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Human Herpesviruses: a Consideration of the Latent State

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INTRODUCTION AND SCOPE OF THE REVIEW

Taken together, herpesviruses that infect humans are responsible for multiple syndromes involving many organ systems. Aside from a similar ultrastructural morphology and some similarities in replication cycles, the only major feature shared by all of these agents is the capacity to establish latent infection, which is defined as a type of persistent infection in which the viral genome is present but infectious virus is not produced except during intermittent episodes of reactivation (R. Ahmed and J. G. Stevens, *in* B. N. Fields, ed., *Virology*, 2nd ed., in press). Following initial infection, the viruses can be reactivated from the latent state, producing episodes of significant, sometimes serious disease. Latency is central to the natural history of herpesviruses; however, although the phenomenon has been appreciated for years, basic mechanisms involved in the processes of establishment and maintenance of and reactivation from this state are only now being understood in detail. The studies described below are based largely upon the conceptual and technical tools of contemporary molecular biology and their application to both cellular and the more complex whole-animal systems infected with herpesviruses. It is the purpose of this review to document and analyze significant investigations carried out over the last decade. All six presently known herpesviruses are considered, but herpes simplex virus type 1 (HSV-1) and Epstein-Barr virus (EBV) are stressed, because they are examples of systems of neurotropic (HSV-1) and lymphotropic (EBV) viruses that have been investigated in considerable detail. The other agents are compared with these prototypes. To provide a perspective concerning the natural history of these agents and a background for the studies of latency, each section is introduced by a brief discussion of acute infections and diseases.

HSV

Acute Infection

Initial, acute infections by HSV principally involve ectodermally derived tissues, and, if there is some associated disease, the site of infection is often the site at which the lesions appear. Thus, skin, mucous membranes, and corneal epithelium are all commonly involved, with gingivostomatitis being the most common clinical manifestation of HSV-1 disease following initial infection. Often, however, there is an initial subclinical infection of one of these areas (usually the mouth) and the subsequent appearance of a protective, specific immune response which includes neutralizing antibodies, which are directed against glycoproteins present in the viral envelope, and cytotoxic T cells, which (if the human infection mirrors the murine model) are directed against both envelope and nonstructural proteins (10, 68). The events occurring with HSV-2 are similar, but the skin of the genital area and the vaginal mucous membranes are the sites of initial infection.

There are, of course, unique, potentially serious syndromes which may follow infection with either agent. HSV-2 infections of newborns (caused by delivery through an infected birth canal) may result in a fulminating disease in which virus replicates in virtually all the organs of the infant. In this instance, the brain is commonly involved, and survivors often suffer severe neurologic sequelae. In adults, HSV-1 is responsible for acute encephalitis, also a life-threatening disease with serious neurologic sequelae. Although this disease presents as an acute process resembling a primary infection, many victims have preexisting antibody, and it is probable that in some instances the disease results from a reactivated latent infection, perhaps involving viral deoxyribonucleic acid (DNA) harbored in the brain itself (31). Finally, three relatively recent reviews (19, 92, 133) offer detailed considerations of the epidemiology and pathogenesis of acute herpetic disease.

Latent Infection

General considerations. After initial infection and many rounds of viral replication at the body surface, the following series of events ensues. The virus (or at least a subviral particle with an associated viral genome) ascends by retrograde axonal transport in nerve axons to associated somas in sensory ganglia. There, one of two mutually exclusive events occurs: either viral replication with neuronal destruction or establishment of a latent infection and neuronal survival. In cases of neuronal survival, the viral genome is reactivated in later episodes and the genome (in a form as yet unidentified) passes anterograde in axons, crossing from axon to epithelium, where a productive infection and lesions develop. As was the case in the initial infection, the reactivated infection may not produce obvious disease.

Specific considerations. Viral genetic information passes from surface to ganglion intra-axonally, and it is probable that the mechanism involves fast axonal transport (15, 59, 84, 133). As mentioned above, the form in which the virus travels has not been firmly established, but there is some ultrastructural evidence that it passes as a nucleocapsid (67; M. L. Cook and J. G. Stevens, unpublished data). This mechanism makes general sense, since there is now good evidence that infection of cultured cells involves fusion of the viral envelope with the cellular plasma membrane, followed by entry of the nucleocapsid into the cytoplasm (32, 50, 84). In the case of neurons in vivo, the envelope would be left at the site of entry into the cell, most probably at intraepithelial nerve endings. It is now universally accepted that sensory neurons harbor the latent virus (14, 57, 71) and, although these particular neurons have been of greatest interest, the fact that many neural tissues have been shown to harbor latent virus (16, 131) suggests that a variety of neurons can become latently infected.

Interest continues in the possibility of latent infections in nonneuronal sites, particularly in cells at the body surface, and there is evidence in experimental systems for persistence of infection for extended periods in both footpads and corneas. In one recent study, for example, HSV-2 could be reactivated 12 weeks after initial infection by in vitro cultivation of skin and subcutaneous tissues from feet of mice in which the foot had been denervated 1 week prior to explantation. To decrease the possibility that the animals harbored replicating viruses, acycloguanosine was administered in drinking water beginning 6 weeks after infection (13). In a later study, virus was reactivated by in vitro cultivation of corneas from rabbits (1) and mice (142) previously inoculated on this tissue. No virus could be isolated from the eyes at the time of explant. It is difficult to establish whether these systems really are examples of latent infection or merely represent low-level virus replication which is amplified upon explantation of tissues in an "immune-free" environment. Obviously, neither the use of agents that inhibit viral replication nor the lack of detectable virus in tissues at the time of explant constitutes proof of the presence of latent infection.

Latent infection in sensory neurons has been the object of considerable study ever since initial experiments indicated that the virus was present in these cells. Historically, incisive studies have been difficult to design in this system, since even under the best experimental conditions, latently infected neurons represent a minority (approximately 1%) of cells in a latently infected sensory ganglion. In addition, neurons are difficult to purify from such tissues. These problems have limited the number of biochemical techniques that could be applied to the systems studied. Also, as noted below, the in vitro latency systems that have been developed have not yet contributed information that significantly extends knowledge derived from whole-animal studies. However, at least with HSV-1, significant progress has been made in recent years, and this is documented below.

(i) Viral DNA in latent infection. Studies of the physical state of viral DNA in latently infected neurons are few, with most of the work being carried out in two laboratories. Initially, Rock and Fraser showed that after mice were inoculated on the cornea and the acute active infection had subsided, viral DNA found in the ipsilateral trigeminal ganglion and brain stem was in a different physical state from that observed during the acute infection and was also different from that present in virions (99). These genomes lacked detectable termini, suggesting that they were integrated into multiple sites on the host cell genome or were present in some extrachromosomal form. If the latter were true, multimeric linear or circular molecules or monomeric circles could be possible structures. The results were confirmed by Efstathiou et al. (29), who showed, in addition, that viral genomes with similar properties to those described by Rock and Fraser (99) were present in latently infected human trigeminal ganglia and that all four DNA isomers could be found in the latently infected tissues. In a later communication, Rock and Fraser (100) showed that in the mouse system, viral genomic termini were covalently linked, a result compatible with single-copy plasmids or concatemeric circular or linear molecules. More recently, Mellerick and Fraser, by subjecting the DNA present in murine brain stems to buoyant density gradient centrifugation, succeeded in separating the viral from the cellular DNA and indicated that the viral DNA existed in an extrachromosomal state (72). They also suggested that the most likely physical form would be a "circular episome." Finally, Deshmane and Fraser have also indicated that in latently infected murine brains, the viral genome is associated with nucleosomes in a structure similar to that of eucaryotic chromatin (26).

