control uninoculated MDCK and Vero cells, both cell lines inoculated with HSV-2, and purified HSV-2 virions are presented in Figures 1-3. Densitometer scans of autoradiograms of polypeptides labeled in HSV-2 inoculated and uninoculated MDCK cells reveal similar profiles with the notable exception of two large molecular weight polypeptides (denoted A and B, Fig. 1) in the HSV-2 inoculated MDCK cells which are absent in uninoculated MDCK cells. Other qualitative differences between these two sources of polypeptides have not been sufficiently reproducible to warrant identification. Polypeptides A and B commigrated with two polypeptides found in crude lysates of Vero cells inoculated with HSV-2 (Fig. 2). Polypeptide A was found to have a similar mobility as a major polypeptide found in purified HSV-2 virions (Fig. 3). A small peak corresponding to polypeptide B was found in purified virions, but does not warrant identifying this as a structural polypeptide. These results presented in Fig. 1-3 were confirmed in three separate experiments.

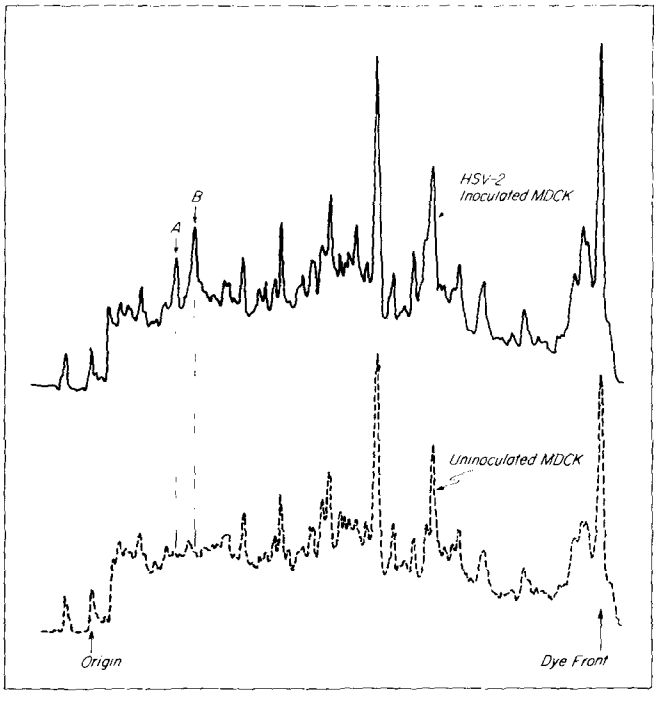

Figure 1. Densitometer tracings of autoradiograms of [^{14}C]-amino acid labeled polypeptides found uninoculated MDCK cells and MDCK cells inoculated with HSV-2 and analyzed by polyacrylamide slab gel electrophoresis. Cell lysates were subjected to SDS-PAGE, the gels were dried, and exposed to x-ray film. The resulting autoradiograms represent a composite of HSV-2-inoculated MDCK cells (solid line) and uninfected MDCK cells (dashed line). Peaks A and B are high molecular weight polypeptides produced only in MDCK cells inoculated with HSV-2.

DISCUSSION

Assay of Vero and MDCK cells inoculated with HSV-2 for the capacity to produce HSV-2 demonstrated that Vero cells produced new virus and MDCK cells were unable to support a complete lytic cycle, resulting in a non-productive or abortive cycle. The RIA data indicate that there are HSV-2 antigens produced in HSV-2 inoculated MDCK cells which react strongly with serum from a human infected with HSV-2 only and weakly with serum from a human infected with HSV-1 only. Data obtained by labeling newly synthesized viral polypeptides and analyzing these polypeptides by SDS-PAGE show that at least two new

