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Papovaviral Persistent Infections

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INTRODUCTION

Variety of Host Responses to Virus Infection

Virologists concerned with the pathogenesis of infection have long been preoccupied with acute self-limited diseases such as measles and poliomyelitis. In such cases, overt symptoms generally appear within several days to several weeks after infection. Subsequently, if the disease is not fatal, the infectious agent is generally eliminated from the host, which is then immune to reinfection.

It is now recognized that viral infection may lead to host responses other than acute illness. In particular, persistent virus infections have become a subject of increasing attention as both their frequency and medical importance have become apparent. Virus infections may also be asymptomatic. In other instances, infection may lead to neoplasia. It is noteworthy that papovavirus infections may give rise to each of the host responses noted above.

Persistent virus infections may be of several types. In one variety, characteristic of infections with herpesviruses, the virus becomes latent after what appears to be a typical acute primary infection. Subsequently, there are intermittent episodes of disease between which virus is usually not demonstrable. Another type of persistent infection, termed slow (slow referring to the disease course, not the virus), is characterized by prolonged periods of infection (often years) before symptoms appear. The disease process then develops over a protracted period, eventually resulting in death. These categories of persistent infection are not strictly alternatives. As seen below, papovavirus infections may display characteristics of each type.

The Papovaviruses

The papovaviruses, in particular simian virus 40 (SV40) and polyoma virus of mice, have attracted great interest primarily because of their capacity to induce neoplastic transformations of some mammalian cells both in vitro and in vivo (see reference 282 for a review). Viral genes persist in transformed cells, and in at least some instances, viral gene products are continuously needed to maintain the transformed cell phenotype. Therefore, these viruses have been extensively studied with the aim of explaining the mechanisms by which the normal controls of cell growth and behavior can be altered to result in neoplasia. Furthermore, the small size of the papovaviral genomes (about 3×10^6 daltons) and the ease with which they can be manipulated account for their usefulness in studying basic aspects of gene expression and regulation in eucaryotic cells.

The interaction of papovaviruses with cells in culture is generally considered to be either productive, resulting in cell death, or abortive, in which case the cells survive and a subset of them undergo neoplastic transformation. The outcome is generally dependent on the species from which the cells are derived. For example, SV40 infection of permissive monkey cells is productive, whereas SV40 infection of nonpermissive mouse and hamster cells is abortive.

An aspect of papovavirus biology which is often overlooked is that the interaction of these viruses with their natural hosts generally results in persistent infection. These infections are usually not associated with any disease, although there is suggestive evidence that they might cause cancers in several species, including humans (61, 164, 258, 272, 303).

JC and BK viruses (JCV and BKV) are two recently recognized human papovaviruses (200). They are highly prevalent in the human population, with infection generally occurring in childhood. After the primary infection, which is either aclinical or mild, these viruses apparently persist indefinitely in their human hosts. Latent BKV and JCV may become active whenever immunity is compromised. BKV has not yet been unequivocally associated with any human disease, but JCV is clearly associated with progressive multifocal leukoencephalopathy (PML), a demyelinating disease of the central nervous system in humans (203, 300). SV40 has also been associated with several cases of human PML (183, 237, 300). A disease with striking similarities to PML also occurs in the Asian rhesus macaque (82), the natural host of SV40. Although PML is a rare disease, it is nevertheless extremely important, since it is the first established instance of a virus-induced demyelinating disease in humans. Furthermore, these viruses have been shown to be active during normal human pregnancy, with as yet unknown consequences to the fetus (32). Both JCV and BKV have oncogenic potential in lower animals, and there is evidence to suggest that BKV may be associated with human neoplasia (61, 213, 328).

With the exceptions of K virus of mice, which has a pronounced tropism for pulmonary epithelium (130), and the lymphotropic papovavirus

isolated from the African green monkey (324), the papovaviruses are known or suspected to persist primarily in the urinary tract. Stump-tailed macaque virus has an unusual biology in that it appears to be transmitted as a congenital infection (252). Similar to the other papovaviruses, stump-tailed macaque virus persists throughout the life of the infected individual. All other members of the papovavirus group appear to be transmitted as postnatal infections.

Importance of In Vitro Model Systems

Despite the importance of currently known persistent virus diseases (see below) and the prospect that persistent virus infections might underlie other important diseases of presently obscure etiology, there is not yet any persistent virus disease of which the basic mechanisms underlying persistence are well understood. Complete understanding is likely to require insight into host factors, such as the role of the immune response and viral preference for certain organ systems or cell types. Nonetheless, persistent infection at the cell level is a requirement for viral persistence in the whole organism. At this level, one must account for the establishment and maintenance of persistent infection despite the fact that productive infections by these viruses always lead to irreversible cell damage. The fundamental molecular and cellular mechanisms which underlie persistence can be more readily studied in easily manipulable cell culture systems than in the whole organism. Consequently, the development and study of in vitro systems has become increasingly important.

Scope of the Review

In vitro systems of persistent papovavirus infections and the interaction of these viruses with their natural hosts in vivo are reviewed below. These in vitro and in vivo papovaviral infections are compared with those of several other viral groups, which in some instances have been more extensively studied than the papovaviral systems. The emphasis of this review is on those factors which might account for the establishment and maintenance of persistent infection both in vitro and in vivo. In addition, those factors which might determine the course of pathogenesis in vivo are considered.

GENERAL CHARACTERISTICS OF PERSISTENT INFECTIONS IN VITRO

Persistent infections of cells in culture fall into two broad categories: steady-state infections and carrier cultures. In steady-state infections, almost all cells are productively infected, and their metabolism and growth are scarcely affected. This class of persistent infections is generally established by noncytopathic ribonucleic acid (RNA) viruses which bud from the plasma membrane. Steady-state infections cannot be cured by growth in antiviral serum. In contrast to steady-state infections, carrier cultures are characterized by productive infection in only a small proportion of the cell population. Cells supportive of viral replication are usually killed as a result. Consequently, factors which limit productive infection in the culture play important roles in maintaining the carrier state. Carrier cultures can generally be cured by cultivation in the presence of antiviral serum.

Infections with the tumor viruses, in which the viral genome may become integrated by covalent bonds into the cellular genome (282), may also be thought of as a kind of persistent infection. However, as noted above, studies of such infections have generally involved nonpermissive cells in which there is only limited viral gene expression. As discussed below, such infections are different in important ways from persistent infections of permissive cells.

The underlying events which lead to persistent infection in systems in which lytic infection is possible are not well understood. Mechanisms that have been advanced to account for these systems include the action of defective interfering (DI) particles, which interfere with the replication of the homologous wild-type (WT) virus (112), temperature-sensitive (TS) viral mutants, which become prominent in several systems of persistent infection and which may also suppress WT replication (216), interferon (see, e.g., reference 115), and other poorly defined host cell factors.

Because of the variety of virus-cell interactions which characterize different systems of persistent infection, the following information must be obtained in an attempt to understand any particular system (modified from references 59 and 291).

(i) The proportion of cells which (a) produce virus, (b) support some viral gene expression but not the complete viral replicative cycle, or (c) contain completely latent viral genomes.

(ii) If viral genomes are present in nonproducing cells, then (a) are those viral genomes integrated or free, (b) defective or nondefective, and (c) can productive infection be activated in those cells?

(iii) Can the culture be cured by growth in antiviral antibody? If so, is the cure permanent, or can the productive infection be reactivated?

(iv) What mechanisms inhibit the productive infection? Do viral variants emerge which interfere with the replication of the WT virus? Do host cell factors such as interferon or other

poorly defined cellular mechanisms play a role in modulating the productive infection?

Several papovavirus systems are discussed in regard to the terms given above to the extent that the relevant information is available.

PAPOVAVIRAL PERSISTENT INFECTIONS: IN VITRO AND IN VIVO

Simian Virus 40

Discovery. SV40 was discovered in the early 1960s as a frequent contaminant of Asian rhesus monkey primary kidney cell cultures, in which it replicated to high titer without producing any notable cytopathic effects (271). The presence of SV40 in the rhesus cell cultures was first recognized when supernatant fluids from those cultures were found to induce cytopathic effect (cytoplasmic vacuolization) and eventually cell death when added to cultures of African green monkey kidney (AGMK) cells.

Before the discovery of SV40, rhesus kidney cell cultures were routinely used for the preparation of viral vaccines. These vaccines were administered to millions of people during the 1950s. Concern over human exposure to SV40 was heightened by the discovery that SV40 is oncogenic in hamsters (51, 52). Largely as a result of those experiences, monkey kidney cultures are no longer used for vaccine production.

Interactions with cultured cells. Because SV40 infections of AGMK cells are acute, resulting in high viral yields, AGMK cell lines have been used in studies of the SV40 replicative cycle. Nonpermissive cells of rodent origin have generally been used in studies of SV40-induced neoplastic transformation. Consequently, despite all that is currently known about the molecular biology of SV40 replication and the transforming response, there have been relatively few studies to characterize the interaction of this virus with the cells of its natural host, the Asian rhesus macaque. Indeed, the fact that the rhesus monkey is the natural host of SV40 is frequently overlooked.

A noteworthy feature of the SV40-rhesus kidney cell interaction is that persistent infections are readily established, with minimal cytopathology after infection over a wide range of input multiplicities (187, 189). Indeed, cytopathic effects were never observed in established SV40-rhesus kidney cell systems. Persistent infections of AGMK cells are also possible. However, those systems are established only after the nearly complete destruction of the cell cultures (88, 158, 189), which then undergo periodic episodes of crisis. Only a small percentage of cells are productively infected in established persistent infections of either cell type. Thus, these are carrier systems.

Establishment and maintenance of persistent infections: importance and modes of action of resistant cells and viral variants. Comparison of the SV40-rhesus cell systems with the SV40-AGMK cell systems underscores the importance of host cell factors in the early course of these infections. One such factor is the transient resistance to SV40 manifested by about half of the rhesus cells in randomly growing cultures (193). This phenomenon is not due to genetic heterogeneity of the cells, since cloned rhesus kidney cell populations displayed the same pattern of susceptibility shown by uncloned cultures. In contrast, nearly all cells in AGMK cultures are susceptible to productive SV40 infection. It may also be important that the plating efficiency of SV40 is more than 10-fold lower on rhesus kidney than on AGMK cells (193).

The block in the refractory rhesus cells is expressed early, at some point before synthesis of the SV40 tumor (T) antigen (193). This early SV40 product is required for viral deoxyribonucleic acid (DNA) replication. The basis for the resistant rhesus cells is not yet clear, but it is related at least in part to the growth state of the cells (unpublished results).

Despite the SV40-resistant subset of rhesus kidney cells and the low SV40 plating efficiency on those cells, the 72-h viral yields per infected rhesus and AGMK cell are equivalent and in each case independent of the input multiplicity of infection (193). Thus, it is somewhat surprising that infected rhesus cells are killed much more slowly than infected AGMK cells (193). For example, 85% of infected AGMK cells (CV-1 line) were killed by 48 h, and 98% were killed by 72 h. Under similar conditions, less than 25% of those rhesus kidney cells (LLC-MK_2 line) which were producing SV40 T antigen at 48 h were killed by day 5. This partial dissociation of viral replication from cell killing might also be a factor which favors the establishment and maintenance of SV40-rhesus kidney cell carrier systems.

As noted above, SV40-rhesus kidney cell carrier systems can be readily established after infection over a wide range of input multiplicities (189). In contrast, SV40-AGMK cell systems are more easily established after infection at high than at low input multiplicities (158,189). This might reflect a requirement for viral-specific autointerference activity. Indeed, SV40 DI particles are rapidly generated in AGMK cells provided that the infection is at high input multiplicity (193a). There is also evidence that high input infections might induce or select for cells which express partial or transient resistence (see below).

SV40-rhesus kidney cell systems initiated at an input of 1 plaque-forming unit (PFU) per cell

contained about 2% of T and V (virus) antigen-producing cells at 48 h (189). The fraction of T antigen-producing cells subsequently increased such that by the third week all of the cells produced T antigen. In cultures infected at an input of 100 PFU/cell, about half of the cells displayed T and V antigens at 48 h. However, the fraction of T antigen-producing cells in those cultures subsequently decreased to 16% by week 3, but by week 11, 100% of the cells produced T antigen. The explanation for the effect of the input multiplicity of infection on the pattern of emergence of T antigen-producing cells might involve the action of interfering viral variants or a multiplicity-dependent induction or selection of cells expressing a transient refractory state, or both (see below). Regardless, whereas the fraction of T antigen-producing cells eventually increased to 100% after infection at each input multiplicity, the fraction of V antigen-producing cells stabilized at about 1% in each instance. This indicates that, whereas all cells appear to contain and at least partially express the SV40 genome, the productive infection is perpetuated by relatively few cells.

Virus-free clonal isolates of the SV40-rhesus cell systems could be obtained by cloning in the presence of antiviral serum (187). This is a general feature of carrier systems but not of steady-state infections. Continuous cultivation of uncloned cultures in SV40 antiserum also resulted in cures. It is noteworthy that virus reappeared in the cured cultures several weeks after the removal of the antiserum, indicating that reactivation of latent viral genomes occurs in this system. The virus which reappeared in the "cured" cultures produced microplaques on AGMK cell monolayers. This might reflect the nature of the latent viral genomes, their state during latency, or the mechanism of reactivation.

The achievement of curing by cultivation in antiviral serum suggests that cell-to-cell transfer of the virus plays a role in maintaining the productive infection, although other interpretations are possible. For example, in measles virus systems of persistent infection, antibody to a V antigen on the cell surface can interfere with intracellular viral events (67).

If cell-to-cell transfer of the virus is required to maintain the productive infection at high levels, then curing also implies that virus-producing cells are either killed or cease producing for some other reason. The finding that some clonal isolates are capable of producing virus (192; unpublished results) indicates that an infected cell (in which the infection is probably latent) can give rise to an infected culture. Virus-producing cells are probably recruited from the population of nonproducing T antigen-positive cells. The nature of the resistant state in the nonproducing cells and the mechanism by which these cells become susceptible is not well understood (see below).

In the SV40-rhesus kidney cell system, a heterogeneous population of viral variants emerged to replace the large-plaque standard SV40 used to initiate the infection (187, 190). The variants included particles which express homotypic interference activity. These were first detected at week 6 (by yield reduction assays) (7a, 190) and were in excess of the plaque-forming virus by week 9 (Fig. 1). Note that the absence of interfering particle activity until week 6 further underscores the importance of host cell factors in the establishment of the SV40-rhesus kidney cell carrier systems.

Restriction endonuclease analysis revealed changes in the carrier culture viral DNA in samples from as early as the second week. Nevertheless, even after a year of persistent infection, most of the viral DNA from the SV40-rhesus kidney cell systems yielded restriction fragments indistinguishable from standard virus fragments (190). In contrast, only a few undiluted passages of SV40 in AGMK cells generated virus stocks containing an excess of grossly altered genomes (193a). These genomes yield greatly simplified restriction endonuclease patterns.

Most of the grossly altered SV40 variants contain reiterations of the viral origin of replication (193a), confering upon them an advantage in replication (145, 146). Whereas these reiterated origin variants become predominant in AGMK cells, they remain a minor component of the viral population in rhesus kidney cells (193a). This might be explained by the relatively low plating efficiency of SV40 on the rhesus cells, since replication of these grossly defective variants is absolutely dependent on coinfection with WT virus. Regardless, the interference activities in these virus stocks do not necessarily correlate with the levels of grossly aberrant variants, since less defective variants with much simpler lesions also express interference (193a). Although the variants with simple lesions express homotypic interference, strictly speaking they are not DI particles, since DI particles are generally considered to contain deletions. Many of these variants produce T but not V antigen (190).