No accurate determination of the number of genomes present per latently infected neuron has been made. From quantitative blot hybridizations, estimates of 0.01 to 0.4 genome per cell in human trigeminal ganglia (29) and 0.015 to 1.0 copy per cell in various mouse neural tissues (9, 29, 99) have been published. Even if one of these extremes represents the number of genomes actually present in a tissue, and assuming that most genomes are nondefective, the actual number per latently infected neuron would have to be much larger. This is because neurons constitute only a minor

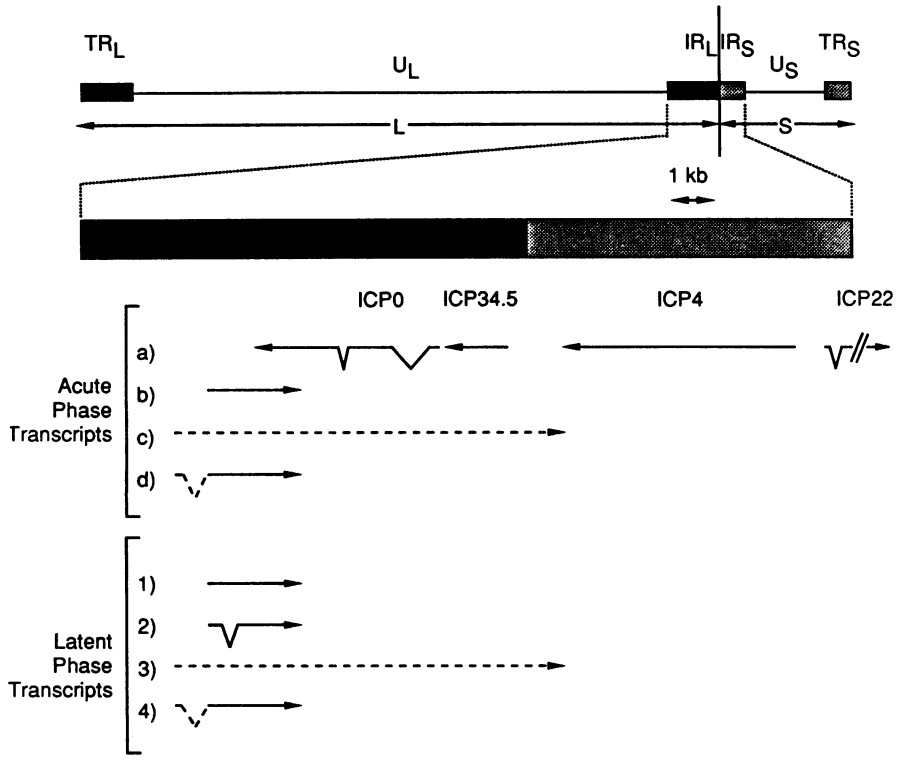

FIG. 1. Transcription patterns of the HSV-1 joint region in productively and latently infected cells. A diagram of the physical structure of the viral genome is presented at the top of the panel. The HSV genome is divided into long (L) and short (S) regions, each terminating in inverted repeat regions (TR_L-IR_L and IR_S-TR_S) separated by extensive unique nucleotide sequences (U_L, U_S). Although not drawn, transcripts originating in IR_L and IR_S are also encoded by TR_L and TR_S. As discussed in the text, in latently infected cells only transcripts arising from the repeat regions can be detected (U_L and U_S are silent). In acutely infected rabbit skin cells in culture, the following several transcripts are seen: (a) transcripts known to be associated with lytic infection; (b) unspliced, nonpolyadenylated 2.2-kb LAT transcripts; and (c) polyadenylated transcripts up to 8.5 kb in length, which overlie the LAT. At least some of the transcripts in line b may possess the short leader shown in line d. In latently infected murine rabbit sensory ganglia, only one unspliced and two spliced derivatives of the LAT are detected. In addition, there is transcription distal to the 3′ end of the LAT which extends beyond the joint. As before, at least some of the transcripts shown in lines 1 and 2 may have the short leader shown in line 4. Dotted arrows represent areas of uncertain length or continuity. Transcripts of sizes comparable to those in lines 1 and 2 have been found in human ganglia.

proportion of the cells populating neural tissues (in spinal ganglia, for example, neurons would probably represent about 10% of the cells). In addition, not all neurons in these tissues would be expected to be harboring latent viral DNA. By scoring neurons expressing the latency-associated transcript (see below) to establish the percentage of neurons harboring latent infection, determining the amount of viral DNA present per ganglion by quantitative slot blot procedures, and then assuming that neurons constitute 10% of the cells present per murine spinal ganglion, we calculate that there may be 20 copies of viral DNA per latently infected neuron. It is of interest that when using in situ nucleic acid hybridization techniques, no one has been able to detect this viral DNA in latently infected neurons. In some attempts the methodologies used were sufficient to detect viral genomes an order of magnitude less in complexity and present at one copy per latently infected cell (6). The reason for these negative results is not immediately apparent. Perhaps the DNA is present in some complex that is not accessible when current methods of section preparation are used.

(ii) Viral gene products in latent infection. Specific viral gene products associated with the establishment of the latent infection have not yet been described. Studies defining these products are difficult to perform with existing systems, because acute productive infections and latent infections are established concurrently in the ganglion. Recently, however, viral genetic expression during the latent phase of infection, both in experimental animals and in humans, has been the object of considerable study in several laboratories. This followed the initial observation by Stevens et al. (120) that a unique transcript (which we have termed the latency-associated transcript [LAT]) is synthesized in latently infected murine sensory neurons. This limited and unique transcription of the viral genome is now known to take place in latently infected sensory neurons of mice (89, 115, 120), rabbits (101, 128), and humans (22, 38, 117, 119, 132). There is general agreement that the transcripts detected by the in situ nucleic acid hybridization method map exclusively to the long terminal repeat region of the viral genome (Fig. 1) and are largely confined to the nucleus (Fig. 2A). On Northern (ribonucleic acid [RNA]) blots, the major transcript (50 to 90% of the total, depending on the virus strain or experimental animal studied) is approximately 2.2 kilobases (kb) in length. Another transcript (10 to 50% of the total) is a spliced derivative of the first and is about 1.5 kb long (128). As a group, the transcripts are minimally polyadenylated (115) or nonpolyadenylated (128). In addition to these two transcripts, a third, very minor transcript some 500 bases shorter than the second has been described by workers in at least one laboratory (115) and probably represents yet an-

