Molecular Localization of Abortive Infection of Resident Peritoneal Macrophages by Herpes Simplex Virus Type 1

PAGE S. MORAHAN,* SAIFUDDIN MAMA, FARVARDIN ANARAKI, AND KATHRYN LEARY

Department of Microbiology and Immunology, The Medical College of Pennsylvania, 3300 Henry Avenue, Philadelphia, Pennsylvania 19129

Received 19 October 1988/Accepted 18 January 1989

Mononuclear phagocytes exhibit different patterns of intrinsic resistance to herpes simplex virus type 1 (HSV-1) that are related to the heterogeneity of macrophage populations and may reflect the particular differentiation or maturation state of the macrophages. In this study, we characterized the molecular basis for the block in HSV-1 replication in resident peritoneal macrophages from B6C3F1 mice. Infected resident peritoneal macrophages were analyzed for the presence of virus-specific mRNA by Northern (RNA) blotting and in situ hybridization and for proteins by immunofluorescence. The data were compared with those obtained in HSV-1-infected permissive Vero cells. The immediate-early genes ICP4, ICP0, ICP22, and ICP27 were transcribed in resident peritoneal macrophages, as was the early gene *tk*. Virus-specific mRNA for the major DNA-binding protein ICP8 was barely detectable, and that for another early gene, the viral DNA polymerase, was not detected. In addition, transcripts for the delayed-early gene glycoprotein D and the true late gene glycoprotein C (gC) were not detectable in resident peritoneal macrophages. In situ hybridization and immunofluorescence studies confirmed that transcripts and proteins for the immediate-early and some early HSV-1 genes were present. These data also established that 14% of the resident peritoneal macrophages were positive for RNA and polypeptide specific for the immediate-early gene ICP4 and that 7 to 11% were positive for RNA or polypeptides specific for the early genes *tk* and ICP8. The fact that only a few cells expressed viral products emphasizes the heterogeneity that exists even in this relatively homogeneous resident peritoneal macrophage population. Consistent with the Northern blot analysis, no RNA specific for the early DNA polymerase gene or the late gC gene was detected by in situ hybridization nor could the polypeptide for the gC gene be seen by immunofluorescence. Thus, while early transcriptional events were initiated in some resident peritoneal macrophages, there was a block in replication localized at the level of expression of the early to delayed-early viral genes.

The fact that mononuclear phagocytes play a pivotal role in cellular defense against viral infections through both nonspecific and specific resistance mechanisms is well established (reviewed in references 22 and 23 and by P. S. Morahan and D. M. Murasko, in D. S. Nelson, ed., *Natural Immunity*, in press). However, it is also evident that macrophages exhibit considerable heterogeneity in antiviral resistance that may be related to their differentiation and maturation stages. For example, differentiation of human macrophagelike U937 cells alters their intrinsic resistance to herpes simplex virus type 1 (HSV-1), in that the early block in HSV-1 replication in undifferentiated U937 cells is released by differentiation (37). In addition, maximal replication of visna virus in infected monocytes only occurs after these cells have differentiated into tissue macrophages (35). The cellular and molecular mechanisms involved in these differing patterns of macrophage-virus interaction are as yet poorly defined. In part, this is due to the present lack of understanding of the origins of macrophage heterogeneity (24a).

The various relationships between macrophages and HSV have been extensively documented since the seminal observation of Johnson (18) that resident peritoneal macrophages from adult mice were resistant to HSV while macrophages from suckling mice were more permissive (reviewed in reference 24). In general, the intrinsic resistance of the resident peritoneal macrophages to HSV is characterized by an abortive infection, no cytopathic effect, and low or

negligible yields of infectious virus. The exact block in HSV replication in resident peritoneal macrophages or other types of macrophages and the underlying mechanisms involved have been addressed in only a few studies (11, 20, 34). Early work established that resident peritoneal macrophages cultured in vitro for 2 days in calf and rabbit serum to enhance spreading resulted in some viral protein and DNA synthesis, declining titers of infectious HSV, and considerable cytopathic effect (33). Our recent work has shown that HSV-1 production in freshly harvested resident peritoneal macrophages is restricted at a point prior to viral DNA synthesis but after expression of some viral proteins encoded by immediate-early or early genes (20, 31).

Further definition of the block at the molecular level requires a detailed analysis of the expression of HSV-1 genes in these nonpermissive cells. In permissive cells, viral infection proceeds in a cascade manner in which the expression of the three broad HSV-1 gene classes, immediate-early, early, and late, is coordinately regulated and dependent on the expression of genes from the preceding class (16, 17). There are five known immediate-early genes, ICP4, ICP0, ICP27, ICP22, and ICP47 (1), and of these, ICP4, ICP0, and ICP27 play an important regulatory role in viral gene expression (7, 8, 13, 27, 29a, 30). The early genes encode many functions required for viral DNA replication and depend for their expression on functional immediate-early polypeptides (6, 7, 17, 40). Delayed-early genes are expressed prior to viral DNA synthesis, but maximum expression requires DNA replication, and finally, the true late genes require viral DNA replication for initiation of transcription (38). While it has

* Corresponding author.

been shown that some immediate-early genes are expressed in freshly cultured resident peritoneal macrophages and that viral DNA is not replicated (20, 29a, 31), it is not known precisely where in this cascade cessation of replication occurs.