In addition to the emergence of defective variants, the infectious virus population in the SV40-rhesus kidney cell systems also undergoes changes. As in the cases of many other systems of persistent infection, involving a variety of viruses (see below), the large-plaque virus used to initiate the infection evolved into a population of small-plaque variants (187, 190). In one in-

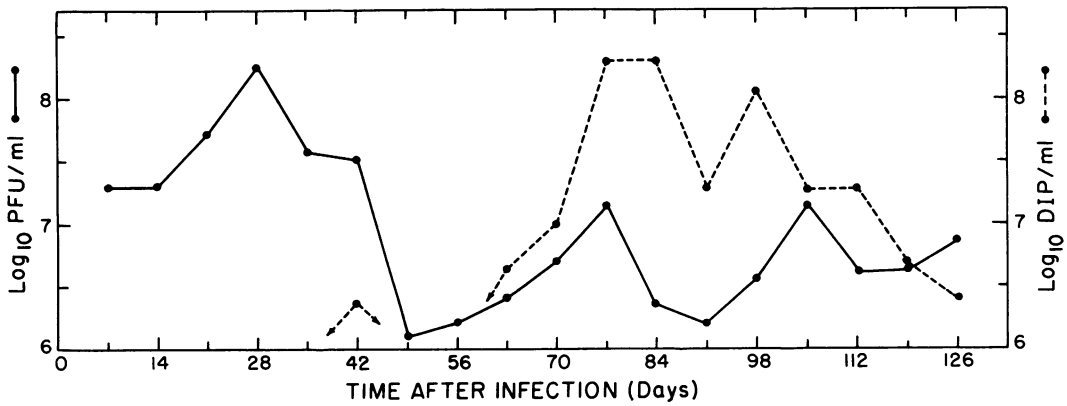

FIG. 1. Extracellular PFU and DI particle (DIP) titers in a culture of rhesus monkey kidney cells persistently infected with SV40. Samples of medium were harvested at the times indicated and titered for PFU and DI particles (as described in reference 190).

stance, small-plaque variants replaced the large-plaque WT virus between weeks 7 and 10 (187, 190). Even though some of these small-plaque variants contain lesions which are detectable by restriction endonuclease analysis, they nevertheless compete very effectively in mixed infections with the large-plaque virus (191). This may account in part for their capacity to replace the original large-plaque virus in the carrier system. Between weeks 14 and 28, the uncloned carrier culture stocks also become somewhat TS for replication (187). Viral temperature sensitivity does not appear to play a necessary role in the SV40-rhesus kidney cell carrier systems, because those persistent infections are stable after transfer to 33°C. Also, note that, whereas virus from the persistently infected rhesus cells is slower than WT virus to produce cytopathic effects in AGMK cells, it still generates a cytopathic crisis in those cells. These results indicate that, despite the emergence of a variant virus population, host cell factors play a major role in the establishment and maintenance of the SV40-rhesus kidney cell carrier systems. The action and role of the viral variants in these systems are discussed below.

Homotypic interference by the SV40 interfering particles is expressed subsequent to adsorption and penetration and requires interfering particle gene expression (190). Interference appears to result from a simple competitive interaction between defective and WT virus. Perhaps a WT viral gene product present in limiting amounts or the limited capacity of the host cell to support virus-directed biosynthetic activity (or both) is partitioned between defective and WT virus.

The interfering particles in these carrier cultures limit the number of cells which produce infectious virus and also limit the rate of infectious virus production by the producer cells (187). This was shown by comparative titration of infectious centers, intracellular virus, and viral yields in acutely infected cultures, and in both uncured and cured persistently infected cultures, some samples of which were superinfected with WT virus. There were, on the average, only about 10 cell-associated PFU of SV40 per virus-producing cell in the SV40-rhesus cell carrier systems. In contrast, acutely infected rhesus kidney cells contained on the order of 10^2 and 10^3 PFU per virus-producing cell at 48 and 72 h, respectively.

Virus in the SV40-rhesus kidney cell carrier system tends to remain cell associated (Fig. 2). Consequently, the relatively low levels of cell-associated virus in the carrier system are not explained by rapid release of virus into the extracellular fluid. Rather, the average 72-h total viral yield per productive carrier system cell is 10-fold lower than the total yield per infectious center in acutely infected cultures.

The SV40-rhesus kidney cell carrier system is resistant to superinfection by standard SV40 (187). However, carrier cultures cured by growth in antiserum are as susceptible to infection as are normal cultures. Thus, the low level of virus-producing cells in the carrier system and the diminished virus production by those cells are not explained by selection of genetically nonsusceptible cells, but instead involve the action of the variant virus population. Thus, these variants probably act to further stabilize the carrier system.

As in normal rhesus kidney cell cultures, only about half of the cells in the cured carrier cultures are initially susceptible to reinfection. This implies that the transient host cell resistance displayed by normal rhesus kidney cells continues to be expressed in the carrier system and that it probably plays a role in the maintenance of the persistent infection.

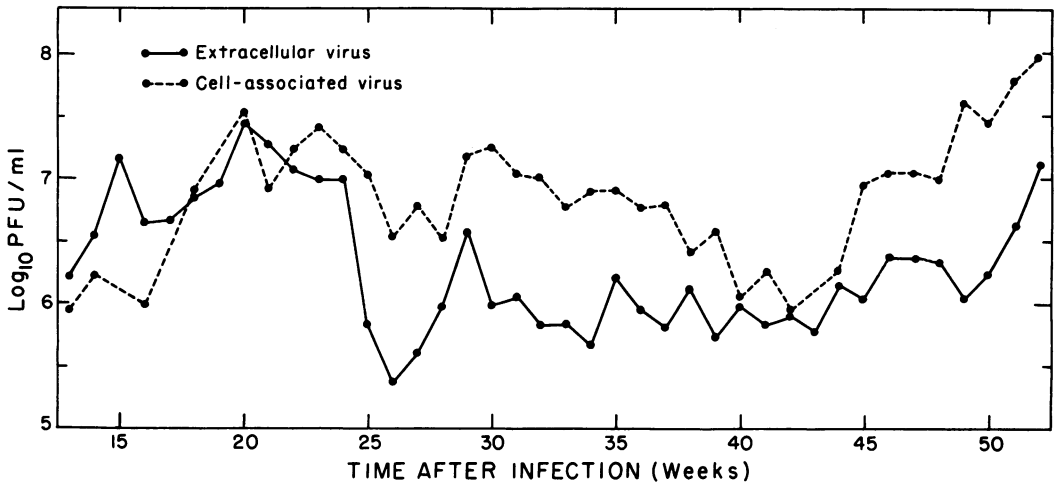

FIG. 2. Cell-associated and extracellular virus titers in an SV40-rhesus monkey kidney cell carrier system. At the times indicated, the extracellular medium was harvested, and the monolayers were washed with Hanks balanced salt solution, resuspended into a volume of medium equivalent to that of the extracellular fluid, frozen and thawed, and sonicated. Titers of PFU were determined by plaque assay on CV-1 cells.

These systems do not produce interferon, as indicated by their susceptibility to direct challenge with vesicular stomatitis virus (187). This is not surprising, since similar rhesus kidney cell cultures, unknowingly contaminated with SV40, were used for the preparation of poliovirus and adenovirus vaccines (271).

As noted above, persistent SV40 infections of AGMK cells are more readily established after infection at high than at low input multiplicities (158, 189). It was suggested that this might reflect a requirement for viral autointerference activity. Indeed, SV40 DI particles are rapidly generated in AGMK cells, provided that infection is at high input multiplicity (193a). There is also some evidence that high-input infections might induce or select cells which express partial or transient resistance (see below).

The probable importance of viral interference activity in the SV40-AGMK cell carrier systems is shown by studies with TS viral mutants (192). These mutagen-induced mutants express interference, like the variants which arise in the SV40-rhesus kidney cell systems, by competing with the replication of WT SV40. Consequently, they promote the survival of AGMK cells coinfected with WT SV40 at 37°C and thereby facilitate the establishment of persistent infection. The TS mutants are also more able than WT virus to establish persistent infections under conditions of single infection. These findings are consistent with the suggestion of Youngner and Quagliano (319a) that TS mutants are defectives of a sort, even though they are not deletion mutants (as DI particles are generally considered to be) and that they might play a role in persistent infections similar to that of DI particles.

Mutants with TS lesions in the early gene which encodes the T antigen and mutants with lesions in late genes which encode capsid proteins each express homotypic interference and promote the establishment of persistent infections (192). Thus, the capacity to express homotypic interference or to establish persistent infection is not restricted to any particular mutant group, and the establishment of persistent infection is not dependent on the expression of any particular SV40 gene product at WT levels. However, not all SV40 mutant groups have been examined in this regard. Also, as discussed below, virus-specific requirements for persistent infection in vivo might be more stringent, as suggested by studies with polyoma virus variants (167).

It is not yet clear why TS mutants are more able than WT SV40 to establish persistent infections of AGMK cells under conditions of single infection at 37°C. Perhaps the relatively slow growth of the mutants at 37°C and their consequent diminished cytopathogenicity (188) might delay cell destruction for a time sufficient to allow for the expression of other factors, such as the emergence of DI particles or cells which express partial or transient resistance (see below). In these systems, the original TS infectious virus population evolved to become temperature independent, although not necessarily WT. Furthermore, DI particles emerged and became predominant. Thus, DI particles play a more important role than TS virus at later times in

SV40-AGMK cell systems established by infection with TS virus.

AGMK cell carrier systems, established after mixed infection with TS and WT virus, stabilized despite the fact that the virus populations in those systems rapidly became temperature independent without the concomitant emergence of detectable levels of DI particles (192). These results might best be explained by the induction or selection (or both) of cells which express partial or transient resistance. The presence of a large fraction of such cells is implied by the following observations. In contrast to the established rhesus kidney cell carrier systems, in which all cells produced SV40 T antigen, only a few percent of the cells in the persistently infected AGMK cell cultures produced T antigen. Furthermore, the fraction of T antigen-producing AGMK cells and the total virus yields were unchanged after superinfection with WT virus at an input of 100 PFU/cell. Thus, only a small fraction of cells in these systems are able to support viral replication, and those cells are apparently doing so at their maximal capacity. Furthermore, whereas homotypic interference by DI particles and TS mutants is expressed as a simple competitive phenomenon during acute infection of AGMK cells, the block to virus production on a per-cell basis in the established persistent infections is expressed in an all-or-none manner.

Host cell resistance in the SV40-AGMK cell systems is transient or incomplete, and new virus-producing cells are recruited from the population of T antigen-negative nonproducing cells. This was indicated by the presence of T antigen-producing cells within clonal isolates of which most cells were T antigen negative. This finding also implies that resistance in the established carrier systems is not due to cellular genetic heterogeneity.

Somewhat similar results were obtained in the SV40-rhesus cell systems when these were superinfected with WT at high input multiplicities (187). That is, there was no increase in the small fraction of virus-producing cells. However, in contrast to the SV40-AGMK cell systems, there was an increase in the viral yields per productive rhesus kidney cell.

It has been claimed that SV40 induces resistance in AGMK cells by an unknown mechanism as a complex function of the input multiplicity (87, 88). However, fluctuation analysis suggests that, rather than being induced by SV40, resistant cells arise "spontaneously" during the growth of normally susceptible AGMK cells (307).

Regardless of whether resistant cells are induced or selected, there is evidence to suggest that the SV40 *A* gene, which encodes the T antigen, might play a role in the emergence of resistant cells (192). AGMK cell carrier systems, initiated by single infection with an *A* gene mutant, did not stabilize until temperature-independent virus emerged. However, carrier systems initiated by mixed infection with an *A* gene mutant and WT virus readily stabilized with relatively little cytopathic effect. In contrast, persistent infections established by single infection with a *B* mutant stabilized much more readily than persistent infections initiated by mixed infection with the *B* mutant and WT virus. Mutants with lesions in the *A* gene might be less able than *B* gene mutants to select for resistant cells, because the block to infection in resistant AGMK and rhesus cells is at an early stage of viral replication (187, 307). However, in at least some instances, the block is expressed before full uncoating and viral gene expression (307).

Note that the cells of the SV40-rhesus cell carrier system display several properties of SV40 transformation, including production of T antigen by all cells (187). Resistance in this system is not related to transformation, since the cured carrier system produces as much virus upon superinfection as acutely infected rhesus cells, despite the fact that all cells of the cured carrier cultures are T antigen positive. Thus, the maximal resistant state in the rhesus cell system depends on the continued presence of virus. This complicates the analysis of host cell resistance factors in the established carrier systems, since it is difficult to know the extent to which interference by defective virus contributes to overall resistance.

The underlying basis of the refractory state and the mechanism by which resistance diminishes in some cells to allow for productive infection are not yet clear. Because of its transitory nature, resistance might be related to the cell cycle or to the growth state of the cells. Viral multiplication and the appearance of cytopathic effects appear to occur more slowly in "resting" than in growing AGMK cell cultures (155a). The rate of viral replication is actually not diminished within virus-producing cells of resting cultures. Rather, the initiation of virus multiplication is seen to occur asynchronously in the cell population of those cultures. Studies with synchronized cell cultures showed that SV40 replication is dependent on a cellular function expressed near the very beginning of S phase (75, 206). Because that critical stage of the cell cycle occurs more frequently in dividing than in nondividing cell populations, dividing cultures should provide a better environment for viral replication. After infection, host cells entering or induced to enter S phase complete a single round of replication and then do not divide again (75). Note that the expression of

integrated viral sequences in SV40-transformed nonpermissive mouse cells is also dependent on events in the cell cycle. Viral transcription and T antigen expression in SV40-transformed mouse cells initiate in the G1 phase of the cycle (322). However, T antigen is produced independently of the cell cycle phase in acutely infected AGMK cells (75).

The relatively low susceptibility of resting cells to SV40 would not seem sufficient to account for the high level of resistance in the established persistent infections. Adaptation through selection of heritable cellular changes is also not a likely explanation, since uncloned SV40-rhesus kidney cell carrier cultures, cured by growth in antiviral serum, are as susceptible to SV40 as are normal rhesus cells. Nevertheless, adaptation through selection could in part underlie host cell resistance if less susceptible cells were also slower growing than the initial cells. Cells in which resistance was due to slow cell growth would lose their selective advantage in the presence of antiviral serum, and their relative frequency would diminish. This model is attractive because it accounts for the relatively slow growth of these carrier systems. For example, despite its expression of several characteristics of SV40 transformation, the established SV40-rhesus kidney cell system has a doubling time of 60 h, whereas normal rhesus cells have a doubling time of about 30 h (187). In this model, virus acts to modulate the productive infection by selecting for less susceptible, slower-growing cells as well as by expressing autointerference activity.

Resistance in some cells might also reflect the presence of overwhelming amounts of interfering virus particles. Resistance due to viral autointerference might also be transient, since the interfering particle activity, as well as the WT viral levels, cycle (Fig. 1). This cycling might be explained by the helper dependency of the defectives on the one hand and their capacity to interfere with WT virus on the other.

Susceptibility to infections may also be affected by changes in the state of cellular differentiation (155) and changes in the cellular chromosome complement (232). Because susceptibility is also related to the growth state of the cells, there probably are several classes of resistant cells. Indeed, some SV40-resistant cells are susceptible to infection with SV40 DNA, whereas others are resistant to infection with DNA as well as virions (18, 254, 307).

I recently isolated a total of 31 clones from two independent SV40-rhesus kidney cell carrier systems (unpublished results). Cloning was done in the absence of antiviral serum. Of the 31 clones, 20 produced SV40 T antigen. However, only two clones produced virus. All of the subclones of a virus-producing clone also produced virus. All 31 clones were resistant to superinfection with WT SV40. In the case of the virus-producing clones, this meant that upon superinfection, even at high inputs, there was no increase in the small fraction of virus-producing cells or in the viral yields. No interference activity was detected in the cellular extracts or extracellular fluid of any of the clones, and there was no induction of virus upon cocultivation or fusion with cultures of the CV-1 line of fully permissive AGMK cells. These results might imply that selection of genetically resistant cells did indeed take place, although further analysis of the clones will be required to establish this point. The failure of most clones to produce virus, even upon fusion to CV-1 cells, suggests that the latent viral genomes in these cells are defective. If such cells are present in vivo, then the expression in them of an incomplete SV40 genome might yet have important consequences for the organism.

Latent infection: state of the viral genomes and reactivation of the productive infection. Viral latency involves not only its establishment and maintenance, but reactivation of the productive infection as well. To understand the mechanisms of reactivation, it is necessary to know the state of the viral genomes during the nonproductive phase of the infection. The state of the viral genomes in our persistently infected rhesus kidney and AGMK cells has not yet been determined, although preliminary results show that some nonproducing clones of the SV40-rhesus kidney cell system contain episomal viral genomes (V. Steinberg and L. C. Norkin, unpublished results). Studies of other papovavirus-permissive cell carrier systems, discussed more thoroughly below, revealed viral genomes exclusively in an episomal state. The persistence of free viral DNA in these carrier systems is noteworthy because of the well-known presence of integrated viral DNA sequences in SV40-transformed nonpermissive cells of rat, hamster, and mouse origin, in which free viral DNA is generally not detected.

Defective as well as WT SV40 genomes can persist as episomes in both permissive and nonpermissive cells, provided that the viral sequences contain an intact early region. This was shown by the persistence in transfected rat cells of a hybrid episome containing the origin of replication and the entire early region of SV40 and an *Escherichia coli* sequence encoding a supressor transfer RNA gene in place of a portion of the SV40 late region (see reference 287, which shows that permissive monkey cells may carry free replicating defective SV40 genomes).

The percentage of cells persistently infected with an episomal viral genome must continually

diminish if the cell population were dividing and viral DNA synthesis did not occur or did not keep pace with cellular replication. This was demonstrated in a system of nonpermissive muntjak cells infected with SV40 in which each cell division reduced the fraction of T antigen-producing cells by half (76). In contrast, T antigen continued to be synthesized by most cells of a nondividing culture. These findings may be very important with respect to the negative results obtained in the screening of human tumor cell lines for BKV (see below), because the viral genome might persist in the tumors as an episome in a continually decreasing fraction of cells at a level below that detectable by current techniques (76). These results also indicate that episomal viral genomes might persist and remain biologically active in nonproductive nondividing cells, such as in the central nervous system (CNS).