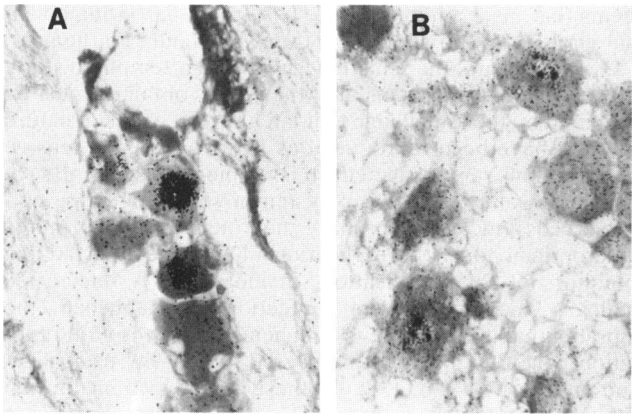

FIG. 2. In situ hybridization of latently infected neurons in sensory ganglia with ^{3}H-labeled HSV-1 DNA probes. The method specifically detects RNA. (A) Latently infected murine spinal ganglia hybridized with a LAT-specific *Hpa*I R fragment (0.766 to 0.785 map units) specific for the LAT. Two neurons demonstrate strong signals restricted to the nucleus (magnification ×260). (B) Latently infected murine spinal ganglia hybridized with a 1-kb *Sma*I fragment (0.823 to 0.830 map units) downstream from the LAT. Two neurons demonstrate weak signals restricted to defined areas of the nucleus (magnification ×260).

other splicing variant. There is general agreement that all the RNAs are transcribed from the DNA strand opposite that encoding the transcript for the immediate-early polypeptide infected-cell polypeptide 0 (ICP0) and that they initiate 3′ to the ICP0 gene and extend approximately one-third of the distance into the transcribed region of this gene. There is one significant open reading frame which is not interrupted by the splice in the two major species of RNA (128), but there is no evidence that a protein is encoded by these sequences. Indeed, despite intensive searches in many laboratories over the years, virus-encoded proteins have not been reproducibly demonstrated in neurons latently infected with HSV. Small amounts of the unspliced transcript (which we also find to be nonpolyadenylated) are present in lytically infected cultured cells (58, 115, 120, 128), and the transcript does not appear to fit any of the kinetic classes of HSV transcripts previously described (116). Finally, a rough calculation indicates that the LATs, as a group, are present at 2×10^4 to 5×10^4 molecules per latently infected murine neuron (127).

More recent evidence indicates that the transcription pattern is considerably more extensive than that understood at present, and we now know that there are transcriptionally active regions adjacent to both ends of the LAT (A. T. Dobson et al., J. Virol., in press). With respect to the area 5′ to the LAT, present data clearly indicate that nominal polymerase II binding and regulatory sequences present some 700 bases upstream from the previously determined 5′ end are involved in transcription during the latent state. Some possible models for this are shown in Fig. 1. With respect to transcription distal to the 3′ terminus of the LAT, by in situ nucleic acid hybridization techniques we have observed transcripts in latently infected neurons which hybridize to several probes extending sequentially across the joint into the short terminal repeat region of the genome. An example of the in situ nucleic acid hybridization pattern seen with the most rightward probe used (covering map units 0.823 to 0.830 in the viral genome) is shown in Fig. 2B. Others (58, 117) have also detected "minor" transcripts 3′ to the LAT in latently infected human neurons. Fewer neurons contain these transcripts than express the LAT, and there are fewer copies of them per neuron. In addition, transcription in the area is linked to the LAT, since a deletion mutant unable to synthesize the LAT does not express these transcripts (49). We have not yet identified additional LAT-related transcripts in Northern blots prepared from latently infected ganglia. However, when used in in situ nucleic acid hybridization experiments, probes specific for the LAT and the flanking sequences define a LAT transcription unit which starts at the upstream TATA box in the long terminal repeat, crosses the joint, and ends in the vicinity of a canonical polyadenylation sequence in the short terminal repeat just downstream from the ICP4 gene (identified in reference 70). From this primary transcript, a variety of stable and unstable RNAs may be derived.

Clearly, the transcription pattern of the LAT-encoding region is complex. Physical characteristics of transcripts change between acutely and latently infected cells, and there are transcriptionally active areas adjacent to both ends of the LAT. A complete physical characterization of these transcripts, particularly during reactivation, is likely to yield important clues concerning their function.

(iii) Biologic function of viral gene products present in latently infected neurons. With the discovery of the LAT, considerations of its role in the latent infection logically follow. Theoretically, the transcript could function in any of the stages of establishment of, maintenance of, or reactivation from the latent state. To date, one paper dealing with this overall subject has appeared (49) in which it was reported that a spontaneous deletion mutant of an HSV-1–HSV-2 recombinant virus that did not synthesize the LAT was able to establish, be maintained in, and reactivate from the latent state (reactivation being defined here as induction of viral replication initiated by in vitro cultivation of ganglia from mice). These results indicate that expression of the LAT is not an absolute requirement for completion of any of the phases in the latency cycle.

More recent studies employing an HSV-1 in which the deletion was engineered gave identical results, and quantitative studies further indicated that the transcripts played absolutely no role in establishment or maintenance of the latent infection in mice (F. Sedarati et al., J. Virol., in press). These results show that HSV-1 infection may be maintained in neurons in the complete absence of viral genetic expression; no virus-encoded transcripts can be detected in the ganglia of mice infected with these deletion mutants. Of course, the sensitivity of the assay is important. With our methods, it can be calculated that approximately 100 molecules of a transcript with the complexity of the LAT would be needed in a neuron for detection.

The results found with deletion mutants suggest that if the LAT plays a role in the latent state, it does so by facilitating reactivation, a phenomenon that may well be different in vivo from in vitro. During reactivation in vitro, mature infectious virus is produced by the neurons. As discussed elsewhere in greater detail (118), synthesis of virions in all systems studied (including acutely infected ganglionic neurons) results in cell death, which also occurs in reactivating neurons maintained in vitro. This destruction of neurons is difficult to reconcile with multiple reactivations involving similar if not identical anatomic sites and no increasing anesthesias over time of the area where lesions appear. As an alternative to replication of virions and neuronal destruction, it may be suggested that reactivation in vivo involves expression, in neurons, of a subset of viral genes (with

replication of the viral DNA obviously being essential) and that these gene products do not destroy the neuron. The DNA would then pass intra-axonally to the epithelium, infect epithelial cells by transfection through intraepithelial nerve endings, and initiate a replication cascade, leading ultimately to vesicles. Whatever the physical form taken, it seems almost certain that the viral genome does pass intra-axonally to the neuroepithelial junction, where infection of cells of the body surface follows.

Studies of the LAT function, investigations of the viral form reactivated in vivo, and a definition of the overall phenomenon of reactivation demand a dependable and predictable animal model. Fortunately, the rabbit system in which iontophoresis of epinephrine through previously infected corneas is followed by reactivation and appearance of virus in the tear film (45, 60, 61) satisfies all requirements. Although the cost of experiments with this model is not inconsequential, rabbits have considerable advantages over mice and guinea pigs. Mice are notoriously poor and undependable "reactivators" with any of several manipulations used to date (reviewed in reference 102), and guinea pigs, although known to reactivate HSV-2 virus spontaneously in a genital model (102), are not known to react to induction procedures used in other systems.

With the rabbit model, we have recently shown (J. M. Hill et al., submitted for publication) that the LAT does, in fact, function during epinephrine-induced reactivation. In these studies, animals were inoculated on both corneas either with the spontaneous deletion mutant used in the murine system (49) or with a recombinant in which the LAT function was restored to this agent in transfection experiments. The viruses were shown to replicate similarly in rabbit tissues, and they established latent infections with equivalent efficiencies. After epinephrine had been iontophoresed, virus reappeared in at least one eye in 18 of 20 rabbits inoculated with the LAT-restored agent but in only 3 of 22 animals harboring the deletion mutant. These results indicate that although the LAT is not absolutely required for reactivation in this system, it greatly enhances the process.