The goal of this study was to precisely establish the transcriptional and translational events in HSV-1-infected resident peritoneal macrophages from B6C3F1 mice to definitively determine the block in HSV-1 replication at the molecular level. Immediate-early and some early viral transcripts were present in resident peritoneal macrophages. However, we were unable to detect mRNA for the viral DNA polymerase, the mRNA for the major DNA-binding protein ICP8 was barely detectable, and mRNA for the true late gene for glycoprotein C (gC) was completely absent. The results demonstrated that there is a gradual decline in expression of HSV-1 genes along the cascade of transcriptional events from the immediate-early to early to late classes of genes in resident peritoneal macrophages.

MATERIALS AND METHODS

Mice. Barrier-raised female B6C3F1 mice (Taconic Farms, Germantown, N.Y.) were received in filter crates and housed in sterile microisolator cages. Sera of mice were tested and shown to be free of seroconversion to common inapparent infections as previously described (9, 31).

Virus and cells. HSV-1 strain KOS was propagated and assayed as described previously (20). Vero cells and resident peritoneal macrophages were cultured as described previously (31, 37). After overnight incubation, cells were washed twice with phosphate-buffered saline. The number of cells plated was determined by counting nuclei after cetrimide treatment, and cells were infected with multiplicities of infection of 3 to 5 to ensure a single-cycle infection (20, 31).

Detection of viral antigens. Infected and uninfected cells grown on 12-mm-diameter cover slips were fixed in acetone for 10 min. Immunofluorescence for HSV-1 antigens was assayed indirectly (31). The antibodies included monoclonal antibody (MAb) to the HSV-1 immediate-early protein ICP4 (courtesy of L. Pereira, University of California-San Francisco), MAb 39s to the HSV-1 early protein ICP8 (courtesy of M. Zweig, National Cancer Institute), MAb to the late protein gC, and polyclonal antisera to the HSV-1 late proteins gB, gD, and gC (courtesy of G. Cohen, University of Pennsylvania). The secondary antibody for the MABs was fluoresceinated rabbit anti-mouse immunoglobulin G (Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.), and for the polyclonal sera, it was a fluoresceinated goat anti-rabbit immunoglobulin G Fab' fragment (Organon Teknika, Malvern, Pa.). Results are expressed as the percentage of positive cells from a minimum of 500 cells counted. Controls included uninfected cells and cells stained with secondary antibody only. Cells were visualized in a Leitz Aristophan microscope, and photography was performed with a Leitz Orthomate E camera with Tri X Pan 400 film (ASA 800, factor 1) with exposures ranging from 2 to 38 s.

DNA probes. Plasmids were constructed to contain a portion of the coding region for each gene and as little additional viral sequences as possible. ICP4 was a 1.9-kilobase (kb) *Bam*HI fragment from pSG28K/B, coordinates 0.79 to 0.86 (30); ICP0 was a 2.0-kb *Nco*I-to-*Nru*I fragment from pRS-1, coordinates 0.77 to 0.80 (29), modified by the addition of *Hind*III linkers; ICP22 was a 2.0-kb *Eco*RI-to-*Hind*III fragment from pSG353, coordinates 0.86 to 0.89,

which is an *Eco*RI-to-*Bam*HI subclone of *Eco*RI fragment H (15); for ICP27, the *Hinf*I site in pSG130B/S, coordinates 0.745 to 0.762 (30), was converted to a *Bgl*II site, and a 2.1-kb *Bgl*II-to-*Sst*I fragment was used. These four fragments were cloned into the appropriate sites in pGEM-2 (Promega Biotec, Madison, Wis.). *tk* was a 2.3-kb *Eco*RI fragment from pX1, coordinates 0.0299 to 0.0317 (12), inserted into the *Eco*RI site in pGEM-1 (Promega Biotec); ICP8 was a 780-base-pair *Xho*I-to-*Bam*HI fragment, coordinates 0.397 to 0.403 (32), cloned in pUC18 (kindly provided by R. M. Sandri-Goldin); DNA polymerase was pKOSBamQ containing the strain KOS *Bam*HI Q fragment, coordinates 0.413 to 0.431 (kindly provided by D. Coen). This plasmid contains approximately 90% of the *pol* gene and no known other genes. gD was an 840-base-pair *Hind*III-to-*Acc*I fragment, coordinates 0.908 to 0.914, cloned into pUC18 (kindly provided by R. M. Sandri-Goldin); and gC was a 950-base-pair *Pst*I-to-*Eco*RI fragment, coordinates 0.626 to 0.623, cloned into pGEM-1.