Latent SV40 genomes may also reside in an integrated state in carrier systems of permissive cells, as shown by the following example (F. J. O'Neill, personal communication). Carrier systems of the TC7 line of AGMK cells were established by infection with SV40 inocula rich in DI particles. Several of these systems displayed T antigen in up to 65% of the cells and produced virus. However, after 8 to 10 months, the cultures began to lose T antigen, infectious virus, and free viral DNA. In each of the cultures, the unique defective present before the loss of WT virus and DNA could be reactivated upon superinfection with WT virus. Thus, the defective genomes not only effectively competed with and eliminated WT virus genomes, but they persisted in an apparently integrated state in cultures free of WT virus.

Both free and integrated SV40 genomes were detected in another system of transformed AGMK cells, of the CV-1 line in this instance (98a). The free viral DNA probably resulted from excision, since the cells were resistant to superinfection and were very inefficient at rescuing SV40 upon fusion with SV40-transformed mouse cells (126). Viral defectiveness and host cell resistance each play a role in the maintenance of this system.

Details on the arrangement of integrated SV40 genomes have been obtained from studies of SV40-transformed nonpermissive cells. These findings are considered below because they might be relevant to some persistant infections of permissive cells and because SV40-transformed nonpermissive cells also represent a kind of persistent infection.

The structure and organization of integrated SV40 genomes, as well as host integration sites, were revealed by Southern blot hybridization analysis (244) of transformed cell DNA samples cleaved with different restriction endonucleases, some of which do and some of which do not cleave SV40 DNA. These studies demonstrated that transformed cells may contain less than one, one, or several copies of complete or partial SV40 genomes integrated at different chromosomal locations (12, 129, 140). Also, there tends to be a disproportionately high representation of early gene sequences (10). In addition, the junction points between viral and cellular DNA contain viral sequences which map at different positions on the viral genome. Thus, the insertion of SV40 DNA into cellular chromosomes is not mediated by a site-specific recombination event, as occurs in bacteriophage λ lysogeny. After transformation, selection, and subsequent cloning, the chromosomal locations of the SV40 sequences in any cell line appear to be stable upon further cell passage (12, 129, 140).

Although the mechanism of the insertion event is still obscure, it probably does not occur by a simple reciprocal recombination process, since this would conserve the cellular sequences flanking the integration sites. Actually, both viral and cellular sequences are deleted or inverted by the integration event (12, 267). A transposon-like process is not likely, since the cellular DNA sequences adjacent to the integrated SV40 DNA do not display the direct repeat structures characteristic of transposition (266). Integration does not appear to involve long stretches of homology between the viral and cellular genomes, as suggested by the nucleotide sequences across the recombinational joints of defective SV40 variants which contain cellular DNA segments (84, 290) and by the sequence of the junction of an integrated SV40 genome with the cell genome in an SV40-transformed rat cell line (267). However, there are several examples in which short sequence homologies of 3 to 5 base pairs may have played a role in the formation of virus-virus and virus-cell recombinant junctions (84, 267, 311).

As noted above, one should not presume that SV40 latency in carrier systems of permissive cells involves integrated viral genomes. Nevertheless, integration probably occurs during productive infection, as implied by the generation of defective viral genomes which contain substitutions of cellular DNA (see, e.g., reference 144). Also, large quantities of SV40 DNA are associated with cellular DNA during productive infection (89, 109). Although more recent studies with electron microscopic and restriction endonuclease mapping techniques indicate that this viral DNA is organized into large polymeric molecules of wholly viral origin (230), they do not exclude the likelihood that a low level of integration occurs during productive infection.

Induction and rearrangement of integrated vi-

ral genomes. Productive infection may be activated when heterokaryons are formed between SV40-transformed rat cells and normal AGMK cells (134, 295). This probably reflects excision of viral DNA from rat cell chromosomes rather than the amplification of a persistent infection or of a viral plasmid carried within the transformed cells. The possibility of a persistent infection is eliminated in those nonpermissive cell lines which do not support SV40 DNA replication. Second, with the Southern blot technique, no free viral DNA could be detected in the transformed cells, even though those procedures would have detected one free viral genome per 100 cells (13). The transformed rat cells are not genetically heterogeneous for the capacity to yield replicating viral DNA, since they cannot be cloned to subclones which either yield or do not yield viral DNA.

A model for the excision event, which has as its first step the repeated initiation of viral DNA replication at the level of an integrated copy (the "onion-skin" or polythene model), followed by excision via any of the several plausible mechanisms, is suggested by the following observations (13). First, upon heterokaryon formation between SV40-transformed rat cells and permissive AGMK cells, there is an increase in the amount of viral DNA still associated with high-molecular-weight DNA before the appearance of free viral DNA. Second, the excision process is dependent on the expression of the SV40 *A* gene, which encodes the T antigen. SV40 mutants which contain TS lesions in the *A* gene are temperature sensitive for the initiation of DNA replication, and cells transformed by these mutants are temperature sensitive for the excision event (6, 13). The polytenized SV40 DNA sequences, created by in situ replication of the integrated viral DNA, are thought to form an unstable structure within which the excision event occurs.

The SV40 gene products that are required for excision are contained exclusively within the early region, as shown by rescue studies with cells transformed by restriction fragments of SV40 DNA (13). Whereas the *A* gene product (the large T antigen) is apparently required for excision, small t antigen, the other known product of the early region, is not required. Cells transformed by SV40 mutants that do not produce the small t protein can be induced to yield infectious virus upon fusion with AGMK cells (256).

In general, cell lines that contain tandem insertions of viral DNA yield infectious virus more readily than cell lines which contain single insertions. The latter cells tend to yield a heterogeneous population of noninfectious molecules (12, 261). Although tandem insertions of SV40 DNA may not be required for the excision of infectious DNA, this structural feature probably greatly increases the likelihood of precise excision, presumably by providing for recombination between homologous segments (13, 128).

Ordinarily, the structure and chromosomal locations of SV40 sequences in transformed nonpermissive cells appear to be stable during cell passage. However, alterations of the original integration structures can be detected after certain selection procedures. For example, phenotypically normal revertants of SV40-transformed rat cells, which initially contained a single copy of the viral genome, were isolated by exposing the cells to agents (e.g., 5-fluorodeoxyuridine) which are cytotoxic to replicating but not quiescent cells under conditions which favored the growth of the transformed cells (261). The revertants were found to fall into three classes with respect to the SV40 sequence: (i) the SV40 DNA was retained with no detectable alteration; (ii) the SV40 DNA was retained but underwent a deletion within the coding region for T antigen; and (iii) the SV40 DNA was lost, generating a cured cell. All three classes of revertants are T antigen negative. Also, all three classes of revertants can be retransformed, although in the case of class (i) this has only been accomplished by microinjection of viral DNA (78, 261). One might expect the superinfecting viral genomes in the retransformed cells to at least occasionally insert into the already integrated viral genome. However, the generation of a unique new insertion is apparently an invariable feature of retransformation.

In a related study, SV40-transformed mouse cells containing one viral genome per cell were injected into immunocompetent syngeneic mice (141). This resulted in the selection of tumor cells which no longer expressed the SV40 T, surface, and transplantation antigens. These antigenic activities reflect different sites on the same protein. A tumor cell line selected in this way and its subclones were found to have undergone a rearrangement of viral DNA sequences resulting in a deletion of most of the viral early region.

Somewhat related experiments were based on the dependence of the transformed cell phenotype on the activity of the SV40 *A* gene product in many (but not all; see reference 282 for general review) SV40-transformed cell lines (100). Clones of SV40 *tsA* mutant-transformed mouse embryo cells, which displayed temperature-independent transformation, were selected from a TS parental cell line. The integrated SV40 DNA sequences in these temperature-independent isolates were found to have undergone amplification and rearrangement, whereas clones isolated under nonselective conditions

maintained the integration pattern of the parental line. Furthermore, the new integration patterns of the temperature-independent isolates were found to be stable upon subsequent cell passage. Thus, in these instances the rearrangement of viral DNA sequences appeared to be specifically associated with the selection of temperature-independent clones, and the integration patterns were otherwise stable. Note that the temperature-independent clonal isolates generated patterns of integrated SV40 DNA which were characteristic for each clone. Most (9 of 12) also retained the original parental integration sites. Free viral DNA could not be detected in either the parental line or any of the clonal derivatives.

Several plausible mechanisms could be suggested for the amplification and rearrangement of viral DNA sequences as observed in the study described above. Among these is excision via the onion-skin model (13), which could generate free viral DNA molecules (capable of reintegrating) without necessarily destroying the original integration structure. Regardless, the investigators make the interesting suggestion that such processes might play an important role in vivo in the progression of a virus-induced benign tumor to an apparently virus-independent one with metastatic potential (100).

Recently, it was found that SV40 mutants with defective origins of replication are more efficient than WT SV40 at transforming semipermissive human fibroblasts (256a). Likely factors which might account for this are that (i) the mutants are unable to replicate, thereby sparing the cell, and (ii) the mutant viral genomes are probably more stably integrated than WT genomes. Regardless, these results suggest that the defective viral variants which arise in a persistent infection in vivo might have greater oncogenic potential than the WT virus in the natural host.

Polyoma Virus

Discovery. Polyoma virus was discovered after the observation that cell-free filtrates of leukemic AK mice induced fibrosarcomas and parotid tumors, as well as leukemia, when inoculated into newborn C3H mice (83). The initial samples were later found to also contain a leukemia virus. Upon subsequent isolation in tissue culture, polyoma virus (so named by Stewart and Eddy [264]), was shown to induce a wide variety of histologically distinct solid tumors. Polyoma virus is endemic in populations of feral and laboratory mice. Nevertheless, it is not associated with any disease, and tumorigenesis is only observed in newborn rodents under artificial laboratory conditions. Polyoma virus-induced tumors do not contain infectious virus or particles.

Interactions with cultured cells. As in the case of SV40, the interaction of polyoma virus with cells in culture is generally thought to result in either of two mutually exclusive outcomes, depending on whether or not the cells are permissive. Permissive cells support productive infection and undergo extensive cell destruction. Nonpermissive cells are abortively infected, and some may become transformed. However, infection of some permissive mouse cell lines results in the establishment of stable carrier systems. Some of these are reminiscent of the SV40-rhesus kidney cell system in that they continually produce virus but do not display cytopathic effects (92). Other polyoma virus carrier systems are more like the SV40-AGMK cell systems, which undergo periodic destruction followed by repopulation by surviving cells (97).

Persistent infection: roles of resistant cells and viral autointerference. A stable noncytopathic persistent infection of Earle's L929 mouse cells with polyoma virus has been described (92, 151). Among its noteworthy features, most cells carry complete infectious virus in their cytoplasm but not in their nuclei, and they display no obvious cytopathology. These cells may enter and complete mitosis, and the cytoplasmic virions are distributed between the daughter cells. Because only 1 to 3% of the cells are in a cytolytic cycle at any moment, the cytoplasmic virus reflects a nonproductive or a noncytolytic process. Its presence has no effect on cellular metabolic activity (i.e., oxygen and glucose utilization and lactic acid production), cloning efficiency, or cell generation time.

The cytoplasmic virus in the nonproductive cells was probably taken up from the medium after being released by the small proportion of virus-producing cells. This was further suggested by the curing of these cultures by cultivation in anti-polyoma virus serum. Thus, as in the SV40-rhesus kidney cell system, cell-to-cell transfer of the virus probably plays an important role in maintaining the productive infection.

Host cell resistance may play a role in the establishment and maintenance of the polyoma L cell system. This was suggested by the finding that the early viral T antigen gene was not expressed in most L cells after high-input-multiplicity infection. The fraction of T antigen-producing cells was equivalent to the fraction of capsid antigen-producing cells, implying that the block to infection is at an early stage in the viral replicative cycle. Furthermore, infection with polyoma DNA was much more effective than infection with whole virus in causing productive infection. These results, as well as the above finding of cytoplasmic but not nuclear virus in most cells, suggest that the block in the viral replicative cycle is at the level of uncoating.

Genetic heterogeneity among the cells is not responsible for the above results, since similar findings were obtained with cloned cell populations. Interferon does not play a role in this carrier system, as demonstrated by the equivalent plaquing efficiency of vaccinia virus on normal and carrier system L cells. Interestingly, pretreatment of normal or carrier system L cells with Newcastle disease virus did induce an interferon activity that interfered with vaccinia virus replication.

These studies suggest that the polyoma virus-mouse L cell carrier system is maintained by a form of host control that limits the viral uncoating process in all but a few cells at any time. However, a few cells relinquish this control and produce virus to maintain the productive infection. Autointerference activity is also present in this system. Although not examined in detail, it too probably acts to protect potentially susceptible cells.

Earlier studies of the susceptibility of clonal lines of mouse embryo fibroblasts to high-input polyoma virus infection and on the reinfectability of cultures derived from the surviving fractions of infected cultures also indicated that the surviving cells are not mutational in origin (302). Instead, resistance probably reflects transient fluctuations in the physiological state of the cells. It is known that the polyoma virus yield from infected 3T3 cells depends on the stage of the cell cycle at which the cells are infected. Yields are maximal when cells are infected at or near the beginning of G1 (281). This is reminiscent of the results obtained when synchronous populations of AGMK cells were infected with SV40 (206).

Integrated and episomal viral genomes. Little is known about the state of the polyoma genomes in carrier systems of permissive cells. In contrast, many studies of polyoma-transformed nonpermissive cells indicate that polyoma genomes are generally integrated into the host genome in a stable manner (282). However, studies of polyoma-transformed semipermissive rat cells (discussed below) suggest that both integrated and episomal viral genomes might play a role in polyoma virus persistent infections.

Populations of polyoma-transformed rat cells may contain on the order of 10 to 50 viral genome equivalents per cell, on the average. The majority of this viral DNA is found in an episomal state, although integrated polyoma genomes are also present. The integrated genomes generally occur in head-to-tail tandem arrangements of full-length and defective viral DNA molecules (6, 9). There is no specific region on the circular viral genome which opens upon insertion into the host DNA.

Only a small minority (0.1%) of the transformed rat cells actually contain nonintegrated polyoma DNA at any moment, as shown by in situ hybridization experiments with ^{3}H-labeled polyoma RNA as a probe (323). The presence of nonintegrated polyoma DNA in these cells is not due to a carrier state, since the cells do not produce infectious virus. However, virus could be rescued from those cells by fusion with permissive mouse cells (214).

Induction and rearrangement of integrated viral genomes: role of the viral T antigen. The above results suggest that the free polyoma DNA molecules in transformed rat cells reflect the spontaneous and periodic induction of viral DNA replication in a minority of the cell population. A higher percentage of the cells are potentially inducible, as indicated by the finding that 90% of all clones and subclones of a line of polyoma-transformed rat embryo cells are inducible, despite the fact that less than 0.2% of the cells spontaneously produce virus (62).

Induction of polyoma viral DNA synthesis in latently infected rat cells requires the functional activity of the viral A protein (T antigen). This was indicated by the finding that Fisher rat fibroblasts, transformed by a polyoma TS *A* mutant (encoding a TS T antigen), produced free viral DNA only at the permissive temperature (6). No free viral DNA was present in cultures propagated at the nonpermissive temperature, although even after months at the nonpermissive temperature, shiftdown to the permissive temperature resulted in the rapid reappearance of free viral DNA molecules. The free viral DNA molecules were probably derived from the integrated ones, since they faithfully reflected the main species of integrated viral DNA present in each of several different cell lines (73). These results suggest that free viral DNA is not maintained in a plasmidic state in this system, but instead always derives from the integrated form.

The requirement for a functional T antigen in the induction of free viral genomes could merely reflect the fact that T antigen is required for replication of the exised DNA. Alternatively, T antigen might actually be required for the excision event. The latter possibility is probably correct, as shown by the following. Revertants of polyoma-transformed rat cells were selected which had regained the normal phenotype (6). Loss of integrated viral sequences indeed occurred in some of these revertants, and, as shown by studies of cells transformed by TS mutants, functional polyoma T antigen was necessary for this to occur. The association of the viral DNA with the host genome appeared to be very stable in the absence of functional T antigen. However, in another system of polyoma-transformed rat cells, integrated viral sequences

were excised from partial head-to-tail duplications in the absence of a functional T antigen (142).

The mechanisms of excision in the above instances differ from the polytenic or onion-skin model, noted above with respect to SV40, since the loss of integrated viral sequences does not occur in the latter model (13, 100). Regardless, integrated viral DNA may also be lost from SV40-transformed cells, generating cured revertants (261). An intramolecular recombination event, involving homology within the head-to-tail tandem arrangement of integrated polyoma genomes, probably accounts for excision, since an integral number of viral DNA molecules are generally lost from the integrated tandem repeats (7). Furthermore, the amount of free viral DNA produced appears to be proportional to the amount of duplicated sequences present, suggesting that excision may be a function of the target size for homologous recombination processes (142).