Now that its function in a stage of the latency cycle has been established, mechanistic studies of the LAT can follow. As one approach, it seems likely that a sustained and systematic study of physical alterations in the molecule during the reactivation process induced by epinephrine in the rabbit will yield useful results. In addition, the notion of limited expression of the viral genome during reactivation can be tested in in situ nucleic acid hybridization experiments involving individual viral gene probes and latently infected trigeminal ganglia taken from animals in which reactivation occurred.

(iv) In vitro systems. To circumvent the obvious problems associated with the studies of latency in sensory ganglia maintained in vivo, an in vitro model system would clearly be useful. Thus, a uniform population of neurons, all latently infected, or capable of becoming so, and capable of being reactivated by a defined manipulation would have great utility. Aside from the historical problems of proving that such cultures are not producing low levels of virus, thereby perpetuating the infection, a theoretical problem with development of such systems is that insufficient information has been available from "real" in vivo systems. That is, there have been no well-described, fundamental characteristics of latent infections which one could attempt to reproduce in cultured cells. Without such guidelines, the relevance of any system to latency is unclear. This problem has now at least been blunted, and from the discussion just presented it seems that, as a minimum, studies of cells harboring episomal viral DNA and expressing the LAT should be the goal.

Over the years, there have been several attempts to study latency in in vitro systems, and the results obtained must be tempered by the knowledge that latency as it exists in nature may not have been reproduced as yet. The most extensive investigations are reflected in the pioneering efforts of Wigdahl et al., whose basic system involves infecting cultured cells (including primary neurons) at temperatures superoptimal for virus replication (40°C) and then adding inhibitors of viral replication, including DNA nucleoside analogs. Once the "latent" infection is established, the supraoptimal temperature is sufficient to prevent viral replication; when temperature is lowered to 37°C, virus replication ensues (134–136, 138). In these systems, transcription patterns have not been reported, and the virus appears to persist in some linear form (137).

Investigations of an additional model have been initiated by Russell and co-workers, and this system represents a variation on the theme just described. HSV-2 was converted to a "latent" state in human fetal lung cells by increasing the temperature of incubation to 42°C. Viral replication was usually not induced by a downshift to 37°C but could be induced by superinfection with temperature-sensitive mutants of HSV-1 or human cytomegalovirus (CMV) (104). Neither potential macromolecular syntheses instituted from the viral genome nor determinations of the physical state of the viral DNA were reported. To date, studies have concerned genes involved with establishment of and reactivation from latent infection (105). It was found that superinfection at 42°C with a virus defective in expression of the immediate-early gene ICP4 resulted in the rescue of "latent" virus (presumably by complementation), suggesting that ICP4 expression is not necessary for reactivation. In addition, HSV-1 mutants defective in expression of immediate-early genes ICP0 and ICP4 were shown to persist, but the ICP0 mutant could not "reactivate" a virus already present when it was used in superinfection experiments. The latter result suggests that expression of ICP0 is necessary for reactivation in this system. Another model developed by Cooke and Brown involved the use of supraoptimal temperatures and addition of acycloguanosine to corneal cells infected with HSV-1. Upon downshift and removal of the drug, virus reappeared in cultures where it could not be detected earlier (18).

In the last system developed, latent infection (in which no viral antigens could be detected) was established by incubation of HSV-1-infected primary rat sympathetic neurons in the presence of acycloguanosine. When anti-nerve growth factor antibody was added to the cultures, virtually all the cells began producing viral antigens, and virus appeared (139, 140). Again, neither the state of the viral genome nor the extent of viral transcription was established. The contribution of these systems to an understanding of latency may or may not be a direct one. To paraphrase from a recent review by Roizman and Sears (102), such systems may not be particularly informative with respect to latency, but they will certainly contribute to an understanding of viral gene expression in nonpermissive cells.

VZV

Acute Infection

The pathogenesis of varicella (chickenpox) is not adequately defined, but as it is presently understood, it involves

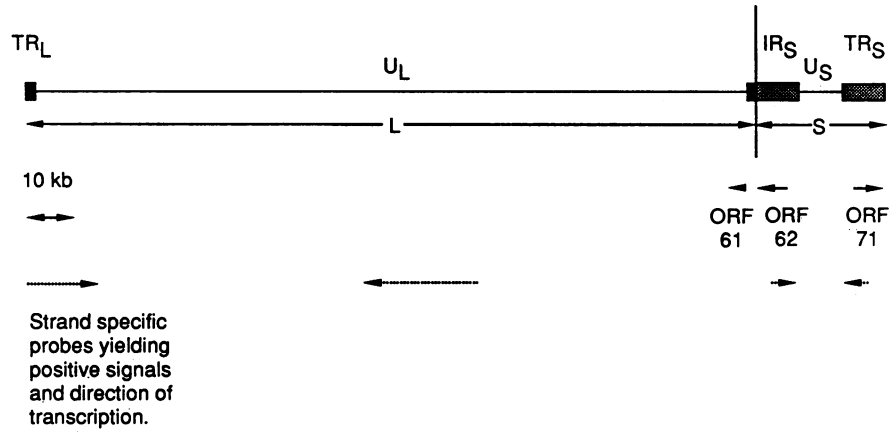

FIG. 3. Transcription pattern of VZV DNA during the latent state of infection in satellite cells populating human trigeminal ganglia. A diagram of the physical structure of the viral genome is presented at the top of the figure. The VZV genome is divided into long (L) and short (S) regions, each terminating in inverted repeat regions (TR_L-IR_L and IR_S-TR_S) separated by extensive unique nucleotide sequences (U_L, U_S). The locations and direction of transcription of open reading frames 62 (ORF-62), 71 (ORF-71) (the IE-175 genes), and 61 (ORF-61) are shown. The length and direction of transcription of individual probes giving positive hybridization signals are shown by dotted arrows at the bottom of the figure. Note that these studies do not place ends on the transcripts. In addition to those described, at least two other areas of the genome are known to be transcribed, since groups of probes approximating a left one-third and middle one-third of the viral genome detected signals not identified by the individual probes shown. For a more detailed description, see reference 23.

a primary asymptomatic infection of the oropharynx or conjunctiva, followed by viremia, replication in cells of the reticuloendothelial system, and a secondary viremia followed by seeding of the skin and mucous membranes subsequent to passage of the virus through capillary endothelium (reviewed in reference 33). Development of classic lesions follows. The pathogenesis of zoster (shingles), the reactivated infection, is also not well documented, although there is now strong evidence that the virus does, in fact, establish latent infections in sensory ganglia (see below). This finding is a fundamental one that establishes on solid experimental grounds the earlier theories that the pathogenesis of zoster involves a neural circuit resembling that associated with HSV. From analogies with HSV, it is reasonable to think that the ganglia are seeded by virus traveling in nerves from the site of varicella lesions and that a latent infection follows. However, exposure of ganglia to virus leading to establishment of latent infection from the viremia remains a definite possibility. Whichever the case (the possibilities are not mutually exclusive), because of the dermatomal distribution of lesions, it seems a surety that the virus reactivated from the ganglion travels in nerves to the periphery, where acute lesions characteristic of zoster develop. The lack of a suitable animal model and the continued inability to replicate cell-free virus in cell culture are the principal reasons for our relative lack of knowledge concerning the pathogenesis of these two syndromes.