Northern (RNA) hybridization. RNA was isolated by the method of Chomczynski and Sacchi (5). Gel electrophoresis of 20 μ g of total cell RNA was performed in a 1% agarose-formamide gel, and Northern blot analysis was done essentially as described by Maniatis et al. (21). A ladder of poly(A)⁺ RNA molecular weight standards (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) was run in parallel. RNA was transferred to Gene Screen (Dupont, NEN Research Products, Boston, Mass.) in 10 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate). Prehybridization and hybridization were performed at 42°C for 18 to 24 h. Filters were prehybridized in 50% formamide-6 $\times$ SSPE (1 $\times$ is 0.15 M NaCl, 0.01 M NaH₂PO₄ · H₂O, 1 M EDTA [pH 7.4])-7 $\times$ Denhardt reagent (1 $\times$ is 2% polyvinylpyrrolidone, 2% Ficoll, 2% bovine serum albumin)-0.1% sodium dodecyl sulfate (SDS)-heterologous nucleic acid mixture [250 μ g of salmon sperm DNA per ml, 100 μ g of tRNA per ml, 4 μ g of poly(A) per ml, 4 μ g of poly(C) per ml]. The hybridization buffer was 50% formamide-5 $\times$ SSPE-1 $\times$ Denhardt reagent-0.5% to 1% SDS-5 to 7% dextran sulfate plus twice the concentration of the prehybridization heterologous nucleic acid mixture. [³²P]dCTP (Dupont, NEN Research Products)-labeled DNA probes were prepared by using a kit obtained from Bethesda Research Laboratories. The specific activity of the probes ranged from 2 $\times$ 10⁷ to 6 $\times$ 10⁸ cpm/ μ g. After hybridization, filters were washed twice in 2 $\times$ SSC-0.1% SDS and twice in 0.1 $\times$ SSC-0.1% SDS for 10 min each at room temperature, followed by a high-stringency wash in 0.1 $\times$ SSC-0.1% SDS for 30 to 60 min at 55 to 65°C. Autoradiography was performed for 4 to 14 days at -70°C with two Cronex intensifying screens. The blots were stripped of hybridized probe by two to four washes in 0.1 $\times$ SSC-0.1% SDS at 100°C before rehybridization to different probes.

In situ hybridization. Cover slips (22 by 22 mm) were treated with Denhardt solution and acetylated before cells were cultured. At various times postinfection (p.i.), the cells were washed with phosphate-buffered saline, fixed with freshly prepared periodate lysine paraformaldehyde, washed with phosphate-buffered saline, and dehydrated in graded ethanol solution (14). The cover slips were glued onto microscope slides, pretreated in 0.2 N HCl, and rinsed in double-distilled water (ddH₂O). They were incubated in 2 $\times$ SSC at 70°C for 30 min, rinsed in ddH₂O, and incubated for a further 15 min at 37°C in 10 mM Tris hydrochloride (pH 7.4)-2 mM CaCl₂ containing 1 μ g of proteinase K (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) per ml.

They were then rinsed in ddH₂O and dehydrated. Control slides were washed in 2× SSC for 5 min, rinsed in ddH₂O, incubated for 15 min at 37°C with 100 µg of RNase A per µl and 10 µg of RNase T₁ per ml, rinsed in ddH₂O, and dehydrated. A ³⁵S-labeled nick-translated probe was heated at 100°C for 5 min, quenched on ice, and suspended in 2× SSC–10 mM Tris hydrochloride (pH 7.4)–1 mM EDTA–1× Denhardt reagent–10% dextran sulfate–1 mg of salmon sperm DNA per ml–1 mg of tRNA per ml–100 µg of poly(A) per ml–45% formamide. Prior to hybridization, the probe mixture was heated again at 100°C for 30 s, quenched on ice, incubated at 45°C for 1 h, and quenched on ice and dithiothreitol was added to a final concentration of 10 mM. The probe was applied to the cells at 0.5 × 10⁶ to 6 × 10⁶ cpm/5 µl of hybridization mix. The cells were covered with silicized cover slips that had been heated at 180°C for 3 h and were sealed with rubber cement for incubation for 42 h at 48°C in a humid chamber. The cover slips were then removed, and the cells were washed in 1× SSC–10 mM Tris hydrochloride (pH 7.4)–1 mM EDTA–45% formamide for 24 h. They were washed twice in 1× SSC for 5 min and dehydrated in graded ethanol solutions containing 0.3 M ammonium acetate (36). The slides were dipped in Kodak NTB2 emulsion, diluted 1:1 in 0.6 M ammonium acetate, and developed 4 or 5 days later as previously described (3). They were then stained with modified Wright stain counterstain (Geometric Data, Wayne, Pa.). The identity of the slides was concealed until 1,000 cells per sample had been analyzed. Photography was performed with a Leitz Orthomate E camera with Technical Pan film 2415 (ASA 100, factor 2) with exposures ranging from 5 to 25 s.