Whereas rat cells are semipermissive for polyoma virus, they are nonpermissive for SV40. This might explain why SV40-transformed rat cells contain only integrated viral genomes (12, 129). It was therefore of interest to determine whether there is any interaction between polyoma and SV40 which might affect the state of the viral genomes in cells doubly transformed by these viruses. Note that these doubly transformed cells yield either polyoma or SV40 after fusion with the appropriate permissive cells (215). However, only polyoma genomes exist in a free state in the doubly transformed cells. These results are explained if either the association of SV40 DNA with rat cell DNA is much more stable than that of polyoma DNA, or if rat cells are completely lacking the factors necessary for SV40 replication, even in the presence of free polyoma genomes. These factors might be required for excision as well as replication, as noted above.

Because integrated polyoma DNA is not entirely stable and because excision generally leads to the loss of an integral number of viral DNA molecules from an insertion site, it might be expected that amplification of integrated viral DNA sequences might also occur in polyoma-transformed rat cells. This is indeed the case (27). Furthermore, this process is under the functional control of T antigen, which might be acting to promote excision, viral DNA replication, and perhaps reintegration. It is suggested that excision precedes amplification, since the amplified sequences which generally contain the origin of replication correspond to the families of free viral DNA produced by the transformed clones. Curiously, however, the amplified sequences appear only at the initial integration sites. There is no evidence for reintegration in other regions of the host genomes. In this regard, superinfection of SV40-transformed cells with SV40 did not result in integration of the superinfecting viral DNA into the already integrated viral DNA. Instead, DNA of the superinfecting virus was integrated into other chromosomal locations (13). It was suggested that unequal crossover events between homologous chromatids might account for both excision and amplification (174). Such crossover events would be expected to occur at a much higher frequency than the rare initial integration events.

Results somewhat similar to those obtained with polyoma-transformed semipermissive rat cells were also obtained with polyoma-transformed permissive mouse cells (21). A mouse cell line, transformed at the nonpermissive temperature with a polyoma TS mutant, produced virus or viral DNA in large amounts on transfer to the permissive temperature. When the organization of the integrated viral sequences in five subclones of this line were examined, it was found that, whereas the viral sequences of all the subclones could be assigned to a common integration site, the integrated sequences differed markedly from subclone to subclone, as if cell propagation had been accompanied by amplification, recombination, or both within the viral insertion. Unexpectedly, no correlation could be found between the abundance or the organization of the integrated viral sequences in the subclones and the degree of inducibility or the nature of the free viral DNA produced upon induction. One of the least inducible clones carried complete viral genomes integrated in tandems, whereas one of the most inducible clones had no such tandems. Furthermore, the latter did not even carry a complete colinear copy of the viral genome, since the continuity of the inserted genome was interrupted by the repetition of part of its sequences. This suggests the action of a mechanism for the selective production of nondefective viral genomes. Such a mechanism might act during excision or subsequent replication.

It is also of interest that superinfection of these clones with WT polyoma at the nonpermissive temperature resulted in the replication of the superinfecting virus but not of the integrated TS polyoma genomes, despite the ability of WT polyoma to complement the TS mutant in lytic infection (44). A likely explanation is that integrated genomes are not readily available for replication, presumably because they have to first undergo excision, which is a rare event. Another possibility suggested by the above is that excision might require a function distinct from the early viral function involved in replica-

tion. In this regard, it has been suggested that the SV40 T antigen synthesized in transformed cells might not be identical to that initiating the replication of viral DNA (138).

The arrangements of the resident viral genomes in a number of polyoma-transformed rat cell lines displayed an even greater instability when the cells were grown in vivo as tumor cells than when passaged in cell culture (143). In vitro cell lines, containing multiple inserts of polyoma sequences and producing a functional large (100,000 molecular weight [100K]) T antigen, generated tumor cells containing a reduced number of viral inserts. These in vivo-passaged cells no longer produced large T antigen or free viral genomes. In contrast, no changes were observed during in vivo cell propagation if the original cell lines contained only a single insert of viral sequences and did not produce a full-size large T antigen or free viral genomes. Thus, a selection appears to operate in vivo against those cells producing a functional large T antigen and, consequently, free viral genomes. The selection might reflect an antigenic property of the 100K large T antigen, which is associated with the tumor-specific transplantation antigen. The full-size 100K large T antigen is not present in hamster tumors induced in vivo with either polyoma virus or polyoma DNA (117). Perhaps a similar immunoselective process also acts to modulate polyoma virus persistent infections in mice. Note that all the tumor cell lines expressed the polyoma 55K middle and 22K small T antigen species. One or both of these proteins are thought to be responsible for polyoma transforming activity (see, e.g., reference 95).

Selection against T antigen-producing cells also occurs during in vitro propagation of polyoma-transformed rat cells (40). A possible explanation is that T antigen activity is associated with instability of the viral DNA integration. Its action could thus lead to loss of the transformed cell phenotype (6, 40). Note that the polyoma 100K large T antigen is required for the establishment but not the maintenance of cell transformation (282).

Viral variants in vivo. Variants of polyma virus and SV40, which contain lesions as long as 300 base pairs, may nevertheless be viable in cell culture (see reference 191 for additional references). Some of these viable variants contain lesions in coding regions. This is remarkable, because papovaviruses are generally thought to have evolved toward minimal genome size. Yet some of the DNA sequence, including parts of coding regions, appear dispensable. A likely explanation suggested by McCance (167) is that the requirement for viral viability in vitro might not reflect the requirements for viability in the whole animal, where the virus of course evolved. Thus, viable deletion mutants of polyoma virus were examined for their ability to grow and persist in their natural host, the mouse. Indeed, the replication of early region deletion mutants which are viable in vitro was reduced in mouse kidneys by 10- to 10,000-fold as compared with WT polyoma. Furthermore, in vivo the mutants failed to produce the persistent infection observed with WT polyoma. These results underscore the fact that care must be taken in the extrapolation of results from cell culture systems to the situation in the whole animal. It is also noteworthy that polyoma DNA is apparently free in the form I supercoiled configuration in persistently infected mouse kidneys.

A progressive multifocal leukoencephalopathy (PML)-like disease in mice. It is significant that a PML-like disease is associated with polyoma virus infection of nude mice (D. J. McCance, personal communication). This condition followed heterotransplantation with human tumors and was characterized by wasting and posterior paralysis. Polyoma virus, identified by serology and restriction enzyme analysis, was observed by electron microscopy in oligodendrocytes. This cell type appears to exclusively support JC replication in human PML (see below). Intracerebral inoculation of uninfected nude mice also induced a similar symptomology. Demyelination was observed in the CNS, presumably resulting from lytic infection of oligodendrocytes. As in human PML (see below), no inflammatory cells were observed in the CNS, suggesting that demyelination resulted from the direct cytopathic effect of the virus rather than an immunopathogenic process.

BK Virus (BKV)

Isolation. BKV was first isolated by Gardner et al. (72) from the urine of a renal transplant recipient who had undergone immunosuppressive therapy. BKV has since been isolated from the urine of other individuals with impaired immunity caused by prophylactic immunosuppression, genetic defects, or diseases (see references 71, 197, and 200 for reviews). BKV has also been obtained from a kidney specimen (123) and from a brain tumor of patients with Wiskott-Aldrich syndrome (278), a rare genetic disorder characterized by defects in both humoral and cellular immunity.

Epidemiology. BKV is ubiquitous in the human population, as indicated by the presence of anti-BKV antibodies in about 80% of the human inhabitants of the United States and England. The distribution of BKV is apparently worldwide, with the exception of a few remote populations (17, 70, 200). These results, together with

the preferred replication of BKV in humans cells in vitro (161), imply that humans are the natural host of BKV.

BKV is now recognized as one of five known primate papovaviruses. The others are SV40 from the rhesus macaque, stump-tailed macaque virus, SA12 virus from the chacma baboon, and JCV of humans. There are now many data in support of the biological relatedness of these viruses (39, 161, 185).

Despite the ubiquity of BKV, it has thus far been isolated only from immunocompromised individuals. The nature of the primary infection is not known, but it is not likely to be associated with serious illness, since infection is so common. The route of transmission is also not yet known, although BKV must be easily transmitted to maintain such high incidences of infection. In some instances, seroconversion has been associated with respiratory tract infections (156).

It was proposed that BKV might be transmitted congenitally because of the finding of a significant rise in anti-BKV antibodies in the sera of 6 of 80 women during pregnancy. Furthermore, anti-BKV antibodies were found in the immunoglobulin M fraction of cord blood from three of their six babies (272). More recently, it was confirmed that latent BKV infections are reactivated during pregnancy (248). Nevertheless, no BKV-specific immunoglobulin M could be detected in over 300 umbilical cord sera specimens which were examined (248). Thus, whereas reactivation of these viruses is common in pregnancy, infection in utero either does not occur or is very rare. Note that the frequency of congenital infection is very low in other infections which are known to be transmitted congenitally (42). Thus, examination of 300 umbilical cords might not have been sufficient to detect congenital BKV infection occurring at low frequency.

It is possible that serum antibody protects the fetus, as suggested by the finding in mice that polyoma virus can be transmitted to the fetus if infection occurs for the first time during pregnancy but not if pregnancy reactivates a previously latent infection (168, 169). This finding is potentially important, since there has not yet been an opportunity to examine the consequences of a primary papovavirus infection during human pregnancy.

Because in utero BKV infections of humans have not been documented, the possible consequences of such infections to the fetus are unknown. It may be relevant that SV40 (as well as rubella virus) was isolated from the cerebrospinal fluid of a newborn child suffering from neurological and anatomical anomalies (16). The significance of this isolation is not clear.

There are generally no clearly identifiable clinical syndromes associated with seroconversion and excretion of BKV, although several transplant patients developed uretral stenosis with shedding of inclusion-bearing cells (31, 102), and others displayed pancreatitis (22). Thus, the long periods of BKV excretion observed in transplant recipients (30) might be associated with late complications of transplantation. Indeed, rising BKV antibody titers are significantly correlated with gradually deteriorating renal function and transplant-related complications (102). For this reason, the effects of interferon on BKV infection in renal transplant recipients was studied (22). Neither seroconversion nor excretion was reduced by human leukocyte interferon. BKV is also relatively resistant to interferon in vitro. In contrast, SV40 is quite sensitive (312).

Oncogenicity and possible role in human malignancies. BKV is highly oncogenic in vitro. It transforms cultured cells from a variety of animals, including hamster, rat, mouse, rabbit, African green monkey, and rhesus monkey (see reference 197 for a review). Cultured human cells persistently infected with BKV may also express certain phenotypic properties of transformation (276). It is particularly oncogenic in rodents after either intravenous or intracerebral inoculation. The virus expresses a broad cell tropism and, consequently, a broad oncogenic potential, as indicated by its induction of ependymomas, choroid plexus papillomas, malignant insulinomas of the pancreatic islets, osteosarcomas, fibrosarcomas, adenocarcinomas, angiosarcomas, lymphomas, and seminomas (34, 36, 81, 249, 286). Attempts to recover BKV from these tumors have not succeeded.

Despite the oncogenicity of BKV in cell culture and in animals, conflicting reports exist regarding its role in human neoplasia. BKV DNA sequences were detected in the DNA from 5 of 12 human tumors and in 3 of 4 cell lines derived from human tumors (61). However, other extensive efforts, using serological approaches or searches for BKV nucleic acid sequences, did not demonstrate an association between BKV and human neoplasias (35, 116, 250, 309). Because BKV is shed in the urine of immunocompromised individuals and because the related JCV is associated with PML, it is noteworthy that urological tumors, renal tumor cell lines, brain tumors, and brain tumor cell lines were each examined and that negative results were obtained in each instance. Thus, it is unlikely that BKV is commonly associated with human cancer.

Persistent infections of human cells in culture: virus-cell interactions and state of the viral genomes. Although cultures of a variety of human

cell types (e.g., fibroblasts, fetal kidney, fetal brain, and endothelial cells) are usually completely destroyed by BKV infection (58, 277), persistent infections of these cultures may nevertheless be established. For example, a few cells of a culture of human fetal brain cells (containing a mixture of spongioblasts and astrocytes) may survive a massive BKV infection and eventually form visible colonies. From these colonies, permanent cultures can be established which continuously shed BKV (276). These cultures also display several phenotypic properties of transformation and are tumorigenic in athymic nude mice.

The BKV-human fetal brain cell carrier system displays interesting virus-cell relationships not previously observed in other papovaviral systems. For example, unlike previously described papovavirus persistent infections, only a small percentage of the cells express the intranuclear T antigen. The T antigen-producing cells apparently correspond to a similar number of virus-producing cells. The cultures could be cured of T antigen and virus by cloning in the presence of BKV antiserum followed by prolonged growth in antiserum-containing medium. Surprisingly, the T antigen-negative, virus-free clonal isolates continued to display the transformed phenotype. Furthermore, the BKV genome was still present in all of the cured clonal lines, but only in an episomal state. Restriction endonuclease digests of the free viral genomes were indistinguishable from that of the standard BKV used to initiate the infection. Thus, a remarkable feature of this system is the persistence of latent homogeneous and apparently nondefective, free BKV genomes, even after several years in culture. This probably accounts for the "spontaneous" release of virus by some of the clones after removal of antiserum. It is not clear why some of the clones release virus in antiserum-free medium whereas others do not, although it is likely that cell factors might be important in this regard.

Transformation in the BKV carrier system described above is unique in that it is associated with episomal rather than integrated viral genomes and is not dependent on T antigen, as detected by immunofluorescence or complement fixation. In this regard, a BKV-induced hamster tumor cell line was recently described in which the early region of the single resident viral genome was separated into two portions by the integration event (213). Consequently, production of full-size large T antigen was precluded in that cell line. The expression of the small t antigen is apparently also unnecessary for transformation by BKV, since the MM strain is able to transform cells (162) despite its inability to encode small t antigen (245). These results indicate that expression of a complete BKV early region is not required for transformation by this virus. Perhaps a portion of the large T antigen, which does not react with anti-T serum but which retains transforming capability, is produced in these systems. Thus, it would be of interest to know the extent of early viral gene expression in the T antigen-negative transformed cell lines.

A persistent BKV infection of secondary human embryonic kidney cells has also been described (218). As in the case of the glial cell system described above (276), infection of the kidney cells initially generated massive cell destruction. This was followed by the emergence of surviving colonies of virus-producing transformed cells, which could be maintained in culture without any noticeable cytopathic effects. In contrast to the BKV-glial cell system, which is deficient in T antigen production, all cells of the BKV-kidney cell system expressed the BKV T antigen. As in other papovavirus carrier systems, only about 1% of the cells produced V antigen. The BKV-kidney cell system was also resistant to superinfection with BKV. Nonintegrated, superhelical BKV DNA was present in uncloned cultures. This was expected, since the cultures were producing virus. The viral DNA was essentially wild type, as suggested by restriction endonuclease digestion analysis. Integrated viral DNA sequences could not be detected in uncloned cultures by using the Southern technique. However, in contrast to the BKV-glial cell system (276), analysis of T antigen-producing clonal isolates revealed the presence of integrated BKV DNA sequences corresponding to the early region of the BKV genome. Free viral DNA could not be detected in the clones, again in contrast to the situation in the BKV-glial cell system. The small fraction of virus-producing (V antigen-positive) cells might account for the free DNA in the uncloned cultures (approximately 20 copies of BKV DNA per cell, on the average). The remaining nonproducing cells probably contain only integrated sequences, which might not be detectable in the uncloned cultures for several possible reasons (218). Regardless, comparison of the BKV-kidney cell system with the BKV-glial cell system again reveals the capacity of the host cell to determine important parameters of the persistent infection.

State and expression of the BKV genome in BKV-induced tumors and in cells transformed in culture. Several lines of hamster cells, transformed either in vitro or in vivo with BKV, display a variety of situations with respect to the state and expression of the resident viral genomes. In a line of T antigen-producing fibrosarcomas, the great majority of the BKV DNA

appeared to be present as free molecules, all apparently lacking a portion of the late region (280). As expected, virus could not be rescued from this line. Thus, free viral DNA persisted in a system in which the DNA was defective and the cells were nonpermissive.

In a line of BKV-transformed baby hamster kidney (BHK)-21 cells, BKV genomes persisted in an apparent integrated state (194). These genomes could be rescued by fusion with permissive human embryonic kidney cells, indicating that they were nondefective, as had been implied by restriction endonuclease analysis. Spontaneous virus release from the nonpermissive hamster cells did not occur, indicating that a carrier state did not play a role in the perpetuation of the viral DNA. Despite the presence of nondefective viral genomes in this system, no T antigen production was detectable by immunofluorescence, immunoperoxidase staining, or immunoprecipitation. The basis for the lack of expression of T antigen by the nondefective integrated BKV genomes is not clear. In a system of BKV-induced hamster choroid plexus carcinoma cells, the early region of the integrated viral genome was split by the integration event (313), thereby precluding T antigen production. A similar BKV integration structure might exist in the BKV-transformed BHK-21 cell line. Regardless, the findings with that system imply that the presence of a complete T protein in the cells before fusion is not necessary for successful BKV release. As noted above, similar findings were obtained in a study of polyoma-transformed rat cells (142). Likewise, the complete BKV large T protein is not required for transformation in vitro or in vivo (see also reference 313).