Latent Infection

The base of knowledge concerning latent infection with varicella-zoster virus (VZV) is very small and is just emerging. It is now clear that sensory ganglia do harbor latent VZV, and two lines of evidence establish this fact. First, both in situ and Southern blotting nucleic acid hybridization techniques, with probes specific for VZV nucleotide sequences, performed on human sensory ganglia have shown that such sequences are present (23, 35, 36, 48). Second, explantation and in vitro cultivation of such ganglia is followed by the appearance of VZV-encoded immediate-early RNA and several proteins (126). Parenthetically, it is of interest that, unlike the usual situation with HSV, no detectable amounts of infectious virus were induced in the ganglia by this procedure.

A controversy exists concerning the cell type involved in harboring latent virus. Using in situ nucleic acid hybridization technology, workers in two laboratories (35, 48) incriminated neurons, and, very recently, another group (23) has presented evidence for satellite cells. The initial investigators did not study the extent of viral transcription, but later results indicated that, as was found in HSV infections, there is restricted expression of the VZV genome in latently infected cells. A diagram representing several features of the transcription pattern recently reported by Croen et al. (23) is presented (Fig. 3). LAT-like molecules were not ruled out, and although no counterpart to the HSV ICP0 gene has yet been found in the VZV genome (open reading frame 61 is a candidate [86]), there is a counterpart to the immediate-early ICP4 gene (open reading frame 62, or IE-175). One transcriptionally active region in satellite cells latently infected with VZV covers the strand opposite open reading frame 62, and may also cover open reading frame 61 (Fig. 3). In addition to this area, at least four other widely scattered regions of the VZV genome were found to be transcribed. These results indicate that certain basic features of the latent infection may differ from those of latent infection by the biologically similar HSV, in which, as detailed above, only one region of the viral genome appears to be transcribed during latency.

The physical state of the latent viral DNA is unknown, and, as with HSV, VZV DNA has not yet been detected by in situ nucleic acid hybridization methods. Although the molecular basis for reactivation is unknown, the difference in reactivation patterns between this virus and HSV (for example, lesions are more extensive in zoster and appear much more frequently in herpes simplex) indicates that a major difference exists either in the nature of the latent state itself or in the process of reactivation. Croen et al. (23) suggest that these differences can be explained by the difference in latently infected cells. For example, reactiva-

tion from satellite cells could result in active VZV infection in many adjacent neurons and could then ultimately result in lesions over larger areas of the body surface than would be seen with HSV.

EBV

Acute Infection

Most EBV infections, particularly in childhood, are asymptomatic, and whatever the syndrome induced, primary infection with EBV follows infection and replication of virus in the nasopharynx. At least some of the cells involved in producing virus in the nasopharynx are epithelial cells (39, 109–111, 147). The active infection may be prolonged, with virus shed for weeks or months. Indeed, up to 60% of seropositive individuals may be shown to be shedding virus when sampled at any time during their lives (34, 75, 143).

The one presently appreciated, clinically apparent acute infection induced by EBV is heterophile antibody-positive infectious mononucleosis. Following the initial asymptomatic infection, the virus ultimately infects B lymphocytes in the nasopharynx (tonsils or adenoids). These lymphocytes become latently infected and then circulate, and, if subsequent events are correct, heterophile antibody-positive infectious mononucleosis is the result. The latently infected lymphocytes demonstrate the presence of EBV nuclear antigen (EBNA; see below). When cultivated in vitro, some cells produce virus which immortalizes other lymphocytes in the culture, whereas other cells become capable of prolonged replication themselves (46, 55, 95, 96). During week 2 of clinically apparent mononucleosis, the number of EBNA-positive cells falls, but they are never eliminated, as shown by the fact that culture of buffy-coat cells from seropositive individuals again results in establishment of immortalized EBNA-positive viral genome-containing B lymphocytes.

During the acute disease there is a vigorous cellular response (consisting of the characteristic atypical lymphocytes), which includes suppressor and cytotoxic T cells and an accompanying antibody response to some, but not all, virus-encoded antigens. Although the subject is outside the purview of this review, the recent discussion by Rickinson (94) makes it clear that these immunological responses have some unique characteristics that must contribute somehow to the clinically apparent features of infectious mononucleosis. Whatever the precise roles of virus and the immunopathological consequences of the immune response in the pathogenesis of disease, clinical recovery ultimately follows this characteristically protracted disease. The recovery is accompanied by a fall in the number of reactive lymphocytes and the appearance of protective virus-specific cytotoxic T cells and neutralizing antibody. Detailed considerations of the pathogenesis of acute primary EBV-related diseases are presented in references 74 and 94.

As with HSV, neutralizing antibodies are directed against virus-encoded membrane glycoproteins, and in this case the 350/220 component appears to be a prominent antigen (82, 123). The virus-encoded antigens seen by class 1-restricted cytotoxic T cells are not completely defined, but the latent membrane protein (LMP) is an example (79, 80, 124) and probably either represents or influences the virus-specific, human lymphocyte antigen-restricted surface component operationally termed lymphocyte-detected membrane antigen (LYDMA). There is also evidence that the EBNA-2 antigen serves as a T-lymphocyte target (78, 80). Given the general knowledge that viral proteins not normally considered to be membrane structural proteins may serve as targets in several viral systems (including, as discussed above, HSV systems), it seems not unlikely that additional EBV-encoded proteins will ultimately be shown to provide target epitopes for cytotoxic T lymphocytes.

To summarize, productive infection of an undetermined cell type (presumably an epithelial cell) in the oropharynx results in a subclinical disease and the seeding of B lymphocytes. These cells circulate, and the resultant overall infection may be severe enough to induce the disease infectious mononucleosis, with its associated vigorous and complex cellular immune response. This protracted disease resolves, but B lymphocytes latently infected with the virus may still be detected in the blood, and infectious virus is shed from the oropharynx at intermittent intervals for extended periods.

Latent Infection

B lymphocytes. In this discussion, the latent infection is of primary interest, and specific phenomena now generally thought to relate principally either to the neoplastic transformation or immortalization or to the property of the virus as a polyclonal B-cell activator are not stressed. As mentioned above, circulating, latently infected B cells can be induced to produce virus when cultivated in vitro, and they can also be cultivated as immortalized, virus-episome-containing, EBNA- and LYDMA-expressing lymphocytes. Because of this, it has been classically thought that in most individuals persistently infected with EBV there is a compartment of latently infected, dividing B lymphocytes which persist for long periods. Since LYDMA-expressing lymphocytes are destroyed by cytotoxic, class 1 human lymphocyte antigen-restricted, cytotoxic T cells which are present in significant amounts in EBV antibody-positive, latently infected individuals, an alternative to the concept of a complement of long-lived, latently infected B cells was recently proposed (94). It was suggested that there could be a site of active chronic virus infection in the pharynx, which constantly reinfected B lymphocytes in the local area. These reinfected cells would then become latently infected and circulate, but would be destroyed by the pool of specific cytotoxic T cells and would be constantly replaced by new latently infected B cells originating in the pharynx. Two pieces of evidence subsequently gathered by the group presenting the alternative viewpoint argue against this explanation. First, latently infected cells (both B lymphocytes and epithelial cells) which do not express latent membrane protein or other latency-associated antigens present in cell membranes were found to exist (103, 148). Although these cells are neoplastic, their existence indicates that translation patterns may in some situations be more restrictive than those characteristically described for latently infected cells and that the virus-associated antigens seen by cytotoxic T cells are not necessarily present. More recently, it was shown that individuals receiving high levels of acycloguanosine for prolonged periods (28 days) stopped shedding EBV in the oropharynx, but that latently infected, circulating B cells in these patients remained at constant levels over the entire period of study (142). Although the final mechanism for generating circulating EBV genome-containing B lymphocytes is unknown at present, it is clear that such cells do exist. This latent infection has been extensively studied ever since the virus was initially discovered, and considerable information is available concerning molecular aspects of this cell-virus interaction.