RESULTS

We have previously reported that infection of B6C3F1 or CD1 resident peritoneal macrophages with HSV-1 results in an abortive infection during which no infectious virus is produced and the viral DNA is not replicated (20, 31). To precisely determine the block in HSV-1 replication at the molecular level, we carried out a detailed analysis of the extent of viral gene expression in these cells using Northern hybridization, in situ hybridization, and immunofluorescence techniques.

Synthesis of HSV-1 virus-specific mRNAs. Total cell RNA was isolated at various times from mock-infected and HSV-infected resident peritoneal macrophages and Vero cells and was analyzed by hybridization to probes specific for individual HSV-1 genes. In each case, three or more experiments were performed, and data representative of each are presented in Fig. 1. Equal quantities of RNA were loaded in each well; therefore, the amounts present in Fig. 1 reflect differences in the levels of mRNA expressed in resident peritoneal macrophages. Northern blot hybridization with cloned probes encoding HSV-1 immediate-early genes ICP4, ICP0, ICP27, and ICP22 revealed that transcription of these genes occurred in resident peritoneal macrophages (Fig. 1; Table 1). Antisense-specific single-stranded RNA probes were generated by in vitro transcription of the coding sequences of ICP4, ICP0, or ICP22 cloned under the T7 promoter in the plasmid pGEM-2. These hybridized efficiently to the RNA shown in Fig. 1, confirming that the mRNAs for these genes were transcribed in resident peritoneal macrophages rather than sequences that were antisense to these mRNAs (data not shown). We consistently observed less viral mRNA in infected resident peritoneal macrophages than in the permissive Vero cells. These data

were also confirmed by slot-blot hybridization (data not shown) and by in situ hybridization (Fig. 2).

To determine whether the early class of viral genes was also transcribed in resident peritoneal macrophages, we hybridized infected cell RNA to probes for *tk*, ICP8, and DNA polymerase. Vero cells exhibited high levels of all three transcripts (Fig. 1) consistent with published results (39). Infected resident peritoneal macrophages contained readily detectable mRNA specific for *tk*, while mRNA specific for ICP8 was present at a much reduced level. Surprisingly, in contrast to the data obtained from infected Vero cells, DNA polymerase-specific mRNA was not detectable in resident peritoneal macrophages by Northern hybridization (Fig. 1) or in situ hybridization (Fig. 2). mRNAs specific for the delayed early gene *gD* (data not shown) and the true late gene *gC* (Fig. 1) were also present at high levels in Vero cells and were not detected in resident peritoneal macrophages by Northern analysis or, for *gC*, by in situ hybridization (Fig. 2).

In situ hybridization analysis. In situ hybridization was performed to establish the percentage of the cell population expressing mRNA transcripts for the representative HSV-1 genes. Representative data are shown from two or more experiments for each HSV-1 gene investigated. In addition, in every case, a series of time points were examined, ranging from 4 to 20 h p.i. The data presented reflect the time of maximum expression of these genes or are representative of a negative result that was observed at all times.

Transcription of the immediate-early gene ICP4 was evident in both Vero cells and resident peritoneal macrophages; however, over 95% of the Vero cells expressed ICP4-specific mRNA, in comparison with 12 to 15% of the resident peritoneal macrophage population (Fig. 2; Table 1). Grains were distributed over both the nucleus and the cytoplasm in both cell types. RNA for the early gene *tk* was evident in 2% of the resident peritoneal macrophages at 6 h p.i. and was maximally expressed in 7% of these cells at 12 h p.i. (Fig. 2; Table 1). Over 95% of the Vero cells were positive for *tk* at both these time points. Grains could be detected in only 1 to 3% of infected resident peritoneal macrophages probed with DNA polymerase, while again, over 95% of the Vero cells were positive (Fig. 2; Table 1). When uninfected cells were counted in our blind analysis, we obtained the same result (1 to 3%) and therefore concluded that the DNA polymerase gene was probably not transcribed in these resident peritoneal macrophages. Identical results were obtained for the true late gene *gC*. Collectively, these results agree with those obtained during the Northern analysis, suggesting that there is a tapering off of expression of the HSV-1 early genes.

HSV-1 polypeptide synthesis. To compare the number of cells expressing viral RNA with those expressing viral polypeptides, we performed indirect immunofluorescence using MAbs or polyclonal antibody specific for viral polypeptides. The data shown in Fig. 3 are representative of three individual experiments and are in general agreement with the data obtained from the hybridization experiments. Similar to the in situ data, these data reflect the time of maximum expression or are representative of a negative result that was obtained at all times. ICP4 was present in 14% of resident peritoneal macrophages and in over 95% of Vero cells (Fig. 3; Table 1). It was distributed mainly in nuclear compartments, being maximally expressed at 6 h p.i. and completely absent by 24 h p.i. The early polypeptide ICP8 was first detected at about 6 h p.i. and remained weakly positive up to at least 14 h p.i. Maximal expression was at 8 h p.i., when 6 to 11% of the resident peritoneal macrophages