Other lines of BKV-induced hamster tumor (314) and transformed (8) cells have been described in which the viral DNA is present in a free form. The presence of nonintegrated genomes in these lines is again not the result of a carrier state, since the viral DNA was either defective, or virus could not be detected by electron microscopy. Free defective viral genomes can apparently replicate in these systems, provided that they contain the origin of replication and an intact early region (314). T antigen seems to be produced in those systems in which free genomes persist.

The host cell type appears to be a factor in determining the state of the resident viral genomes in BKV-induced tumors in rodents. For example, tandem integrations of full-size linear BKV DNA are a feature so far common to all ependymomas, whereas osteosarcomas and insulinomas generate Southern blot patterns which suggest individual integrations (23). Furthermore, free viral genomes were also present in the osteosarcomas and insulinomas, but these could not be detected in the ependymomas.

The viral integration patterns of a number of BKV-induced tumors were each different, indicating that viral integration could take place at many different sites on the cellular and BKV genomes. Similar findings in the SV40 and polyoma systems are noted above. Also note that the rearrangement of integrated viral sequences takes place in cells cultivated from the BKV-induced tumors. Furthermore, free viral DNA appeared to be lost upon cultivation of the osteosarcomas.

BKV neurotropism. There is an extremely high incidence of ependymomas among BKV-induced tumors. Indeed, 72% of the tumors induced by BKV in hamsters are ependymomas, even when the virus is injected intravenously (34). A factor in this regard might be the possible tendency of BKV DNA to persist in the brain. Although BKV DNA sequences are generally detectable only in neoplastic tissues, traces of BKV DNA have also been detected in an apparently normal portion of the brain of an inoculated hamster that had developed an ependymoma (23). Viral DNA was also detected in the brain, but not the liver, of another animal 15 days after inoculation (23). BKV DNA sequences were not detected in various organs, including brains, of noninoculated animals.

An unconfirmed report of BKV DNA in a human cerebella-spongioblastoma (61) suggests a possible causative relationship between BKV and human brain tumors. Nevertheless, as noted above, other extensive efforts failed to demonstrate an association between BKV and human neoplasias.

JC Virus (JCV) and PML

Etiology and course of PML. Although PML is a rare disease, it is of great interest because it represents the first reported instance of a virus-induced demyelinating disease in humans. Furthermore, convincing evidence that PML is caused by papovaviruses (203,300) provided the first indication that these viruses can cause disease in humans.

The etiology of PML was somewhat complicated by the isolation of a virus very similar or perhaps identical to SV40 from two PML patients (237,300) and JCV from all other cases (183,203). BKV has not been associated with PML. The diseases of patients from which SV40 was isolated differed in no significant way from JCV-associated PML (165,183; see below). Note that a papovavirus-associated disease with striking similarities to human PML occurs in the rhesus monkey (82).

PML usually arises as a complication of a grave underlying illness associated with states of

cellular or combined cellular and humoral immunodeficiency. In most patients, immunodeficiency results from lymphoproliferative disease (e.g., Hodgkin's disease, chronic lymphocytic leukemia, and lymphosarcoma). Other cases have been associated with other malignancies, sarcoidosis, tuberculosis, prophylactic immunosuppression of renal allograft recipients, or therapy for systemic lupus erythematosus or rheumatoid arthritis. The consistent deficiency appears to be one of cell-mediated immunity. However, PML may rarely be observed in patients not displaying any recognized deficiencies in either the humoral or cell-mediated systems (see, e.g., reference 233). In this regard, note that viral infections are normally eliminated by cellular immune mechanisms and that humoral immune mechanisms are inadequate for their control. Recent in vitro tests of lymphocyte responses to antigen and mitogen stimulation suggest that PML is associated with a specific defect in cell-mediated immune responsiveness to JCV which is part of a general but variable depression in cellular immune function (306). A selective loss of cellular immune responsiveness to JCV might account for those cases of PML in which there was no established underlying disease.

PML generally develops only in adults, usually during the fifth to seventh decades of life. However, a case of PML associated with JCV infection was seen in an 11-year-old boy suffering from severe immunodeficiency disease (327).

The course of PML is relentlessly progressive, with death generally occurring 3 to 6 months after the onset of symptoms. Because of the unusually long survival of the two patients from which SV40 had been isolated (300), it was suggested that the SV40-PML viruses might be less virulent than the JC-type virus (53). However, patients with JCV PML have also had longer than usual survival periods. Furthermore, there were no obvious differences between the SV40- and JCV-associated cases with respect to either histopathological changes or viral concentrations in the brain (165, 183).

PML symptoms may include visual disturbances, progressive mental deterioration, focal motor weakness, atoxia, and aphasias. Symptoms are variable, apparently depending on the size and sites of the lesions. The lesions are widely disseminated, being most often found in both the gray and white matter of the cerebrum, although other parts of the neuraxis may also be affected.

PML lesions: differential cell susceptibility in the disease process. PML lesions are plaque-like, representing foci of demyelination. Oligodendrocytes, the cells which maintain the myelin sheaths, appear to be lytically infected. They are depleted within demyelinated foci and are packed with virus at the periphery of the lesions. Note that papovavirus concentrations reach extraordinary levels in PML brains, exceeding 10^7 to 10^{10} particles per g of tissue (see reference 48, which identifies the cell types in PML lesions in which JCV DNA is found).

Astrocytes, which are the other major glial cells of the white matter, are also found to be affected within the foci of demyelination. They become enlarged and distorted and display bizarre nuclei and mitosis. Furthermore, they resemble the malignant astrocytes of glioblastomas (328). The aberrant astrocytes are apparently abortively infected, since they occasionally contain virus in their cytoplasms but only rarely in their nuclei (165). Thus, lytic infection of oligodendrocytes and abortive transforming infection of astrocytes might account for the demyelinating foci which display the bizarre astrocytic proliferation characteristic of PML. Also note the simultaneous occurrence of brain tumors in a few PML cases (20, 229). Two patients had gliomas, which in one case appeared to correspond topographically to the demyelinating foci (20). Neither neurons nor cerebrospinal fluid are affected in PML.

The hypothesis that oligodendrocytes are productively infected, leading to demyelination, whereas astrocytes are abortively infected, thereby acquiring aspects of the neoplastic phenotype, is supported by in vitro studies. When cultures of human fetal glial cells were infected with SV40, spongioblasts, but not astrocytes, underwent lysis (253). Spongioblasts are believed to be precursors of oligodendrocytes. The astrocytes in those cultures became transformed. Similar results were obtained in another study in which cultures of fetal brain cells of either simian or human origin were each infected with either SV40 or BKV (196).

The premise that oligodendrocytes are productively infected in PML whereas astrocytes are transformed is also supported by studies in which ^{3}H-labeled JCV RNA was hybridized in situ to brain sections from a PML patient (48). Autoradiography was then used to identify those cells which contained JCV DNA. The complementary JCV RNA specifically hybridized to cells in and around the demyelinated lesions. This is the area in which virus particles are observed by electron microscopy. The majority of the positive cells were oligodendrocytes, whereas only a minority of the astrocytes displayed silver grains. The small percentage of positive astrocytes probably corresponds to the astrocytes which contain virus in the disease.

Autoradiographic analysis also revealed a positive reaction in vascular endothelial cells (48). This may have important implications with

respect to the pathogenesis of PML. In this regard, note that the host range of JCV in vitro appears to be extremely limited (198), a fact which delayed its isolation until 1971 (202). The virus was isolated in primary human fetal glial cell cultures in which the most susceptible cell type is the spongioblast. JCV has not been grown to any useful extent in any other cells except secondary human amnion cells (275) and perhaps human vascular endothelial cells (58). Thus, the possible ability of JCV to replicate in the vascular endothelium might account for its ability to enter the CNS and also for the general occurrence of the foci of demyelination around blood vessels. Of related interest, a study of brain sections of Sindbis virus-infected mice showed that infection of endothelial cells of the small vessels of the brain preceded infection of the surrounding neurons and glial cells (119).

At present, nothing is known about the extraneural sites of JCV replication or, for that matter, of its mode of transmission and the nature of the primary infection. However, the importance of blood-borne virus and the maintenance of adequate viremia in the invasion of the CNS have been demonstrated for a number of other viruses (59).

Cellular infection and cytopathogenesis in PML. Although the first stages of viral replication in PML brains are not known, the ultrastructural characteristics of papovavirus adsorption and penetration into the oligodendrocytes of PML lesions have been described (166). The mechanism by which the virions penetrate the brain cells appears similar to that previously characterized as "pinocytosis" in cell cultures (5, 114). Virions appears to sink one after the other between the deeply invaginated oligodendrocyte cell membranes. The sides of the hollows above the virions then close, thereby forming vacuoles. Thus, the majority of the virions are initially found either in a system of labyrinthine cisterna or in vacuoles, but always surrounded by membranes. The morphological features of the subsequent uncoating phase have not yet been demonstrated, although studies of cell culture systems indicate that uncoating occurs in the nucleus (5). Perhaps virions enter the nucleus through the cisterna system or by reverse pinocytosis (163).

An immune mechanism of demyelination in PML is highly unlikely, since the patients do not display any signs of an inflammatory response, have normal cerobrospinal fluid, and usually have an associated immunological deficiency. Thus, demyelination is considered to result directly from cytolytic infection of the oligodendrocytes and the consequent loss of their cytoplasmic extensions which make up the myelin sheaths.

The mechanisms by which viruses damage and kill cells are still largely unknown (see reference 4 for a review). Nevertheless, there are reasons to believe that changes in the permeability and function of cellular membranes are major factors leading to cell killing by the papovaviruses (56, 57, 188). This was indicated by the SV40-induced release of cellular enzymes, the shutdown of membrane-associated electron transport, an increased permeability to deoxyglucose, an increase in the intracellular Na^+/K^+ ratio, and swelling of cells and organelles. These cytopathic effects are considered to be antemortem because they precede the loss of ability to exclude trypan blue dye, which provides a valid assessment of lethal (i.e., irreversible) cell damage (239, 284). Furthermore, these cytopathic effects preceded any decline in cellular levels of ATP and protein synthesis. Thus, the severe alterations in cellular membranes which these cytopathic effects reflect could be, at least in part, responsible for subsequent cell death. Also, altered membrane permeability appears to be a major determinant of lethal cell injury in several nonviral systems of cytopathology. Furthermore, a common sequence of ultrastructural alterations occurs during lytic SV40 infection and in the nonviral systems of cytopathogenesis (57, 284). The sequence includes swelling of endoplasmic reticulum, followed by condensation of mitochondria and finally swelling of most other membrane systems, including mitochondria and nuclear membrane. Note that lysosomes appear to be relatively stable structures which remain intact until cellular injury has progressed to postmortem stages (56, 57).

Studies of PML biopsies showed that affected oligodendroglia undergo a course of cytopathogenesis similar to that observed in SV40-infected AGMK cell cultures (57, 165, 166). These oligodendroglia, at the periphery of the demyelinated areas, became swollen, and many myelin sheaths in these areas were vacuolated (165, 166). However, their axons were generally well preserved. The affected oligodendroglia also had enlarged nuclei which were filled with both icosahedral and filamentous forms of the virus. Furthermore, swelling of mitochondria and cytoplasm and the breakdown of endoplasmic reticulum and polysomes were observed. As the progeny virions in the nuclei increased in number, the vacuolization of the cytoplasm and breakdown of polysomes became more severe. The breaking up of the membranes of the cytoplasm was followed by disintegration of the nuclear membrane, releasing virus into the severely damaged cytoplasm or directly into the extracellular space.

The above findings are consistent with the premise that altered membrane permeability,

leading to loss of cellular and intracellular compartment volume regulation, is a major determinant of cell killing by papovaviruses both in vitro (57) and in PML. In this regard, note that there were no ultrastructural differences which could distinguish an SV40 case from a JCV case of PML (165). Also note that there were no major differences in the cytopathic effects induced by SV40 and BKV during in vitro infections of cultures of fetal brain cells of either simian or human origin (196). Although cytopathogenesis progressed more slowly in the BKV-infected cultures, cytoplasmic vacuolization (the classic SV40 cytopathic effect) developed in all BKV-infected cultures.

In the infections of fetal brain explant cultures noted above, SV40 and BKV were each able to infect and lyse not only oligodendrocytes, but also neuroblasts and mesenchymal cells as well. This is in contrast to PML, in which only oligodendrocytes and, rarely, astrocytes appear to support productive infection. It was suggested that less differentiated or dedifferentiated cells in culture might be more susceptible to infection than their in vivo counterparts. Regardless, these results, as well as the fact that BKV is not associated with PML, underscore the importance of both viral and host factors in slow virus disease. These factors are presently not well understood.

Papovavirus particles have been observed in intimate relationship with the periphery of the myelin sheath (207, 326). More recently (166), virions have been observed between the separated lamellae of the myelin sheath. These virions, which are in an extracellular position, probably reflect the attraction of papovaviruses to cell membranes and are not considered to play any role in demyelination.

JCV persistence: roles of differential cell susceptibility and viral variants. The factors which underlie persistent papovavirus infections in vivo may be similar to those which are most likely to account for persistent infections in vitro. As described above, these factors include the presence of a subpopulation of restrictive cells and the interfering activity of defective viral variants. The role of differential cell susceptibility in PML, although still a matter of conjecture, is suggested by the findings in both PML brains and in cell cultures that virus grows in and selectively destroys oligodendroglial cells, whereas it undergoes highly restricted replication in astrocytes, which may become transformed. Even the spongioblasts appear to display differential sensitivity to JCV. For example, 5 days after JCV infection of primary human fetal glial cells, only 2% of the cells contained V antigen, whereas 60% of the cells contained T antigen (198). JCV infection of human amnion cells gave somewhat similar results (275). Note, however, that these results might also be explained by the presence of T antigen-producing defective virus in the inoculum.

Other cells which have been tested in vitro for their susceptibility to JCV include primary human embryonic lung, kidney, intestine, and liver, primary human adult testes, two human diploid cell lines (L-809 and WI-38), a human heteroploid cell (Chang conjunctiva), and several primary and continuous cell cultures of simian, hamster, mouse and mink origin (198). Negative results were obtained in each instance. Nevertheless, it is likely that in vivo JCV grows in sites other than the brain, since it is a ubiquitous virus and infection with it is a common childhood event (see below). Thus, the restricted host range of JCV in vitro might reflect properties of cells in culture and not be indicative of host cell susceptibility in the whole organism. More recently, it was reported that, although human embryonic kidney cells are quite resistant to JCV, the virus can be adapted to growth in those cells (178).

Other aspects of the virus-cell interaction, as observed during in vitro infection of primary human fetal glial cells, might also play a role in vivo. For example, even in permissive spongioblasts, viral replication and the development of cytopathic effects occur slowly. Furthermore, the virus tends to remain cell associated, thereby limiting its spread (198).

It is not yet possible to assert whether or not defective virus plays a role in PML. Light viral particles, which contained a reiteration of about 40% of the SV40 genome in a molecule about 80% as long as standard SV40 DNA, were present in an SV40 PML isolate after three passages in human fetal brain cells and one passage at high multiplicity of infection in AGMK cells (see reference 160, which describes SV40 variants which may have arisen in the PML lesion). It is not known whether these variants were present in the original isolate or arose during the subsequent passages. Similarly, JCV DNA samples from JCV PML isolates passaged in primary human fetal glial cells have also been found to be heterogeneous (65, 111, 195). JCV virions grown in human amnion cells (275) or human embryonic kidney cells (178) also contained heterogeneous DNA. Findings of this kind, obtained by using plaque-purified virus as inocula, indicate that JCV, like SV40 and polyoma, is unstable upon passage in vitro. Thus, JCV might very well also generate defective particles in vivo. Unfortunately, this instability also implies that one cannot use stocks of PML isolates which have been amplified by in vitro passages to determine whether defectives were present in the initial isolate. In recognition of

this problem, JCV DNA was purified directly from the brains of two PML patients and cloned in *E. coli* by using the vector pBR322 (228). Uncloned and unpassaged JCV DNA from one of the isolates showed a minor band upon electrophoretic analysis, indicating that it might be heterogeneous in size. No minor species of DNA were detected in the other isolate. Comparison of the cloned samples with each other and with the laboratory-adapted prototype JCV (Mad-1 strain) revealed only slight differences between strains, limited to the noncoding region located to the late side of the origin for DNA replication. Note that extensive variations in this region do not necessarily affect the viability of either polyoma or SV40 and perhaps JCV as well, although they might affect the viral host range (127, 191, 225, 246a, 317).

In another study, JCV particles were purified directly from human brain (48). The single peak of dense virus obtained in cesium chloride suggested that most, if not all, of the virus particles in that brain were complete. However, analysis of the DNA was not possible, because not enough material was available.