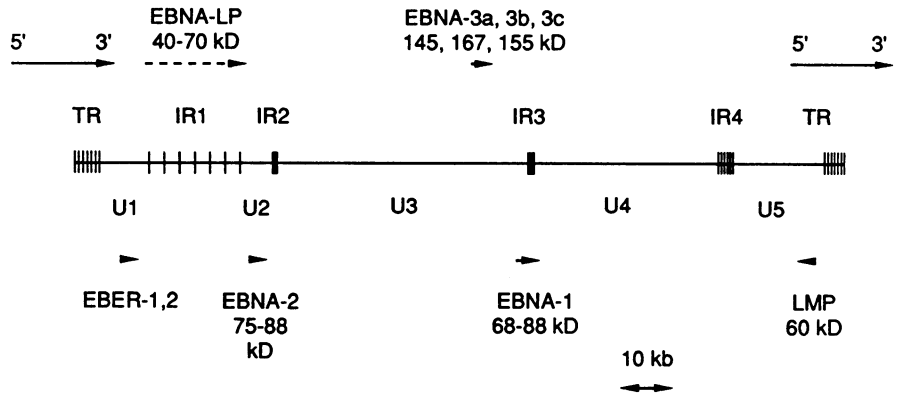

FIG. 4. Genes expressed in B lymphocytes latently infected with EBV. The physical structure of the viral genome is diagrammed. Five regions of unique nucleotide sequences (U1 to U5) are separated by areas composed of direct tandem repeats (TR and IR1 to IR4). The sizes of proteins and the location of genes and direction of transcription (arrows) are shown. The locations of EBV sequences and the 5' and 3' boundaries of the gene encoding the highly spliced RNA transcribed from circularized genomes are also depicted.

(i) Viral DNA and gene products in latent infection. Latent EBV genomes are characteristically present in multiple copies per latently infected B cell (56, 81, 149). They are present as single-copy episomes (63) joined by covalent linkage of the repeat sequences which are present at each end of the DNA (24). In culture, at least, the number of plasmids per cell remains remarkably constant over time, which implies a tightly controlled replicative and distributional system that is coordinated with cell division. Viral and cellular elements contributing to this phenomenon are by no means completely identified and understood. There is an origin of viral replication that is very likely to be specific for the latent phase (65, 144, 146). It is present in a different portion of the viral genome from the site identified in lytic infection (41). The DNA is replicated by the host cell DNA polymerase, and one of the virus-encoded intranuclear proteins (EBNA-1) is somehow involved in maintenance of the plasmid in proliferating cells (see below). Integrated EBV DNA has been found in at least two cell lines derived from patients with Burkitt's lymphoma (69). In one cell line (Namalwa) there is a single copy of viral DNA per cell, apparently integrated from a linear molecule, and the integration is accompanied by a 15-kb deletion of host cell DNA. In the other cell line (SB4), three or four episomes are also present in each cell, and the one integrated genome is thought to have arisen from an episomal intermediate, since the left and right ends of the viral DNA (as it exists in the virion) are linked. Clearly, the physical characteristics of viral DNA in these two cell lines are very different. Whether other cells harbor integrated DNA and whether there is a particular biological significance to these integrated molecules are not known.

An ongoing and increasingly detailed definition of transcription and translation products encoded by the latent genomes has followed the original finding that cells harboring EBV genomes specifically express an intranuclear antigen termed EBNA. It is now known that this "antigen" consists of six or more different polypeptides encoded from three different regions of the viral genome (Fig. 4). The first of these, EBNA-1, is encoded by an area in the center of the viral genome including the right end of the areas designated U3, IR3, and the left end of U4. The messenger RNA (mRNA) is a 3.7-kb RNA with the coding sequences following an exceptionally long leader of some 1.5 kb (reviewed in reference 25). There is evidence that this leader arises from long-range splicing events involving as many as 100 kb (8, 114). The protein itself varies in size from 68 to 88 kilodaltons (kDa) depending upon the cell line from which it is isolated. As with other members of the EBNA group of proteins, EBNA-1 is chromatin associated (25). Functionally, this protein is involved in maintenance of the episome (65, 146) and probably with regulation of transcription. It interacts physically with the putative plasmid origin of replication (90). For the purposes of this review, it is important to note that this is the only EBNA protein shown to be expressed in virtually all cells harboring latent EBV genomes. Presumably, then, viral DNA cannot be maintained in the latent state without the EBNA-1 protein.

EBNA-2 is a 75- to 88-kDa protein encoded within the U2 region and translated from a primary transcript encompassing the right end of the U1, the IR1, and the U2 regions. Depending upon the number of tandem repeats in the IR1 region, the primary transcript for EBNA-2 can be larger than 20 kb. From this RNA, a principal spliced derivative of 3.0 kb is produced, with approximately three of these molecules per latently infected cell (reviewed in reference 25). As with the EBNA-1 mRNA, there is extensive and long-range splicing relative to the EBNA-2 mRNA. In fact, it is now believed that all EBNA-encoding mRNAs, under certain conditions, at least, have the same transcription start site (which initiates in the U1 region of the viral genome [107]), each mRNA arising by differential long-range splicing events. Clearly, much remains to be learned about the genesis of mRNAs for these proteins in latently infected cells: there is evidence both for alternate transcription initiation sites and for alternate splicing patterns. These complications have obvious significance with respect to the regulation of the lytic and latent cycles in B lymphocytes and in epithelial cells.

There is strong evidence that EBNA-2 contributes to the process of immortalization. Probably most compelling is the finding that a natural deletion mutant (P3HR-1), which lacks coding sequences for EBNA-2, is incapable of transforming cells (77, 92). Recombinants between this agent and "transforming viruses" reacquired the EBNA-2 gene (112). In addition to this evidence, it is known that one of the two EBNA-2 alleles (type A) is represented by an 82- to 87-kDa protein that transforms cells to a rapidly growing phenotype. The other (type B) is represented by a 75-kDa protein that is antigenically distinct from the type A protein. Cells expressing the type B protein are associated with a lower growth

rate, a different pattern of growth, and lower saturation densities (97).

The remaining EBNA genes and their products are not as well understood, and their functions have not been defined. To date, three additional areas of the genome have been shown to be responsible for EBNA proteins (43, 54). EBNA-3 (three different proteins termed either 3A, 3B, or 3C [87] or EBNA-3, EBNA-4, and EBNA-6 [98]) is encoded in the U3 region near the middle of the genome (51, 87), and, as with EBNA-1 and EBNA-2, there is evidence for long-range transcript splicing (85). There are three long open reading frames in U3, each preceded by a shorter open reading frame. The protein product of the 3A and 3C genes is translated from an mRNA containing the short and long open reading frames spliced in frame (44, 51, 88). It is likely that 3B is translated from a similar spliced mRNA, although this has not been proved (87). The proteins are 145, 165, and 155 kDa (3A, 3B, and 3C, respectively) in size (44, 51, 87, 88, 98), and there is presently no information concerning their potential function in latently infected cells.