FIG. 1. Northern blot hybridization of HSV-1 KOS-infected and mock-infected resident peritoneal macrophage (RES) and Vero cell RNA. Cells were infected with HSV-1 at a multiplicity of infection of 3 to 5. RNA was extracted, electrophoresed, and hybridized as described in Materials and Methods. The numbers at the top of each autoradiogram are the times (in hours) p.i. that RNA was extracted. Lanes C, RNA extracted from uninfected cells. The probes used are indicated to the left of each autoradiogram, and the relative size of each mRNA species to host rRNAs is indicated. DNA POL, DNA polymerase.

and over 95% of the Vero cells were positive. Immunofluorescence for the delayed-early polypeptides gB and gD could barely be seen in <2% (gB) and 3% (gD) of resident peritoneal macrophages, while more than 80% of the Vero cells were positive. Finally, consistent with the results obtained previously, the true late gene gC was not seen in resident peritoneal macrophages but was evident in over 80% of infected Vero cells.

DISCUSSION

It has been shown previously that infection of resident peritoneal macrophages with HSV-1 results in an abortive infection in which the viral DNA is not replicated and no infectious virus is produced (20, 31). In this study, we identified at the molecular level the restriction in replication of HSV-1 in infected resident peritoneal macrophages. We

showed that immediate-early genes are expressed, as are some early genes, and that the true late genes are not expressed. While the infection proceeded normally in the permissive Vero cells, it declined in resident peritoneal macrophages, appearing to taper off at the early to delayed-early gene expression level in the cascade. There was a significant and progressive decline in the amount of viral gene products and in the number of cells expressing those gene products.

It has been shown in permissive cells that activation of HSV-1 immediate-early genes occurs through the interaction of an incoming virion structural protein (Vmw65) with cellular factors (25, 26) and upstream enhancerlike elements (2, 4). Presumably, these interactions function normally in the subpopulation of resident peritoneal macrophages that were shown to contain immediate-early mRNA and protein. By

TABLE 1. Summary of Northern blot analysis, in situ hybridization, and immunofluorescence of Vero cells and B6C3F1 resident peritoneal macrophages (PMO) infected with HSV-1

Viral gene class	Northern analysis ^a		In situ hybridization ^{a,b}		Immunofluorescence ^{a,b}	
	Vero	PMO	Vero	PMO	Vero	PMO
ICP4	+	+	+ (>95%)	+ (15%)	+ (>95%)	+ (14%)
ICP0	+	+	ND	ND	ND	ND
ICP27	+	+	ND	ND	ND	ND
ICP22	+	+	ND	ND	ND	ND
ICP8	+	+	ND	ND	+ (>95%)	+ (11%)
<i>tk</i>	+	+	+ (>95%)	+ (7%)	ND	ND
DNA polymerase	+	—	+ (>95%)	— (<3%)	ND	ND
gB	ND	ND	ND	ND	+ (>80%)	— (<3%)
gD	+	—	ND	ND	+ (>80%)	— (<3%)
gC	+	—	+ (>80%)	— (<3%)	+ (>80%)	— (<1%)

^a +, Positive; —, negative; ND, not done. The numbers in parentheses represent the percentage of cells expressing those viral genes. <X%, Below background levels.

^b These results are expressed as percent positive for at least 1,000 cells counted.

Northern hybridization, the immediate-early transcripts were of the correct size, and their time of appearance was the same as that in the permissive Vero cells. The fact that we consistently observed less viral immediate-early gene-specific RNA in resident peritoneal macrophages can be attributed to the reduced number of cells that expressed these genes. It was therefore surprising that among the early genes, only *tk* was expressed to a level comparable to that of the immediate-early genes. mRNA for the major DNA-binding protein ICP8 was barely detectable, and we saw no evidence of expression of the DNA polymerase gene.

Although we identified the location of the block in HSV-1 replication in resident peritoneal macrophages the mechanism(s) underlying the inability of these cells to support virus replication remains an enigma. Our evidence, however, does

suggest that in resident peritoneal macrophages, either the regulation of transcription of HSV-1 early genes is dramatically different from that found Vero cells (perhaps owing to decreased levels of immediate-early proteins) or the mRNA specific for DNA polymerase and ICP8 is being rapidly degraded compared with that for *tk*. In this regard, the very detailed examination of the cascade of transcription of HSV-1 genes in Vero cells reported by Weinheimer and McKnight (39) is informative. They showed that regulation of expression of HSV-1 genes is achieved not only through mechanisms that control the initiation of transcription but, in addition, by mechanisms that control the accumulation of viral mRNA. They observed that transcription of the genes for thymidine kinase, DNA polymerase, and ICP8 occurred at the same time during infection, albeit at slightly different

FIG. 2. In situ hybridization of HSV-1 KOS-infected and mock-infected resident peritoneal macrophages (RES) and Vero cells. Cells were infected with HSV-1 at a multiplicity of infection of 3 to 5. Cells were processed for in situ hybridization as described in Materials and Methods. The probes used for hybridization are depicted at the bottom of the figure. ICP4 was used to probe the mock-infected (UN) cells. The times p.i. for each set were as follows: ICP4 and TK, 6 h; DNA polymerase (POL) and gC, 8 h.