The results discussed above suggest that the heterogeneity of the JCV DNA, observed when PML samples have been passaged in cell culture, is largely, but probably not entirely, the result of in vitro passage. The minor species of smaller supercoiled DNA obtained directly from one patient (228) indicates that viral heterogeneity might also be a factor in PML. Nevertheless, although viral defectiveness might in part account for slow viral replication and cytopathogenesis, more brain samples will have to be analyzed directly to determine whether viral heterogeneity plays a role in the pathogenesis of PML. Furthermore, biological studies, as well as DNA analysis, should be carried out because papovavirus DI particles may indeed have lesions too small to detect by restriction endonuclease digestion analysis (190).

Epidemiology of JCV infection. Although JCV has thus far been isolated only from PML patients, its epidemiology is similar to that of BKV. For example, both viruses have been detected in the urine of normal pregnant women (32) and renal allograft recipients (102). Seroepidemiological studies indicate that JCV infection is common in humans, with about 70% of adults seropositive for JCV antibodies (199, 200). Seroconversion appears to occur most frequently in 10- to 14-year-olds, 65% of whom had antibodies to JCV. Humans also appear to be the natural host of JCV, as there is no known animal reservoir for this virus (198). The distribution of JCV is also worldwide, with the exception of a few remote populations (17, 71, 200).

Because infection with JCV is so common, it must be readily transmitted. Nevertheless virtually nothing is known about its route of transmission. Similarly, the primary infection has not been characterized, although it is probably not associated with any serious illness, since infection is so widespread. The extraneural sites of viral replication have not been identified, although the virus probably replicates in sites other than the urothelium, because rises in antibody titer are not consistently related to urinary excretion (102).

JCV persistence and the origin of PML. It is likely that primary infection is followed by lifelong persistence of the virus, since a high proportion of adults maintain consistent antibody levels decades after a childhood primary infection (197). Periodic reactivation of the latent infection probably provides continual antigenic stimulation.

It is likely that PML results from the reactivation of papovaviral infections that have been latent in the brain or other tissue since childhood infection. Alternatively, the patient who gets PML might be one of the few who failed to acquire immunity to JCV during childhood and who first became infected during a period of impaired cell-mediated immunity.

Results of a serological study of pregnant women imply that JCV may indeed remain latent after primary infection and that reactivation may occur as a consequence of immunological impairment (42). One hundred paired samples of sera from pregnant women were examined for JCV antibodies. The first sample in each pair was obtained at the first prenatal visit and the second at the time of delivery. Of the 100 pregnant women, 57 were seropositive at the time of the first prenatal visit. JCV infections, as indicated by a fourfold or greater rise in serum antibody titer, were seen in 9 of the 100 women. All nine instances of increased serum antibody levels were in women who were seropositive at the first prenatal visit. Primary JCV infection was not observed in any of the 43 seronegative, presumably nonimmune, mothers. Another aspect of this study was the failure to detect congenital transmission of JCV, indicating that such transmission occurs only very rarely, if at all. In another study (D. J. McCance, personal communication), direct evidence was obtained for the persistence of JCV DNA in healthy kidneys. These kidneys, removed from the cadavers of individuals who had apparently been normal, were examined by DNA-DNA filter hybridization. Of the 14 kidneys examined, 4 contained JCV DNA.

Results of another study suggested that immunosuppression might predispose some patients to primary JCV infection (102). In this study of 61 renal transplant recipients, it was found that

rises in antibody titer against JCV occurred in persons who did not have significant prior antibody levels. In contrast, most increases in titer against BKV occurred in persons with preexisting antibodies. Thus, among immunosuppressed transplant recipients, BKV infections may often represent reactivations, whereas JCV infections may more often be primary. Primary infections might occur via transplantation of an infected kidney, as suggested by a study of the transplanted kidney as a source of cytomegalovirus infection (101).

Whereas PML is the only serious disease clearly associated with JCV infection, recent studies associate JCV, as well as BKV, with urethral stricture and an increased frequency of transplant-related complications (102).

Oncogenicity and possible role of JCV in human malignancies. JCV is of interest not only because of its association with PML, but also because of its oncogenicity. This oncogenicity is of further interest because of its possible neurotropism. As noted above, some of the cells in PML lesions become indistinguishable from the malignant astrocytes of glioblastomas. Furthermore, two patients with PML had coexisting gliomas (20, 229), which in one case appeared to correspond topographically to the foci of demyelination (20). Although both JCV and SV40 induce tumors in hamsters, intracranial inoculation with SV40 leads to papillary ependymomas (74), whereas JCV usually produces medulloblastomas, glioblastomas, and unclassified primitive tumors (294, 328).

Despite the above, there is as yet no confirmed evidence that JCV is an etiological agent of cancer in humans. Nevertheless, it may be relevant that medulloblastomas, although rare, are among the most common brain tumors in children, accounting for about 20 to 25% of such tumors. No viral agent other than JCV has yet been shown to produce medulloblastomas. Furthermore, JCV is the first virus shown to induce neuroblastomas (289) and pineocytomas (204), in these instances in hamsters. Neuroblastomas are also among the most common tumors in children. JCV was also shown to induce brain tumors in owl monkeys (152), making it the only known human virus able to cause brain tumors in a primate.

To determine whether the neurooncogenicity of JCV is actually characteristic of the virus rather than a peculiarity of the first isolate, three more recent JCV isolates, from each of three PML patients, were also tested (204). Each isolate produced malignant brain tumors in a majority of the inoculated Syrian hamsters. However, inoculation with one of the isolates resulted in a strikingly higher incidence of tumors arising from the pineal gland and a lesser incidence of medulloblastomas. The basis for the difference in neurooncogenicity between these isolates is not known. No differences were noted in their other biological characteristics or in the pattern of the restriction endonuclease digestion fragments that they generated.

JCV genomes in JCV-induced brain tumors and in cells transformed in culture. The states of the JCV genomes in two clonal cell lines, established from a JCV-induced hamster brain tumor, were determined (308). Neither clonal line contained free viral DNA detectable by the Southern blotting procedure. Instead, each line contained multiple JCV genomes integrated in tandem head-to-tail arrangements. Also, there were multiple sites of JCV integration in each line. Thus, JCV resembles both polyoma virus (9) and SV40 (12) with respect to the state of its genome in transformed nonpermissive cells. An interesting finding was that, although both clonal lines were established from the same hamster tumor cell line, after 50 passages in culture most or all of the integration sites on the cellular or the viral genomes, or both, were different in each clonal line. This shows that the integration pattern of the viral DNA was not stable in those tumors.

JCV is also able to transform primary hamster brain cells in tissue culture (66). Comparison with SV40 infection of these cells showed that at approximately equal inputs of virus, only SV40 induced extensive cytopathic effects. This might be explained by the 10- to 100-fold higher plating efficiency of SV40, compared to JCV, on hamster brain cells, as shown by the fractions of T antigen-producing cells.

Human amnion cells in culture have been transformed with full-length JCV DNA (111). The viral DNA had first been cloned in *E. coli* by using the plasmid vector pBR322. This was done to circumvent the limited replication of JCV in cell culture. The cell lines established from the transformed foci were positive for the JCV T antigen and, interestingly, contained full-length JCV DNA sequences in a circular episomal state. These lines were not productively infected. The presence of episomal viral DNA in this instance probably reflects the capacity of the cells to support viral DNA replication, thereby perhaps eliminating the selective advantage of transformants containing integrated sequences (see below).

PAPOVAVIRAL PERSISTENT INFECTIONS COMPARED WITH THOSE OF OTHER VIRUSES: IN VITRO SYSTEMS

General Factors Common to a Variety of Systems

Although there is an extensive literature describing a variety of virus-cell systems of persistent infection, carrier systems of DNA viruses

have generally been less well characterized than those of RNA viruses (e.g., see review by Rima and Martin [231]). Nevertheless, many persistent infections, involving a variety of RNA viruses in numerous cell lines, display important common properties which are also in evidence in the papovavirus systems described above. These common features include the following. A large majority, and in some instances possibly all, of the cells contain and express viral genetic information, although only a small percentage of cells produce virus at any moment. In some systems, the amount of virus produced varies in a cyclical manner. Many of these systems can be cured by cultivation in antiserum or by cloning. Furthermore, many of these cured cultures continue to produce viral antigen, as demonstrated by immunofluorescent staining. Persistently infected cultures also tend to be resistant to superinfection by the homologous standard virus, although they are susceptible to infection by heterologous virus.

Viral Variants

During the course of persistent infection, there is also a common tendency for the original infectious virus population to be replaced by small-plaque or TS variants as well as for a population of DI particles to emerge. The emergence of either DI particles (112) or TS mutants or both (216, 318) could, at least in part, account for the other common properties of a variety of systems, as noted above.

Some of the defective viral variants persist because they have a replicative advantage in the presence of standard virus. Since the defective variants can use the proteins supplied in trans by the WT helper virus, some may gain a selective advantage by amplifying those portions of the genome which are required in cis. Thus, grossly aberrant SV40 variants frequently contain reiterations of the unique replication origin (146). For a similar reason, most DI particles of vesicular stomatitis virus (VSV) acquire the same sequence at the 3′ ends of their negative-stranded genomes as at the 3′ end of their plus-stranded homologs (209, 224, 240). This sequence is found at the 3′ end of the WT plus-strand homolog, but not on the WT genome. Since WT VSV makes much more genomic RNA than its complement, those DI particles with rearranged 3′ ends have exchanged a low affinity replicase-binding site for a high affinity one. The selective affinity for this sequence by the viral replicase favors replication of the DI particles to the extent that the replication of parental VSV is virtually excluded. Other defective genomes acquire a selective advantage by becoming shorter than the standard genome.

TS mutants, although defective, are not considered to be DI particles, since DI particles are generally defined as lacking a portion of the genome. Nevertheless, TS mutants are capable of competing and interfering with WT virus (319a). This is so in the SV40 systems (192) and in a wide variety of RNA virus systems, including those of Newcastle disease virus, Sendai virus, measles virus, Sindbis virus, Semliki Forest virus, VSV, and reovirus (reviewed in 216 and 318). For this reason, and because of the greater rate of forward mutation to variant than back mutation to WT (190), defective variants with simple lesions can become and remain predominant in a persistent infection. The relatively slow growth of these variants and their consequent diminished cytopathogenicity, as well as the interference effect, might promote cell survival and thereby further the establishment and maintenance of the persistent infection (188, 192, 318).

Interference in at least some systems probably results from a competition between WT and variant virus for the limited capacity of the cell to support virus-specific biosynthetic activities and for those *trans*-acting viral products which the WT, but not the variant virus, is capable of generating (see, e.g., reference 268). Since competition may be occurring independently at a number of points in the viral replicative cycle, the yield of standard virus is diminished as the product of the fractional reduction at each level (28). Consequently, the interference effect can be quite striking.

In some systems, interference by DI particles appears to be expressed in an all-or-none manner on a per cell basis (33, 157) rather than as a simple competition. In some instances, this might be explained by the activity of DI particles with genomes containing replicase-binding sites of much higher affinity than those of WT virus (209, 224, 240). Those DI particle genomes might express sufficient competition for replicase to exclude the replication of WT genomes. DI particles might also express all-or-none interference by activating the interferon system (246). Note that there is no evidence to indicate that interferon plays a role in the papovavirus systems described above. Although interferon does not appear to play a role in most of the carrier systems which have been described (231), it has been shown to stabilize a Sindbis-L cell system (115) and a VSV-L cell system (186, 223).

Little is known about the production and possible role of viral variants in other DNA virus systems of persistent infection. There is some evidence that herpesviruses generate DI particles which might play a role in the establishment of herpesvirus persistent infections in vitro (98, 180).

Host Cell Factors

Properties of the host cell, which vary from one system to another, also appear to play important roles in persistent infection. For example, SV40 persistent infections are much more readily established in rhesus monkey kidney cells than in AGMK cells (158, 187, 189, 192). Furthermore, persistent SV40 infections are more stable in the former than in the latter cell type. This host cell effect apparently reflects differences in cellular susceptibility to productive infection at the population level and differences in the rates at which productively infected cells are killed (187, 189, 193).

The ability of monkey kidney cells to support SV40 replication is dependent on the physiological state of the cells or the stage of the cell cycle (75, 206). Similarly, in a measles virus-hamster cell system, proliferating cells gave rise to lytic infections, whereas non-proliferating cells tended to become persistently infected (133). In other systems, including mumps virus-human conjunctiva cells (292), parainfluenza virus 3-human conjunctiva cells (29), and Sindbis virus-BHK-21 cells (241), virus production and cytopathogenesis correlated with a nonproliferating cellular state. Infectious particle production by VSV mutants, containing TS lesions in the viral polymerase, was much more depressed at nonpermissive temperature in stationary than in proliferating cultures (64). Since TS mutants of other VSV complementation groups were not so affected by the cellular growth state, and because polymerase mutants have been specifically implicated in virus persistence (319a), these findings might be relevant to persistent infection in vivo, where cells exist in growth states ranging from quiescence to rapid proliferation.

Cellular mutants which are absolutely refractory to virus growth are not selected in the SV40-rhesus kidney cell system. This was indicated by the finding that curing by growth in antiviral serum renders the cultures as susceptible to infection with SV40 as cultures of normal rhesus cells (187). Furthermore, in the SV40-AGMK cell system, cell destruction was noted in some regions of otherwise healthy clones (192). Likewise, uninfected cells, as indicated by immunofluorescent staining, were recovered from a Newcastle disease virus-L cell system (234), a measles virus-HeLa cell system (177), and a mumps virus-human conjunctiva cell system (293) and were found to be as susceptible to lytic infection as control cells. Also, cell destruction occurred in the centers of colonies derived from a Sindbis virus-BHK-21 system (241). Because cells at the centers of colonies tend to be more growth restricted than cells at the periphery, this finding also suggests that the growth state of the cells regulates viral replication and consequent cell destruction. The periods of crises in the SV40-AGMK cell system (192), as well as in many other carrier systems, also indicate that resistant host cell mutants are not a factor in the maintenance of many of these persistent infections.

As noted above, kidney cells of rhesus monkey and African green monkey origin differ in ability to amplify the levels of SV40 DI particles in the viral population (193a). In other systems of persistent infection, the host cells also display a marked effect on the generation and replication of DI particles. For example, Semliki Forest virus is able to generate DI particles by the second passage in 3T3 cells, whereas in a line of HeLa cells, DI particles are not apparent, even after 200 passages (260). Nevertheless, although the host cell determines when the Semliki Forest virus DI particles appear and the rate at which they become predominant in the virus population, the size and nucleotide sequence of the final DI RNA is the same in nearly every cell type, suggesting that the properties of the DI RNA are determined by the virus. In contrast, different cell lines appear to selectively amplify different DI particles of VSV. Because VSV DI particles are generated at random, this suggests that the host cell plays an important role in the selection of the VSV DI particle population (107). Interestingly, some cell lines do not support VSV DI particle replication, although DI particle activity is expressed within them (107). This is somewhat similar to the SV40-rhesus kidney cell system in which grossly aberrant DI particles are generated but not amplified to predominant levels (193a). There is also evidence that the particular host cell can determine the properties of the papovaviral DI particle population. For example, whereas monkey kidney cells generate SV40 DI particles which contain reiterations of only those SV40 sequences at the replication origin, a line of human glioblastoma cells may select for SV40 variants with reiterations of other SV40 sequences as well (19). The species of host cell has also been shown to be a significant factor in the synthesis of defective influenza virus (25) and vaccinia virus (69).

A host cell factor, required for the generation of VSV DI particles, was implied by the finding that pretreatment of host cells with actinomycin D prevented the emergence of DI particles (124). Replication of DI particles already present in an inoculum was not affected by the actinomycin D treatment. This study thus revealed a host cell factor required for the origination, but not the replication, of VSV DI particles. It was suggested that this factor might be part of a host mechanism operating at the cellular level to protect the host from a highly cytolytic virus (124). However, these findings

have not been repeatable and may be incorrect.

A striking example of the capacity of the host cell to influence the outcome of infection was seen in studies with mouse hepatitis and measles viruses (153). Almost invariably, persistent infection with these viruses correlated with TS virus replication. However, the viruses did not actually become TS. Instead, thermal restriction was host controlled, and there was an absolute correlation between the ability of a cell type to be persistently infected and the thermosensitivity of viral replication in that cell type.

Another way in which the host cell can determine whether a cytocidal or persistent infection develops is suggested by studies of influenza and paramyxoviruses (105). In these cases, the failure of some cells to cleave viral precursor glycoproteins may inhibit viral maturation to a pathogenic form, thereby promoting persistent infection.

It is clear that DI particles and TS viral mutants play a role in many systems of persistent infection. Nevertheless, as discussed above, defective virus is not generated or amplified in several carrier systems. The reasons are not entirely clear, but the following generalizations have been noted with respect to RNA virus persistence (104). (i) Those cells which are most susceptible to infection and which produce the highest viral yields also tend to most readily generate and amplify DI particles. (ii) Those cells which are most susceptible to infection and which most readily generate a population of DI particles give rise to the most stable persistent infections. In other systems which are poor DI particle producers, the stability of the persistent infection is dependent on interferon or attenuation of the virus or both.