EBNA leader protein, also called EBNA-5, is encoded by the IR1 region of the viral genome contained in the leader sequence of the EBNA-2 mRNA, and the mRNA has been termed bicistronic (27, 130). The protein synthesized is 40 to 70 kDa in size and has a unique, punctate nuclear distribution. The functional activity of the protein is presently unknown.

The final protein described in latently infected cells is the LMP encoded by the LT3 gene, located just inside the terminal repeat at the extreme right-hand end of the genome. The LMP mRNA is spliced (three exons), 2.8 kb long, and present at a relatively high copy number, about 60 per latently infected cell (reviewed in reference 25). The protein, which has been extensively studied and characterized by Hennessy and co-workers, is approximately 60 kDa in size and is present in the cell membrane (42). It has several remarkable features, which led Hennessy and co-workers to suggest that both termini are present inside the cell and that the molecule traverses the membrane lipid bilayer six times. In this regard, it resembles proteins such as the rhodopsins, the acetylcholine receptor, and the calcium pump proteins. When DNA encoding this protein is transfected into continuous lines of mouse and rat cells, growth characteristics of the transfectants are altered (129), suggesting that the protein plays a role in cellular transformation.

The biological property of the LMP which is important to this review is its potential role as the target for class 1 human lymphocyte antigen-restricted cytotoxic T cells. This interaction, as discussed above, would terminate the latent infection. Although the matter is not settled, it seems likely that LMP either is the LYDMA or is responsible for a change in the B-cell surface which functionally defines the LYDMA. At present, no other virus-encoded antigens are known to be consistently present in the cellular membrane of latently infected cells.

Finally, there are at least three other RNA species in latently infected cells. One very interesting transcript was recently discovered in an EBV complementary DNA library (62) made from latently infected cells. This RNA is transcribed across the terminal repeat units (it is encoded by circular DNA) and possesses an open-end reading frame capable of encoding a 53-kDa protein. As yet, the predicted protein has not been found. In addition to this RNA, two small RNA species of unknown function are present in latently-infected cells. These (termed EBER-1 and EBER-2) are encoded in the *Eco*RI J fragment, are 166 and 172 nucleotides in length, and have structures uncharacteristic of mRNAs. They are transcribed by RNA polymerase III and resemble adenovirus VA-RNAs in several other aspects (discussed in references 25 and 74).

(ii) Events and viral gene products associated with establishment of and reactivation from latent infection. Phenomena related to the establishment of the latent infection have not been the focus of sustained efforts, but are now being studied. For example, in a recent paper, Hurley and Thorley-Lawson (47) indicated that replication of the viral genome is not a prerequisite either for establishment of the latent state or for immortalization. These two phenomena do, however, require circularization of the incoming genomes.

Reactivation of infectious virus from latently infected cells has been studied more extensively. Reactivation is induced at low levels by cultivation of latently infected cells in vitro. The efficiency of the process may be increased by a variety of manipulations, including use of tumor promoters (tetradecanoyl phorbol acetate, for example), butyrate, anti-immunoglobulin G immunoglobulins, nucleoside analogs, and calcium ionophores (discussed in references 30 and 121). It has been suggested that calcium ionophores may function by supplying calcium for activation of protein kinase C, a molecule which is likely to be required for reactivation (30). The mechanism by which protein kinase C induces the expression of the viral genome is unclear, but it most certainly involves inducing transcription from a gene studied in continuing detail by Miller and colleagues (20, 21, 40, 76). This gene was initially studied in experiments involving superinfection of latently infected cells with EBV strain P3HR-1, a virus which always induces lytic infections. When Miller and colleagues examined the genome of certain defective variants of P3HR-1 that induce the lytic infection most efficiently (76), they found that the capability could be transferred to fused lymphocyte-epithelial cells and to lymphocytes by transfection with a 2.7-kb, extensively rearranged, *Bam*HI fragment, designated WZ het, fused to an appropriate promoter (21, 40). Takada et al. (121) showed that the standard *Bam*HI Z fragment also could induce lytic infections from latently infected cells. This fragment encodes a 43-kDa polypeptide called ZEBRA (*Bam*HI fragment Z Epstein-Barr replication activator), a protein that functions in *trans* to activate the replication cycle of the virus (20). Furthermore, the protein was found to be encoded by the BZLF open reading frame (*Bam*HI Z leftward open reading frame) initially identified when the DNA was sequenced (3). Perhaps most importantly, although synthesis of the protein appears to be up-regulated in the P3HR-1 strain, similar proteins have been found to be induced by other EBV strains, and it now appears that this gene product is a crucial early regulator (inducer) of the reactivation process. It has been suggested that this is accomplished by transactivation of additional genes which are themselves transactivators of EBV gene expression early in the lytic cycle (reviewed in reference 4). The understanding of initial events that follow inductions with the various manipulations described above, as well as the relationship of the events to ZEBRA and genes with which it interacts on the EBV genome, will provide key information relative to the herpesvirus reactivation processes. In addition, the mechanism that prevents expression of the gene that encodes ZEBRA under usual conditions will be important to define.

To summarize and extend the foregoing sections, B cells latently infected with EBV DNA express several proteins. In addition, one protein active in promoting the reactivation

cycle has been identified. Only one of the proteins expressed in latently infected cells (EBNA-1) has so far been shown to play a role in the latent infection, and it functions in maintaining the viral DNA. Considering the fact that LAT$^-$ HSV variants are maintained in the latent state in the apparent absence of viral genetic expression, it is possible that the only viral gene requiring expression for perpetuation of the latent state in EBV-infected cells is EBNA-1. The EBNA-1 function is not needed in HSV, since there is no need to replicate and distribute plasmids in terminally differentiated neurons. The other proteins present in EBV-infected cells may function exclusively in the immortalization process.

Epithelial cells. Consideration should be given to the potential of epithelial cells somewhere in the nasopharynx to harbor the latent virus. Although, as discussed above, it is clear that productive infection can be induced in such cells, there is as yet no evidence that normal epithelial cells harbor latent infection. However, malignant epithelial cells populating nasopharyngeal carcinomas certainly harbor the virus, and a subset of the viral gene products described above for B cells is synthesized in these cells. Therefore, when tumor biopsies from 24 patients with nasopharyngeal carcinomas were examined, EBNA-1 was detected in all 24; EBNA-2, EBNA-3, and EBNA-LP were not detected in any tumor cells; and LMP was found in 9 (148). This result clearly shows that the translation pattern in epithelial cells is different from that in most latently infected B cells, and it will be of interest to determine whether this relates to the neoplasia or to latency in epithelial cells, or both. When all of this has been resolved, it seems likely that latently infected epithelial cells will be found to be crucially involved in the pathogenesis of EBV.