FIG. 3. Immunofluorescence in HSV-1 KOS-infected and mock-infected resident peritoneal macrophages (RES) and Vero cells. Cells were infected with HSV-1 at a multiplicity of infection of 3 to 5 and processed for immunofluorescence as described in Materials and Methods. The antibodies used for each set are indicated at the bottom of the figure. Antibody to ICP4 was used for the mock-infected (UN) cells. The times p.i. were as follows: ICP4, 6 h; ICP8, gB, gD, and gC, 8 h.

rates. In addition, they demonstrated that transcription of the delayed-early and true late genes could be initiated prior to the replication of viral DNA; however, the RNA remained unstable until after DNA replication had begun. Thus, the lack of expression of the delayed-early and late genes that we observed in resident peritoneal macrophages could be the result of the general instability of these transcripts prior to DNA replication, since viral DNA is not replicated in resident peritoneal macrophages (20). However, this explanation cannot account for the dramatic difference in the apparent accumulation of *tk*-specific mRNA compared with that for DNA polymerase and ICP8. It is likely that other as yet unknown factors of either viral or cellular origin also play a role. In permissive Vero cells, regulation of DNA polymerase expression occurs in part through a posttranscriptional mechanism (41). As we were unable to detect any DNA polymerase-specific transcripts in resident peritoneal macrophages, clearly the normal mechanism by which this gene is expressed is dramatically altered in these cells; these results further emphasize the extreme importance of the role of the cell type in the replication of viruses.

Another testable hypothesis concerning the role of the cells is that the immediate-early proteins are nonfunctional in resident peritoneal macrophages, perhaps owing to the absence or reduced levels of required macrophage cellular transcription factors. In this case, the early and late genes would not be expressed and the DNA, since it is not part of an active transcription complex, could become degraded by host cell nucleases. We found that for ICP4, approximately the same percentage of cells was positive for viral protein by immunofluorescence as for virus-specific mRNA by *in situ* hybridization. For ICP8, although mRNA for this gene was barely detectable by Northern hybridization, we were able to determine that 6 to 11% of the cells were positive by immunofluorescence. Therefore, ICP4 and ICP8 are trans-

lated; however, these results do not establish whether the final protein products are themselves functional or capable of normal function (perhaps owing to the absence of required cellular factors) in the macrophage microenvironment. We must assume that there was sufficient ICP4 present to initiate *tk* transcription, since the expression of *tk* is stringently dependent on functional ICP4 (10) and significant levels of *tk* mRNA were detected. However, ICP4 also regulates the expression of late genes (7). These two activities reside in separate domains on the ICP4 polypeptide (8); we cannot rule out the possibility that only one domain is active in resident peritoneal macrophages. Finally, it is likely that ICP4 is fully functional but that its localization in these cells is altered, thus affecting its ability to regulate expression of selected HSV-1 genes (19). Taking all our data together, ICP4-dependent synthesis of certain early viral mRNAs appears to be normal in the semipermissive subpopulation of resident peritoneal macrophages; this lends support to the concept that the early gene RNAs are being degraded at rates that vary for each individual gene.

In regard to the issue of regulation of selected viral genes, it is also possible that other immediate-early gene products, such as ICP0 and ICP27, have altered function in resident peritoneal macrophages. While ICP0 is nonessential for virus replication in permissive cells, mutants in this gene exhibit altered replication properties (28). ICP27 plays a regulatory role during productive infection (27) and has either a transactivating or transrepressing activity on viral promoters when introduced into cells in combination with ICP4 and ICP0 (30). It is likely that the mechanism by which these proteins interact to regulate gene expression is changed in resident peritoneal macrophages. We are currently performing experiments to distinguish between these possibilities to identify the precise mechanism underlying

the tapering off of gene expression that results in restriction of HSV-1 replication in resident peritoneal macrophages.

In addition to localizing the molecular basis for the block in HSV-1 replication in B6C3F1 resident peritoneal macrophages to the expression of the early genes, we demonstrated that among this relatively homogeneous macrophage population of cells, viral products were evident in only a small number (15 to 24%) of cells. Sarmiento (29a) has recently reported similar findings of heterogeneity in macrophages for HSV-1 replication, in association with the organ, inflammatory state, and genetic background of the mouse. The block in replication in the approximately 80% of infected resident peritoneal macrophages that did not express viral gene products must occur at a very early stage—at the level of entry, at uncoating, or at the earliest transcriptional events. It is important that not all resident peritoneal macrophages support virus replication to the same extent. This may be due to the presence of a subpopulation of cells that vary in their differentiation state in an as yet undefined way (24a). Our findings have major implications in studies involving virus-macrophage interactions. Since the expression of HSV genes is well defined in permissive cells (38, 39), we are now in a position to examine the cellular effects on HSV-1 replication, particularly in regard to the differentiation or activation states of the macrophages and the possible effects that these have on the expression of other viruses that may be latent in macrophages.