Point (i) above applies to SV40 systems of persistent infection. For example, whereas SV40 DI particles emerge in both AGMK and rhesus kidney cells, the more susceptible AGMK cells more readily generate viral populations in which DI particles predominate (193a). This is probably explained by the relatively low plating efficiency of SV40 on the rhesus cells, since the replication of the grossly defective DI particles is absolutely dependent on coinfection with WT virus.

Point (ii) above is not applicable to the SV40 systems. Indeed, the rhesus cells, which do not generate viral populations in which DI particles predominate, nevertheless establish more stable persistent infections than the AGMK cells, which do produce a preponderance of DI genomes. Other cellular factors, such as those which determine viral plating efficiency, transient resistance, and the rate of development of cytopathic effects, apparently play important roles in the establishment and maintenance of SV40 persistent infections (187, 189).

Integrated Viral Genomes

It has been suggested that because DNA viral genomes can integrate into cellular chomosomes and thereby persist, there might be no need for DI particles in persistent infections with DNA viruses (104). Nevertheless, although the integration of papovaviral genomes is a well-established phenomenon, it has been documented for the most part in those abortive infections of nonpermissive cells which result in neoplastic transformation. Because (i) transformation is a rare event, (ii) viral genes must persist and be expressed in papovavirus-transformed cells to maintain the transformed phenotype, and (iii) nonpermissive cells do not support the replication of free viral DNA, those cells which express the transformed phenotype and which harbor and express integrated viral genomes may represent only a small selected fraction of the initial cell population. Although integration might be an even more common event in permissive cells which support viral DNA replication, it is not apparent that integrated viral genomes might promote the survival of those cells or confer on them a selective advantage. Indeed, several of the papovaviral systems of persistently infected permissive cells described above appeared to involve only free viral genomes. Nevertheless, integration might play an important role in some persistent infections both in vitro and in vivo (see below).

Adenovirus 2 genomes may persist in an integrated state in adenovirus-transformed semipermissive rat cells. However, the viral DNA sequences in those cells are fragmented and incomplete (68, 238). This might explain why adenovirus-transformed cells, unlike papovavirus-transformed cells, never yield virus upon fusion with permissive cells. In contrast to these results, nonpermissive hamster cells transformed by adenovirus 12 or semipermissive cells transformed at the restrictive temperature by TS mutants may contain intact integrated viral genomes (47). Intact viral genomes may persist under nonpermissive conditions, presumably because they do not pose a threat to the viability of the cell. The above findings suggest that it is unlikely that integration plays a role in adenoviral persistent infections of permissive cells.

Integration apparently plays a role in at least some RNA virus persistent infections. The viruses which cause Visna and equine infectious anemia resemble type C retroviruses in their molecular biology and structure, although they are considered to be members of the distinct exogenous lentivirus group. Visna is a chronic inflammatory demyelinating disease of the CNS of sheep. Equine infectious anemia is expressed as a chronic immunologically mediated destruction of erythrocytes which appear to adsorb virus antigens. In both diseases, proviral DNA is

incorporated into host cell DNA (38, 86), thereby promoting persistence under conditions of significant antibody response. These infections are discussed in greater detail below.

Results of RNA-DNA hybridization and transfection experiments suggest that the genomes of more "typical" RNA viruses, such as measles virus, Sindbis virus, tick-borne encephalitis virus (320), and respiratory syncytial virus (255), may also be integrated into host cell DNA, presumably after being reversibly transcribed. Reverse transcription might be mediated either by cellular enzymes or by enzymes of an endogenous retrovirus. Unfortunately, these exciting findings have not yet been confirmed (108). Persistent infection, as indicated by either virus production or the presence of viral antigen, was not demonstrated in the respiratory syncytical virus system, but it was demonstrated in the other systems.

Regardless of whether reverse transcription and integration of non-retroviral RNA virus genomes is confirmed, this is not considered to be the main molecular mechanism by which the majority of RNA virus persistent infections are established or maintained in vitro (231). Nevertheless, the lysogenic relationship in vivo might provide a protected intracellular site for the virus to escape host defense mechanisms (85). Periodic reactivation of the latent infection and the selection of antigenic variants might account for the spread of the infection in vivo (85, 171; see below).

It is clear from the above that numerous carrier systems, involving viruses which display a variety of strategies of replication at the molecular level, share most of several features involving both viral and cellular factors. These factors include the generation and action of DI particles and TS viral mutants, host cell resistance, interferon, and integration. The main question is then which of these factors act and interact to promote viral persistence and cellular survival. Because of their common occurrence, it is conceivable that all of these factors might, when expressed, contribute to the establishment and maintenance of persistent infection in vitro. Features of persistent infection in vivo are considered further in the next section.

PAPOVAVIRAL PERSISTENT INFECTIONS COMPARED WITH THOSE OF OTHER VIRUSES: IN VIVO SYSTEMS

Determinants of Persistence and Pathogenesis: Three Scenarios

The in vitro studies described above indicated that persistent infection with cytolytic viruses is possible provided that virus multiplication is restricted to a small fraction of the cell population. The development of DI and TS virus and the presence of resistant cells were found to be important factors in this regard. Studies of in vivo systems indicate that these factors might also act in the establishment and maintenance of persistent infection in the whole organism.

In addition to those factors which act to promote viral persistence in vivo as well as in vitro, the immune system would be expected to play a major role in persistent infections in the intact host. Furthermore, in addition to its role in the establishment and maintenance of persistent infection, the host defense system is also a major determinant of pathogenesis in several slow virus diseases. Indeed, slow virus diseases display a variety of scenarios with respect to the mechanisms of viral persistence and pathogenesis when the immune system is taken into account. Three such scenarios are discussed below.

Nondefective virus in immunologically compromised hosts: PML. PML appeared to be characterized by a largely, if not entirely, nondefective virus population in immunologically compromised hosts. Note that PML is the only human slow virus disease in which large numbers of intact virions are seen. Thus, it should be a prime candidate for relating a human slow virus disease to the action of DI particles. Nevertheless, as discussed above, it has been difficult to assess whether DI virus plays a role in PML. It is possible that the virus in PML is largely to entirely virulent. If so, viral replication is probably limited by other factors, perhaps including the differential susceptibility of the various cell types within the CNS and the nondividing state of those cells which are susceptible. Viral spread might be restricted by the dense packing of the cytoplasmic processes which make up the neuropil. These factors might also be important in PML, even if DI particles were active as well. They might also be of significance in other slow virus diseases regardless of the status of the immune system or the virulence of the virus. At any rate, note that there is no inflammatory response in PML, and the cerobrospinal fluid is normal. Pathogenesis thus probably results directly from viral cytopathic effects.

Defective virus in immunologically intact hosts: subacute sclerosing panencephalitis. A different scenario, in which defective viral replication occurs in an immunologically intact host, is found in subacute sclerosing panencephalitis (SSPE), a rare, slowly progressing disease of the CNS which occurs primarily in children and young adults (see reference 279 for a review). Measles virus has been implicated as the etiological agent of SSPE. Presently, SSPE and PML are the only human neurodegenerative diseases known to be caused by conventional viruses. SSPE might be a late complication of an acute measles infection, as suggested by epide-

miological studies (1). As in PML, infection with the etiological agent is common, whereas the disease is rare. Consequently, additional host or viral factors or both are involved in the SSPE disease process. Although SSPE patients appear to be immunologically intact (1), their T cells might have impaired cytotoxicity against measles virus-infected cells (137). This might be explained by a failure of the disease to provoke an effective T cell response. For example, viral antigens which are present in brain tissue might be expressed only rarely, if at all, at the surfaces of infected cells. Indeed, although SSPE patients have elevated titers of antibodies in both serum and cerebrospinal fluid directed against the H, N, and F structural proteins of the virus, there is little or no activity against the M protein (91, 172, 297). The M protein serves as the recognition site for the nucleocapsid at the cell membrane during the paramyxovirus budding process (315). In contrast, antibody against M protein is found in the early convalescent sera from acutely infected individuals (154). These results suggest a defect in M protein synthesis in SSPE. This interpretation is supported further by studies of tissue cultures derived from SSPE brains in which only the H, N, and F proteins of measles virus could be detected (89). Also, hyperimmune sera from rabbits immunized with nonbudding SSPE strains did not have antibodies against measles virus M protein (149). The defect in M protein synthesis in SSPE is apparently posttranscriptional, since messenger RNA from either a lytic or persistent infection directed the synthesis of M protein in a cell-free translation system. However, only the lytically infected cells produced M protein (262). Pulse-chase experiments with BHK cells, persistently infected with the related Sendai virus, demonstrated that the M protein was synthesized at a normal rate but was very unstable compared with the other viral proteins (235).

The absence of M protein in SSPE probably accounts for the observation that, although the brain cells of patients are filled with viral nucleocapsids, they do not shed viral particles (110). This lack of viral maturation in the brain might lead to slow cell-to-cell spread of the infection in addition to the possible effect on the immune response to the infection discussed above. As discussed below, antibody modulation of cell surface glycoprotein might also account for some aspects of SSPE.

Note that DI particles of measles virus, which are not easily separated from complete virus, have not been directly demonstrated in SSPE tissue.

Histologically, SSPE is characterized by an enormous increase of hypertrophic astrocytes and proliferation of microglia cells. Virus-containing inclusion bodies are most frequently seen in oligodendroglia cells. Although the disease is generally classified as a panencephalitis, a variable degree of demyelination might occur as well (301). The encephalytic process in SSPE is characterized by perivascular cuffing consisting of lymphocytes and plasma cells with diffuse mononuclear infiltration of the grey and white matter. If an immunopathological process underlies the pathogenesis of SSPE, as suggested by the above, then this disease would differ from PML in this important respect.

Nondefective virus in immunologically intact hosts: Visna and Theiler's murine encephalitis. A third type of virus-host interaction is found in Visna, a slow neurodegenerative disease of sheep. In Visna, the virus is nondefective, and the host is immunologically intact (see reference 182 for a review). Visna virus is a member of the lentivirus group of exogenous retroviruses and thus replicates via an integrated proviral DNA intermediate. This makes possible a latent viral infection despite the early induction of both a humoral and cellular immune response. The infection is continually reactivated in a small percentage of the cells. However, only those cells producing antigenic variants of the virus, which are unrecognized by the immune system, contribute to a new wave of viral replication and spread. Each reactivation results in exacerbation of the disease. Thus, the virus escapes immune surveillance both by its ability to persist as a provirus and by its ability to generate antigenic variants. DI particles have not been detected in Visna. It is of interest that the process of mutation and selection observed in vivo can be readily duplicated in cell culture (181). An analogous situation of latent viral genomes, mutation, and selection of antigenic variants occurs in equine infectious anemia, a persistent retroviral disease of horses (38).

Recent studies with in situ hybridization showed that, although Visna proviral DNA is synthesized in significant amounts in infected sheep brains, Visna gene expression is blocked at the transcriptional level (14). Studies of another retrovirus, the Moloney leukemia virus, may be relevant in this regard. Cellular sequences flanking the integrated genomes of Moloney leukemia virus regulate its expression by a *cis*-acting mechanism (118). In addition, viral gene activity correlated with a pattern of hypomethylation (268). It is not yet known whether the flanking cellular sequences display a corresponding methylation pattern and whether they are under similar control.

In contrast to PML, the neuropathological changes observed in Visna (severe meningitis, choroiditis, demyelination, and intense infiltration and proliferation of mononuclear cells in perivascular areas) are of an inflammatory nature (184, 244).

Theiler's murine encephalitis is another chronic disease in which apparently normal virus persists in immunologically intact hosts (150). This picornavirus-induced persistent demyelinating disease of mice is biphasic. The first phase, during which virus replicates actively in neurons and much less so in glial cells, is characterized by an acute encephalitis. The second phase, during which only glial cells sustain the infection (15), is a progressive condition characterized histopathologically by patchy lesions of demyelination and inflammatory infiltrates and clinically by a gait disorder. In situ hybridization studies show that the restricted viral replication characteristic of the late phase of the disease reflects both the small number of cells containing viral RNA and the small amount of viral RNA synthesized per cell (81). Acute disease thus appears to result from the death of infected neurons. The spread of the acute infection is apparently terminated by the humoral immune response. The glial cells, which impose a restriction on viral replication, sustain the infection into the late stages. The periodic lysis of infected glial cells is probably responsible for the small amount of virus in the CNS during the late stages and for the characteristic inflammatory lesions. Thus, with respect to the restriction of viral replication to poorly permissive glial cells and the consequent demyelination, Theiler's murine encephalitis bears important similarities to PML. However, demyelination in Theiler's murine encephalitis might involve a cellular immune response.

Other examples of the pathogenic consequences of differential cell susceptibility to viral infection within the CNS have been noted (310). An intriguing situation involves rabies virus, which shows a tropism for neurons of the limbic system with relative sparing of the neocortex. This results in an alert, aggressive animal, driven to bite and transmit virus to other animals. This particular cell tropism may well deserve being referred to as a "diabolical selective adaptation" for the persistence of rabies virus at the population level (310).

Regulation of the Infection

Viral variants. As described above, measles virus neurovirulence and persistence in SSPE is correlated with aberrant viral M protein. In Visna and equine infectious anemia spread of the infection and pathogenesis are dependent on the emergence of antigenic variants. Despite the well-documented role of viral variants in these diseases, there are at present only a few known naturally occurring illnesses in which there is evidence to suggest that DI particles and TS mutants are a factor (see below). Nevertheless, defective viruses are an important factor in several animal model systems of persistent virus diseases, suggesting that defective viruses might play an important role in naturally occurring diseases as well.

Studies with VSV (49), lymphocytic choriomeningitis virus (LCMV) (305), and reovirus (79) show that when DI particles are inoculated into animals together with the corresponding standard virus, an otherwise acute fatal disease may be converted to a slowly progressing one. Other studies indicate that DI particles may indeed arise and persist in vivo. For example, at 30 days after intracerebral inoculation of newborn rats with DI particles and standard reovirus, defective reovirions were detected in the brain of an animal which displayed the characteristic runting syndrome of a chronic infection (79). Also, Junin virus-infected baby mouse brains readily generated both fully infectious virus and particles able to interfere with the cytolytic activity of the standard virus (37). DI particles have also been detected in mice chronically infected with LCMV (211). Also of interest, the ratio of DI to standard LCMV differed in various organs, perhaps reflecting the differential ability of various cell cultures to support the generation and enrichment of DI particles (see e.g., references 107, 193a, and 260).

TS mutants have also been shown to either establish or maintain persistent infection in vivo or to alter the course of disease associated with the corresponding WT virus. For example, intracerebral inoculation of weanling mice with WT VSV produces a fulminating disease leading to death in 2 to 3 days, with minimal histopathological changes in the CNS. In contrast, inoculation with some TS mutants produced a prolonged CNS disease with distinct neurological signs and striking spongiform changes in the grey matter of the spinal cord (41). Similarly, whereas intracerebral inoculation of rats with WT reovirus produced an acute encephalitis resulting in a high fatality rate, injection with TS mutants led to a slowly progressive hydrocephalus (60). Changes in neurovirulence were also observed in mice infected with TS mutants of measles virus (93) and VSV (219). These results suggest that TS mutants might be associated with illnesses significantly different from those resulting from WT infection, particularly with respect to altered neurovirulence.

TS mutants may also be selected during persistent infection in vivo. For example, foot-and-mouth disease virus isolated from chronically infected cattle displayed restricted plaque production at 40 to 41°C, whereas viral isolates from acute infections were not so restricted (265). Also, a virus designated 6/94, isolated from brain

cells of a multiple sclerosis patient, produced much higher yields at 33 than at 37°C (148). In contrast, Sendai virus, the putative WT of 6/94, generated equal yields at both temperatures.

An example of a naturally occurring illness in which TS, and perhaps DI, virus plays a role is Aleutian disease of mink. This disease is caused by an autonomous parvovirus that is TS for replication upon isolation from persistently infected mink (212). Also, the virus particles are distributed over a broad range of densities with the lighter particles showing much-diminished infectivity. It is not yet known whether the lighter-band virions are DI particles.

The Aleutian disease process has an immunological basis. The major lesions (glomerulonephritis and arteritis) are caused by deposition of immune complexes in the tissues and subsequent inflammation (24). It may be significant with respect to Aleutian disease virus persistence that the immune complexes retain infectivity.

Lucke frog renal carcinoma is a naturally occurring persistent infection of the frog, *Rana pipiens*, in which the temperature sensitivity of the causative Lucke herpesvirus clearly plays a role in regulating the infection (80). When affected frogs are either in hibernation during the winter or maintained at low temperatures in the laboratory (<12°C), their tumor cells are free of viral inclusions. In contrast, viral inclusions are present in the tumors of frogs captured in the spring or summer or maintained in the laboratory at temperatures above 12°C. Similar temperature-dependent results were obtained with tumor cells in culture. Temperature shift experiments in either the intact host or in cell culture showed that temperature alone is the inducing agent of the virus. Nevertheless, since viral replication is not induced in every tumor cell at low temperature, some other viral or cellular factors may also act to regulate the infection.