CMV

Acute Infection

The pathogenesis of infection with CMV appears to be similar in major ways to that of infection with EBV. In both instances, productive infection of epithelial cells takes place in the nasopharynx, with shedding of virus in the saliva, followed by generalized cell-associated viremia. In acute generalized disease, CMV appears to have a particular predilection for tubular epithelium of the kidney, where a productive infection with prolonged shedding into the urine is common. An infectious mononucleosis-like syndrome with atypical lymphocytes (but lacking heterophile antibody) may be a consequence of the initial infection. In addition, serious cytomegalic inclusion disease presenting as interstitial pneumonitis, central nervous system disease, or other less life-threatening manifestations is not uncommon in immunosuppressed individuals. Transplacental infection with the possibility of teratogenic effects, with or without accompanying acute viral disease and prolonged viral shedding in the urine, is a complication unique to this agent among herpesviruses. During the acute infection, epithelium-derived cells (e.g., ductal cells in the kidneys, alveolar cells in the lungs, and hepatocytes) are most commonly involved in viral replication. There is also evidence for leukocyte involvement during acute disease, with mononuclear cells being the most prominent. Parenthetically, the epithelial components productively infected in vivo are not susceptible in vitro. In culture, cells of fibroblastic origin are the only ones which are known to consistently produce virus. Finally, a detailed discussion of acute infections with CMV is presented in reference 2.

Latent Infection

The institution of immunosuppressive measures results in the appearance of CMV from endogenous sources, and the infection may be transferred in various organ transplants from seropositive to seronegative individuals in whom infectious virus cannot be detected at the time of grafting (11, 12). Therefore, it is clear that the virus establishes persistent infections, at least some of which are latent. The latent infections are very poorly understood, however, and the lack of an animal model susceptible to the human agent has contributed significantly to this dearth of information. Most importantly, the fundamental question of which cells harbor the latent virus has not been answered. Since various epithelial cells are involved in acute infection, it might be theorized that at least some of these are involved in latent infections. At present there is no evidence either for or against this notion. A little more is known about leukocytes, particularly mononuclear cells. In one study involving in situ nucleic acid hybridization techniques applied to seropositive (but asymptomatic) individuals, RNA from the immediate-early region of CMV DNA was found by Schrier et al. to be expressed in circulating lymphocytes bearing either CD4 or CD8 markers (108). Because the individuals studied had no evidence of disease, this result suggests that the lymphocytes were, in fact, harboring latent viral genomes. Viral RNA was detected in lymphocytes taken from eight seropositive individuals, with the proportion of cells expressing signals varying from 0.035 to 2%. Of 12 seronegative individuals, 11 tested negative in the in situ nucleic acid hybridization examinations; 1 was positive, with 0.1% of cells expressing viral RNA. In a later study involving kidney transplantations, Gnann et al. reported that infiltrating interstitial mononuclear cells in the kidneys were the initial cells demonstrating CMV-specific nucleotide sequences in seronegative recipients (37). These patients then began disseminating infectious virus. Although it was not determined whether the lymphocytes expressing viral genetic information derived from the donor or the recipient, the finding is compatible with the suggestion that mononuclear cells harbor latent infections.

Earlier literature indicating that the virus can be selectively isolated from neutrophils during acute disease has suggested to some workers that these cells could harbor latent virus. However, this phenomenon has recently been explained in another way. Neutrophils contain mature, infectious virus and various degradative forms that have probably been phagocytosed (125), and there is no evidence that neutrophils can be actively infected with CMV. Finally, there is a murine counterpart of human CMV which induces some of the same syndromes in mice. Studies are not yet complete, but they have indicated that B cells (52, 83) and sinusoidal lining cells of the spleen (73) harbor latent virus. There is no information concerning the mechanisms involved.

To summarize, then, there is no firm evidence incriminating any cell type as a reservoir for latent CMV. It follows that there is no information concerning the physical state of the viral DNA or the extent of viral transcription during the latent state. The close biologic relationship of CMV to EBV might predict a latent infection resembling, at the molecular level, the one engendered by EBV. However, since the genomic organizations of the two agents are very different (implying different patterns of genetic regulation), this is not necessarily the case. Whatever the final resolution of these questions, this medically important virus demands increased

TABLE 1. Characteristics of latent infections established by human herpesviruses

Virus	Cell type harboring viral genomes	Physical state of viral genome	Transcription pattern, latent state	Proteins detected, latent state	Permissive cells
HSV	Neurons, particularly sensory neurons	Circular extrachromosomal DNA very likely	Limited, only joint region involved	None yet	Essentially all cell types (epithelial cells important for transmission in nature)
VZV	Neurons and/or satellite cells, sensory ganglia	?	At least 5 widely separated regions of genome transcribed	None yet	Neural and epithelial cells most important
EBV	B lymphocytes, malignant (and normal?) epithelial cells	Usually circular, extrachromosomal DNA, may also be integrated	Complex, with long-range splicing covering areas throughout genome	At least 7 (6 in nucleus, 1 in cytoplasmic membrane)	Epithelial cells
CMV	Unknown, lymphocytes probably involved	?	?	?	Epithelial cells
HHV-6	B cells?	?	?	?	?

and sustained attention with respect to characterization of the latent state.

HHV-6

In 1986, Salahuddin et al., when culturing peripheral blood mononuclear cells from patients with lymphoproliferative disorders, isolated "new" herpesviruses from six individuals (106). The isolates were immunologically related to each other, but not to other known human and simian herpesviruses (106). In addition, Southern blotting experiments indicated that when this agent was compared with human herpesviruses and *Herpesvirus saimirii*, it had some unique genomic features (53). Its ultrastructural features were typical of herpesviruses (5). The virus was initially named human B-lymphotropic virus, a name subsequently supplanted by the more general name human herpesvirus 6 (HHV-6), and similar agents were later isolated in Africa (28, 122). Although the viral genome has been found in neoplastic B lymphocytes taken directly from patients, the in vitro cellular host range is now known to be wider than may have been originally thought, and includes T lymphocytes (64, 66). With respect to pathogenic potential, there is now evidence that HHV-6 is responsible for exanthem subitum, or roseola infantum (141), and its potential significance as a more serious pathogen is the subject of current investigation. With respect to persistence, the apparent induction of viral replication by in vitro cultivation of B lymphocytes and the finding of viral genomes in neoplastic B cells are consistent with a latent state in these cells. Beyond this suggestion of latent infection in B cells, little can be said about the potential latent infection engendered by HHV-6.

EXTENSIONS

A summary of the present state of knowledge concerning important aspects of the latent state associated with human herpesviruses is presented in Table 1. Obviously, gaps in our knowledge are sizable, and several observations can be made from a global reconsideration of the discussion presented above. The technical problems which have historically plagued studies of herpesvirus pathogenesis, including latency, no longer represent severe impediments to investigation of all the agents. Although these viruses have complex genomes with many genes and gene products, a store of significant information concerning these genes is developing. Precise and relevant studies of HSV can be carried out with both mice and rabbits, and contemporary molecular biological methods can be applied to these whole-animal systems. Although much remains to be done, the complex relationship between virus and B cell which characterizes the latent EBV infection has been meaningfully investigated by several groups over several years. The advantages to the EBV system are different from those of the HSV system—a readily manipulable in vitro system for the former was available from the beginning.

The other agents are not so easily exploited. There is no suitable animal model or in vitro system for studying latent VZV and CMV, and considerable development must occur before relatively sophisticated studies concerning HHV-6 latency can be initiated. However, even these systems are yielding to contemporary technologies, and it seems likely that the murine counterpart will serve as a useful paradigm for the medically significant human CMV. Finally, it is clear that herpesviruses continue to be significant causes of morbidity and mortality, and a detailed understanding of the latent state is necessary before herpetic syndromes can be dealt with in meaningful ways.

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