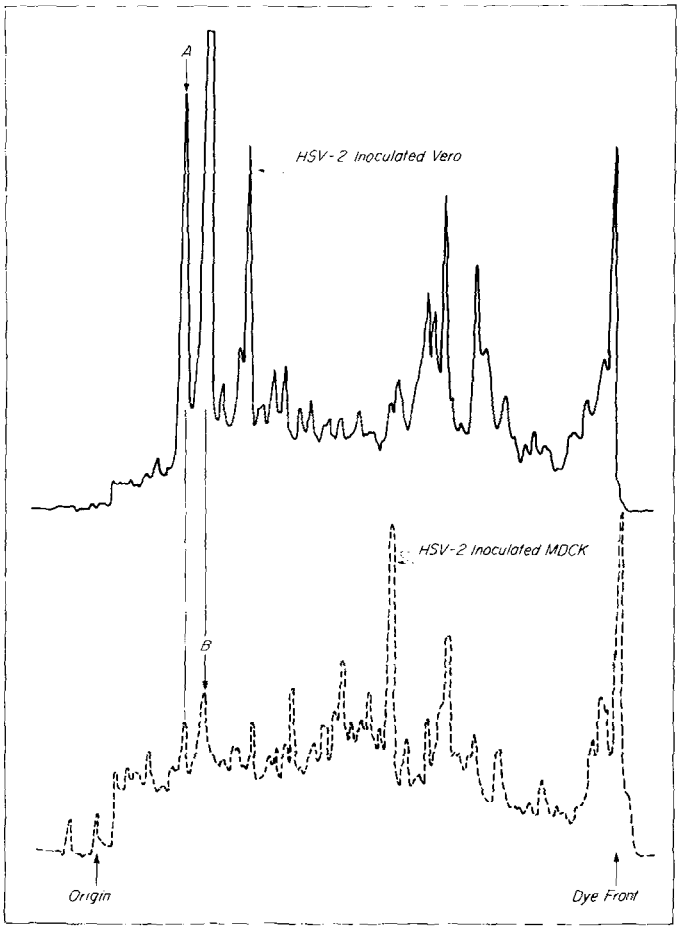

Figure 2. Densitometer tracings of autoradiograms of [^{14}C]-amino acid labeled polypeptides found in uninoculated Vero cells and Vero cells inoculated with HSV-2. See legend to Fig. 1 for details. HSV-2 inoculated Vero cells (solid line), HSV-2 inoculated MDCK cells (dashed line).

polypeptides appear in HSV-2 inoculated MDCK cells. Considering both RIA data and the appearance of newly synthesized polypeptides in HSV-2 inoculated MDCK cells, it is possible that the new polypeptides are responsible for the antigenic reactivity observed by RIA.

The purpose of this study was to find a cell system in which HSV-2 would produce new antigens and little or no new virus. Dog kidney cells were investigated because HSV-1 has been reported to undergo abortive infection in these

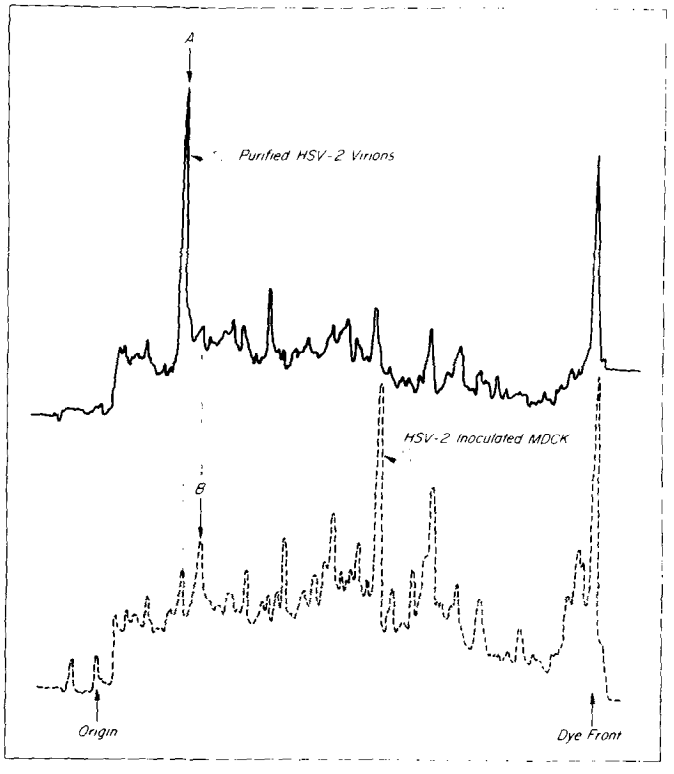

Figure 3. Densitometer tracings of autoradiograms of [^{14}C]-amino acid labeled polypeptides found in purified HSV-2 virions and MDCK cells inoculated with HSV-2. See legend in Fig. 1 for details. Purified HSV-2 (solid line), HSV-2 infected MDCK cells (dashed line).

cells. It has previously been shown that exfoliated cells from patients with cervical carcinoma contain HSV-2 antigens (16), however, infectious HSV-2 can rarely be isolated from these cells (17). One interpretation of this result is that HSV-2 may undergo abortive infection in cells of the human cervix, similar to what is found in abortively infected MDCK cells. Experiments are currently in progress to determine whether sera from women in various stages of cervical carcinoma contain antibodies which react with HSV-2 antigens produced during abortive infection of MDCK cell cultures.

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