Viral persistence is a characteristic of LCMV infections, both in vitro and in vivo (210). Persistently infected cell cultures produce DI particles almost exclusively, and DI particles are found in persistently infected mice as well (211). Studies of cell cultures showed that DI particles strongly inhibited the surface expression of viral antigens, although these antigens were present in the cytoplasm in reduced amounts (304). This might account for the inability of antibody plus complement or T cells to kill LCMV-infected cells. A DI virus-mediated escape from immunosurveillance might operate in vivo as well, as suggested by the finding of antigen-free areas in persistently infected mice (176).

The immune system. The interaction of the immune system with some viral infections might promote latent infection. This is apparently so in the maintenance of latent infection with BKV and JCV, as discussed above. Human herpesvirus infections may be similar to papovavirus infections in this and other ways. After primary infection with any of the known human herpesviruses (e.g., herpes simplex virus types 1 and 2, varicella-zoster virus, cytomegalovirus, and Epstein-Barr virus [EBV]) at any time from in utero to the third or fourth decades of life, the virus may persist for the lifetime of the individual. Persistent herpesvirus infections are characterized by latent viral genomes which may be reactivated to produce overt lesions (120, 205). Spontaneous reactivation is characteristic of herpes simplex virus types 1 and 2 and varicella-zoster virus, but it is not known to occur, clinically at least, with EBV and cytomegalovirus. Nevertheless, reactivation of each of the above human herpesviruses is known to occur as a result of immunosuppression (e.g., in transplant recipients and tumor patients). Similar to BKV and JCV, these herpesviruses are ubiquitous in the human population, and latent infection with them is a common occurrence.

The immune response may promote viral persistence in yet other ways. For example, "capping" and "stripping" of viral antigens from the cell surface occurs when measles antibodies are added to cell cultures either acutely or persistently infected with measles virus (122). This modulation of surface antigen by antibodies might further promote the escape of persistently infected cells from immune surveillance in infections such as SSPE and LCMV, in which there is a high-titer antibody response. Furthermore, the antibody-induced modulation of viral surface glycoproteins interferes with the virus budding process and thereby further restricts the productive infection. That this phenomenon might play a role in vivo is consistent with the observations that cells from SSPE biopsies have a much reduced cell surface viral glycoprotein content and an accumulation of immature nucleocapsids in their cytoplasm. Furthermore, SSPE patients have antibodies to viral glycoprotein (121) and also lymphocytes that can destroy measles virus-infected cells in vitro (136).

Integrated viral genomes. Integrated viral genomes can be important in the maintenance of a chronic infection in vivo, as, for example, in Visna, a retrovirus-induced disease described above. Integration also appears to be a factor in persistent infections involving a variety of DNA viruses. For example, integrated hepatitis B viral genomes have been detected in human hepatocellular carcinomas (55). In this case, both complete and partial viral genomes were found in a lysogenic state. Episomal viral DNA could not be detected in cell lines established from primary human hepatocellular carcinomas.

Note that the integration pattern of the hepatitis B DNA was not stable (285).

Genomes of the adeno-associated virus family of parvoviruses also persist in an integrated state (298). Integration probably plays a necessary role in the life cycle of these viruses, since the WTs are defective and dependent on the helper activity of unrelated adenoviruses for their replication. Consequently, integration permits adeno-associated virus genomes to persist in the absence of helper adenoviruses. Reactivation and spread of the adeno-associated virus infection occurs upon the chance superinfection of the cell by an adenovirus.

It was suggested that EBV DNA persists in an integrated state in Burkitt's lymphoma and in some nasopharyngeal carcinomas (125). However, the large size of linear herpesvirus genomes has made it difficult to conclusively demonstrate integration of those viruses. Furthermore, latent EBV DNA in typical human lymphoid cell lines is mainly present as circular DNA molecules of viral genome length (3).

Integrated viral genomes are not evident in papillomavirus-induced warts and tumors in either humans (see, e.g., reference 139) or animals (see, e.g., reference 2). The presence of papillomavirus DNA in a free circular form rather than in an integrated state and the absence of virus particles and antigens (179) implies that other mechanisms restrict papillomavirus gene expression. These findings might have implications with respect to the in vivo state of latent papovaviral genomes, since papovaviruses are similar in important ways to the papillomaviruses.

Because integrated viral genomes are an important component of some, but not all, persistent infections in which integration ought to be possible, the relative advantages of the integrated versus free state should be considered. Whereas integration can act to maintain a persistent infection, a smoldering plasmid state should provide the advantage of more frequent viral release and perhaps the more frequent selection of viral variants as well. Thus, in a disease such as PML, in which immune surveillance is probably not an important factor, it may be advantageous to the virus to maintain its genome in the free state. Integration might then only occur in those systems in which it reflects a particular need. In the case of retroviruses, integration might reflect the fundamental role of the provirus in the life cycle of that virus group. Retroviral proviruses apparently serve as the exclusive templates for the synthesis of both messenger RNA and progeny viral genomes. Integration probably plays a necessary role in the persistence of adeno-associated virus, since the WT of those viruses cannot replicate their DNA in the absence of an unrelated helper virus. In contrast, integration of papovaviruses, particularly in nonpermissive cells, might represent a laboratory phenomenon having little relationship to the normal life cycle of that virus group in its natural hosts.

Control of nondefective viral gene expression. Regardless of whether nondefective persistent viral genomes are carried in an episomal or an integrated state, the restriction of their expression must be accounted for. The expression of Visna proviral DNA is blocked at the transcriptional level by an unknown mechanism (14). Studies of another retrovirus, Moloney leukemia virus, showed that the cellular sequences flanking the integrated provirus may regulate its expression (118). Furthermore, the inverse relationship between viral genome expression and methylation suggested that this DNA modification might also play a regulatory role (268). An inverse correlation between the levels of DNA methylation and the expression of particular regions of integrated adenovirus genomes has also been found in adenovirus 12-transformed hamster cell lines and in adenovirus 12-induced rat brain tumors (269). Similar findings were also noted in studies of *Herpesvirus saimiri*-induced tumor cell lines (45) in which the viral DNA purportedly exists in a nonintegrated state. *H. saimiri* is an indigenous virus of squirrel monkeys, most of which have a latent infection that persists for life. Although *H. saimiri* causes no known disease in its natural host, it can induce lymphoma and death in other primates. It resembles EBV in that neither virus appears to yield infectious progeny or structural antigens in the tumor cells which they induce. A number of continuous lymphoid cell lines have been established from *H. saimiri*-induced tumors, and it has been found that *H. saimiri* DNA is extensively methylated in nonproducer lines, whereas virus sequences in producing lines are not methylated (45). Thus, it was suggested that DNA methylation might be an important factor in determining permissive versus nonpermissive infection, virus latency, and, in some instances, tumorigenesis (45).

Recently, it was shown that acute measles virus infections of cultivated neural cells can be converted to a chronic state by agents which affect cyclic nucleotide metabolism (175). Increased levels of cyclic adenosine monophosphate (cAMP) resulted in decreased viral replication. This correlated with a marked decrease in M protein, but no significant decrease in other viral proteins. These findings have obvious implications with respect to SSPE. Note that measles virus replicates more efficiently in proliferating than in stationary cells and that low cAMP levels are associated with cell proliferation. A

similar relationship between cell growth and papovaviral replication was noted above, suggesting that results from the measles system might be relevant to the papovaviruses. However, cAMP levels also closely correlate with cellular differentiation, and in at least one system, involving mouse teratocarcinoma cells, papovaviral infection and gene expression were more efficient in more highly differentiated cells (283). This might be relevant to the findings that the cAMP analog, dibutyryl cAMP, markedly increased the frequency of transformation by polyoma and SV40 (257) and also the reactivation of latent EBV genomes (321).

Pathogenesis

Demyelination in PML is believed to result from the direct cytopathic effect of the virus on the myelin-synthesizing and -maintaining oligodendrocytes. In contrast, in all of the other demyelinating infections described above, destruction of oligodendrocytes appeared to result at least in part from immunopathological processes. Thus, note that the demyelination induced in mice by the mouse hepatitis virus, a coronavirus, apparently results from the direct virus injury to oligodendrocytes (94). Infection of immunosuppressed or thymus-less nude mice results in an increase in the severity of the encephalomyelitis. Furthermore, under immunosuppressed conditions, there is no perivascular inflammation, nor are there immunoglobins within the lesions, and mononuclear cells are nearly absent as well (301). Note that mouse hepatitis virus produces a continuum of responses, ranging from an acute fatal encephalitis to a nonfatal primary demyelination (94). The outcome is a function of the age of the animal (older animals are more susceptible to the demyelinating disease) and viral dosage (larger dosages tended to produce fatal disease [301]). Furthermore, TS mutants have been isolated which are associated with either a high or low incidence of demyelination (94).

Neurodegenerative Diseases of Suspected Viral Etiology

Cases of human demyelinating diseases of known viral etiology are relatively rare. Nevertheless, a major reason for the interest in virus-induced demyelination is the possible viral etiology of multiple sclerosis (MS), a human demyelinating disease of considerable importance, with an estimated 100,000 cases in the United States alone. Epidemiological data on the geographical distribution of cases, the risks in migratory populations, and family studies strongly imply that MS results from exposure to an infectious agent early in life. Nevertheless, neither transmission of the disease nor direct evidence of viral replication within the lesions has yet been demonstrated. The pathogenesis of MS has been extensively reviewed (see, e.g., reference 50). In contrast to PML, there is evidence to suggest that the MS disease process is of an autoimmune nature. Antimyelin antibodies are present in MS cerebrospinal fluid, and cell-bound immunoglobulin G and inflammatory cells are present in the lesions.

The pathogenesis of canine distemper of dogs, caused by the canine distemper virus, is similar in important ways to that of MS, adding plausibility to the suggested viral etiology of MS. For example, antimyelin antibodies are also present in canine distemper. Furthermore, no virus or viral antigen is detected in the demyelinating lesions of canine distemper (50). Canine distemper virus is antigenically related to and shares many biological properties with measles virus (131). Interest in canine distemper is thus heightened by those features of its pathology which are reminiscent of MS. Nevertheless, none of the demyelinating diseases currently attributable to viral infection follows the exacerbating and remitting course of MS.

In consideration of (i) the capacity of herpesviruses to remain latent within human neural tissue, (ii) the capacity of herpesviruses to cause human disease characterized by remissions and exacerbations (e.g., herpes labialis), and (iii) the capacity of trauma to precipitate exacerbations of both herpes infections and MS, it was suggested that a herpesvirus might be the etiological agent of MS (135). Although the MS disease process apparently differs from that of PML with respect to the involvement of an immunological component, the filamentous and multilamellated cytoplasmic inclusions in MS brain autopsies (217) have also been observed in the cerebral tissue of two cases of PML (96). These structures do not appear to be paramyxovirus nucleocapsids (50). Their significance is not yet clear.

There is speculation that viral infection underlies a variety of other human neurological diseases of as yet unknown etiology (310). These include amyotropic lateral sclerosis, a chronic neurodegenerative disease characterized by the progressive loss of motor nuerons; Parkinson's disease, a progressive neurological disease involving neuronal loss from the substantia nigra; and Alzheimer's disease, the most common form of presenile dementia, characterized pathologically by neurofibrillary changes. Furthermore, persistent virus infection might underlie some cases of other chronic diseases, such as diabetes, arthritis, and systemic lupus erythematosus.

CONCLUSIONS

Although only a few of the presently known persistent virus diseases of humans and animals have been noted in this review, it is clear that persistent virus infections are responsible for a number of illnesses, including some which are of considerable medical or economic consequence. Furthermore, it is very likely that other significant human and animal diseases of presently unknown etiology will also be shown to result from persistent virus infections. The well-established association of viral infection with the rare human demyelinating diseases PML and SSPE are of particular significance, since they add plausibility to the suggestion that persistent viral infection may underlie other more common human neurodogenerative diseases whose etiologies are presently obscure. The importance of the currently known persistent virus infections and the prospect that other highly important persistent virus diseases will be recognized in the future emphasize the need to understand the basic mechanisms of viral persistence and the associated disease processes.

Consideration of the several in vitro and in vivo papovaviral systems, as well as systems of other unrelated viruses, suggests that persistence may depend on a variety of factors and that there is no single or universal mechanism to account for the phenomenon. Nevertheless, it is clear from the above that a variety of persistent infections share most of several features involving both viral and cellular factors.

The essential prerequisite to the establishment and maintenance of a persistent infection is the continued survival of a subfraction of potentially productive cells. This may be achieved in vitro through the action of DI particles, TS mutants, noncytopathic viral mutants, integration, interferon, and cells which express transient or incomplete resistance. These same factors may also act in the intact host, in which the immune system may also be an important component of persistent infection. Indeed, the greater apparent diversity among the in vivo than the in vitro systems may largely reflect the role played by the immune system.

In PML, the disease process develops in immunologically impaired individuals, suggesting that immunological control mechanisms limit active infection and spread of the virus to the CNS. Nevertheless, the absence of an immune response in the PML disease process underscores the importance of viral and cellular factors in this illness. Consequently, the analysis of papovaviral carrier systems in cell culture as well as PML in humans may provide important insights into those mechanisms which act in vivo at the cellular and molecular levels to promote both persistent infection and pathogenesis.

It is interesting that persistent infections involving a variety of viruses, which employ numerous different replication strategies at the molecular level, all appear to share many of the several features noted above. The main question then is which of these factors act and interact in any particular system to promote viral persistence and cell survival. It might be presumed that any of these factors, when present or expressed, might act to promote persistence. However, the importance of any one of these factors will vary from one system to another. Thus, it is important to avoid generalizations and to analyze each of a variety of systems.

Model carrier systems in cell culture are no doubt oversimplifications of the corresponding persistent infections in the intact host. Nevertheless, persistent infection at the cellular level is a prerequisite for persistence in the whole organism. For this reason, and because the molecular and cellular events which underlie persistence can be more readily investigated in easily manipulatable in vitro systems than in the whole organism, it is important that good cell culture model systems be developed and studied. The papovaviral carrier systems have unique advantages in this regard. First, the molecular biology of their replication and gene expression has been, and continues to be, extensively investigated. Second, the immune response, which underlies the pathogenesis of most of the recognized slow virus diseases, apparently does not act in the PML pathogenic process. Thus, papovaviral carrier systems might more accurately reflect their corresponding in vivo disease (PML) than do in vitro model systems of other slow virus diseases.

Despite all that is presently known about the factors which underlie persistent virus infection, no system is yet adequately understood. Important questions therefore remain to be answered. Those that appear below are listed because of their relevance to the papovaviral systems, but several may be relevant to other viral systems as well.

The state (integrated versus free) and nature (defective versus nondefective) of the viral genomes in nonproducing, but potentially susceptible, cells are still not determined. Also, it is not known where in the viral replication cycle the restriction of viral replication occurs.

The presence of a fraction of cells which express transient resistance appears to play an important role in the establishment and maintenance of papovaviral carrier systems. Nevertheless, neither the nature of this resistance nor the mechanism by which new susceptible cells emerge to perpetuate the infection are well understood. Furthermore, the hypothesis of permissive and nonpermissive infections in differ-

ent cell types of the CNS to account for persistence in PML is attractive but not yet proven.

Because papovaviruses are ubiquitous in humans and because reactivation of latent papovavirus infections are associated with immunological impairment, it is reasonable to presume that PML results from the reactivation of a latent infection in an immunologically compromised host. However, this has not been proven, and the possibility remains that PML results from the first contact with the etiological agent by an immunosuppressed patient. Presuming that PML follows reactivation of a latent infection, it is also not clear whether the CNS represents a site of latency or whether it is a site to which the infection spreads only after reactivation.

The above questions raise several others. For example, the extraneural sites of viral replication have not been identified. To date, human papovaviruses have only been identified in the brain, but their isolation from the urine of immunosuppressed patients indicates that these viruses can be active in tissues outside the nervous system. Thus, the extraneural sites of papovaviral persistence and replication must be identified. It will also be important to know whether active or latent infection of these other sites causes cell dysfunction of any sort that might be manifest as disease. If the CNS is indeed uniquely predisposed to papovaviral persistent infection or, more likely, to chronic disease, then it will be important to identify the factors which account for this. Also, if PML results from reactivation at an extraneural site, what then is the mechanism by which infection invades the CNS? Related questions concern the routes of transmission of the papovaviruses and the nature of the primary infections. Also, virtually nothing is known about viral latency in vivo and the phenomena associated with reactivation both at the cellular level and in the whole host. Clearly, the immune response is a factor in these matters, but the mechanisms by which it acts to maintain latency are unknown.

The role of the papovaviruses in human cancer is outside the main intent of this review. Nevertheless, the neoplastic potential of these viruses in cell culture and in animals and conflicting evidence that they might be involved in human cancer require that they be considered among the potential causes of human tumors.

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