ACKNOWLEDGMENTS

The gifts of MABs from L. Pereira, G. Cohen, and M. Zweig and of plasmids from D. Coen and R. Sandri-Goldin are gratefully acknowledged. We thank Pat Repik for critical reading of the manuscript.

This work was supported by grant IM462 from the American Cancer Society and Public Health Service grants AI 25751 and AI 24004 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

- Anderson, K. P., R. H. Costa, L. E. Holland, and E. K. Wagner. 1980. Characterization of herpes simplex type 1 RNA present in the absence of all de novo protein synthesis. *J. Virol.* **34**:9–27.
- Batterson, W., and B. Roizman. 1983. Characterization of the herpes simplex virion-associated factor responsible for the induction of alpha genes. *J. Virol.* **46**:371–377.
- Brahic, M., A. T. Haase, and E. Cash. 1984. Simultaneous in situ detection of viral RNA and antigens. *Proc. Natl. Acad. Sci. USA* **81**:5445–5448.
- Campbell, M. E. M., J. W. Palfreyman, and C. M. Preston. 1984. Identification of herpes simplex virus DNA sequences which encode a trans-acting polypeptide responsible for stimulation of immediate early transcription. *J. Mol. Biol.* **180**:1–19.
- Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**:156–159.
- Conley, A. J., D. M. Knipe, P. C. Jones, and B. Roizman. 1981. Molecular genetics of herpes simplex virus. VII. Characterization of a temperature-sensitive mutant produced by in vitro mutagenesis and defective in DNA synthesis and accumulation of beta polypeptides. *J. Virol.* **37**:191–206.
- DeLuca, N. A., A. M. McCarthy, and P. A. Schaffer. 1985. Isolation and characterization of deletion mutants of herpes simplex virus type 1 in the gene encoding immediate regulatory protein ICP4. *J. Virol.* **56**:558–570.
- DeLuca, N. A., and P. A. Schaffer. 1988. Physical and functional domains of the HSV-1 transcriptional regulatory protein ICP4. *J. Virol.* **62**:732–743.
- Dempsey, W. L., A. L. Smith, and P. S. Morahan. 1986. Effect of inapparent murine hepatitis virus infection on macrophages and host resistance. *J. Leuk. Biol.* **39**:559–565.
- Dixon, R. A. F., and P. A. Schaffer. 1980. Fine structure mapping and functional analysis of temperature-sensitive mutants in the gene encoding the herpes simplex virus type 1 immediate-early protein, VP175. *J. Virol.* **36**:189–203.
- Domke-Opitz, I., P. Poberschin, S. Mittnacht, and H. Kirchner. 1987. Role of interferon in persistent infection of macrophages with herpes simplex virus. *Virology* **159**:306–311.
- Enquist, L. W., G. F. Vande Woude, M. Wagner, J. R. Smiley, and W. C. Summers. 1979. Construction and characterization of a recombinant plasmid encoding the gene for the thymidine kinase of herpes simplex type 1 virus. *Gene* **7**:335–342.
- Everett, R. D. 1986. A detailed mutational analysis of Vmw 110, a trans-acting transcriptional activator encoded by herpes simplex virus type 1. *EMBO J.* **6**:2069–2076.
- Gendelman, H. E., T. R. Moench, O. Narayan, and D. E. Griffin. 1983. Selection of a fixative for identifying T cell subsets, B cells, and macrophages in paraffin-embedded mouse spleen. *J. Immunol. Methods* **65**:137–145.
- Goldin, A. L., R. M. Sandri-Goldin, M. Levine, and J. C. Glorioso. 1981. Cloning of herpes simplex virus type 1 sequences representing the whole genome. *J. Virol.* **38**:50–58.
- Honess, R. W., and B. Roizman. 1974. Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. *J. Virol.* **14**:8–19.
- Honess, R. W., and B. Roizman. 1975. Regulation of herpesvirus macromolecular synthesis: sequential transition of polypeptide synthesis requires functional viral peptides. *Proc. Natl. Acad. Sci. USA* **72**:1276–1280.
- Johnson, R. T. 1964. The pathogenesis of herpes virus encephalitis. II. A cellular basis for the development of resistance with age. *J. Exp. Med.* **120**:359–374.
- Knipe, D. M., D. Senechek, S. A. Rice, and J. L. Smith. 1987. Stages in the nuclear association of the herpes simplex virus transcriptional activator protein ICP4. *J. Virol.* **61**:276–284.
- Leary, K. R., J. R. Connor, and P. S. Morahan. 1985. Comparison of herpes simplex virus type 1 DNA replication and virus production in murine bone marrow-derived and resident peritoneal macrophages. *J. Gen. Virol.* **66**:1123–1129.
- Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Mogensen, S. C. 1979. Role of macrophages in natural resistance to virus infections. *Microbiol. Rev.* **43**:1–26.
- Morahan, P. S. 1984. Interactions of herpesviruses with mononuclear phagocytes, p. 71–89. *In* B. T. Rouse and C. Lopez (ed.), Immunobiology of herpes simplex virus infection. CRC Press, Inc., Boca Raton, Fla.
- Morahan, P. S., J. R. Connor, and K. R. Leary. 1985. Viruses and the versatile macrophage. *Br. Med. Bull.* **41**:15.
- 24a. Morahan, P. S., A. Volkman, M. Melnicoff, and W. L. Dempsey. 1988. Macrophage heterogeneity, p. 1–26. *In* G. Heppner and A. Fulton (ed.), Macrophages and Cancer. CRC Press Unisciences Series, Boca Raton, Fla.
- O'Hare, P., and C. R. Goding. 1988. Herpes simplex virus regulatory elements and the immunoglobulin octamer domain bind to a common factor and are both targets for virion transactivation. *Cell* **52**:435–445.
- Preston, C. M., M. C. Frame, and M. E. M. Campbell. 1988. A complex formed between cell components and an HSV structural polypeptide binds to a viral immediate early gene regulatory DNA sequence. *Cell* **52**:425–434.
- Sacks, W. R., C. C. Green, D. P. Aschman, and P. A. Schaffer. 1985. Herpes simplex virus type 1 ICP27 is an essential regulatory protein. *J. Virol.* **55**:796–805.
- Sacks, W. R., and P. A. Schaffer. 1987. Deletion mutants in the gene encoding the herpes simplex virus type 1 immediate-early protein ICP0 exhibit impaired growth in cell culture. *J. Virol.* **61**:829–839.
- Sandri-Goldin, R. M., R. E. Sekulovich, and K. Leary. 1987. The alpha protein ICP0 does not appear to play a major role in the regulation of herpes simplex virus gene expression during infection in tissue culture. *Nucleic Acids Res.* **15**:905–919.
- 29a. Sarmiento, M. 1988. Intrinsic resistance to viral infection:

- mouse macrophage restriction of herpes simplex virus replication. *J. Immunol.* **141**:2740–2748.
30. **Sekulovich, R. E., K. Leary, and R. M. Sandri-Goldin.** 1988. The herpes simplex virus type 1 α protein ICP27 can act as a *trans*-repressor or a *trans*-activator in combination with ICP4 and ICP0. *J. Virol.* **62**:4510–4522.
31. **Sit, M. F., D. J. Tenny, J. L. Rothstein, and P. S. Morahan.** 1988. Effect of macrophage activation on resistance of mouse peritoneal macrophages to infection with herpes simplex viruses type 1 and 2. *J. Gen. Virol.* **69**:1998–2010.
32. **Smith, I. L., and R. M. Sandri-Goldin.** 1988. Evidence that transcriptional control is the major mechanism of regulation for the glycoprotein D gene in herpes simplex virus type 1-infected cells. *J. Virol.* **62**:1474–1477.
33. **Stevens, J. G., and M. L. Cook.** 1971. Restriction of herpes simplex virus by macrophages. An analysis of the cell-virus interaction. *J. Exp. Med.* **133**:19–38.
34. **Straub, P., I. Domke, H. Kirchner, H. Jacobsen, and A. Panet.** 1986. Synthesis of herpes simplex virus proteins and nucleic acids in interferon treated macrophages. *Virology* **150**:411–418.
35. **Streicher, H., and R. Joynt.** 1986. HTLV-III/LAV and the monocyte/macrophage. *J. Am. Med. Assoc.* **256**:2390–2391.
36. **Stroop, W. G., D. L. Rock, and N. W. Fraser.** 1984. Localization of herpes simplex in the trigeminal and olfactory systems of the mouse central nervous system during acute and latent infections by in situ hybridization. *Lab. Invest.* **51**:27–38.
37. **Tenney, D. J., and P. S. Morahan.** 1987. Effects of differentiation of human macrophage-like U937 cells on intrinsic resistance to herpes simplex virus type 1. *J. Immunol.* **139**:3076–3083.
38. **Wagner, E. K.** 1985. Individual HSV transcripts: characterization of specific genes, p. 45–104. *In* B. Roizman (ed.), *The herpesviruses*, vol. 3. Plenum Publishing Corp., New York.
39. **Weinheimer, S. P., and S. L. McKnight.** 1987. Transcriptional and post-transcriptional controls establish the cascade of herpes simplex virus protein synthesis. *J. Mol. Biol.* **195**:819–833.
40. **Weller, S. K., K. J. Lee, D. J. Sabourin, and P. A. Schaffer.** 1983. Genetic analysis of temperature-sensitive mutants which define the gene for the major herpes simplex virus type 1 DNA-binding protein. *J. Virol.* **45**:354–366.
41. **Yager, D. R., and D. M. Coen.** 1988. Analysis of the transcript of the herpes simplex virus DNA polymerase gene provides evidence that polymerase expression is inefficient at the level of translation. *J. Virol.* **62**:2007–2015.