Defective Viral Infection: The Rescue of Virus from Antigen-Negative Cells

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There is epidemiologic evidence for a viral infection in multiple sclerosis (MS). Over the years, frequent attempts to rescue virus and demonstrate viral antigens in the brain of MS patients have failed. There have been a number of isolates, but none have been found to play a role in the etiology of the disease. We have been studying an animal model of viral-induced acute and chronic demyelination. The virus, a mouse coronavirus, JHM, is a neurotropic strain of mouse hepatitis virus. In order to better understand JHM virus chronic infection, we have developed an in vitro carrier culture system of persistently infected mouse neuroblastoma cells [6].

In neuroblastoma cells, we are able to study specific cellular functions in relationship to persistent viral infection. Analysis of mouse neuroblastoma JHM-infected carrier cultures (N_J cells) did not detect any changes in the released virus populations even after 40 subcultures of the cells. The properties of the virus continued to be consistent with those of the parental virus which in the experimental animal produces acute demyelination. There was no evidence for the production of temperature-sensitive mutants, defective interfering particles, or interferon [6].

To determine the effect of antibody on the in vitro infection, N_J cells were passaged four times in the presence of antiviral antiserum. The presence of specific antiviral antibody ablated the synthesis of infectious virus as well as the expression of cell surface viral antigen. The majority of the cells retained intracytoplasmic viral antigen as detected by the indirect fluorescent antibody technique [8]. Removal of the antibody did not result in the reinitiation of infectious virus production or expression of cell surface viral antigen, even after ten passages.

The persistently infected cells were cloned in the presence of antiviral antibody in order to get a population of identical cells derived from a single cell. Single cells from the persistently infected cultures were added to individual wells of microtiter plates and grown in the presence of antiviral antibody. Following isolation, the clones were propagated in the absence of antibody. Removal of the antibody did not result in the reinitiation of production of infectious virus or expression of cell surface viral antigen, even after ten passages.

Eighteen clones derived from single cells were established from the original culture. They appeared to be morphologically identical to uninfected neuroblastoma cells. Electron-microscopic examination revealed that the clones contained no viral particles consistent with the morphology of JHM virus, and the doubling times of the cell clones derived from the infected cultures and uninfected cells were similar. Eleven of the isolated cell clones had viral antigen detectable by immunofluorescence.

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The antigen-positive clones were refractory to superinfection by JHM virus. Ordinarily, 24 h after infection of C-1300 neuroblastoma cells with 0.1 plaque forming units per cell (PFU/cell) of JHM virus, 80% of the cells show cytopathic effects, and by 96 h, the culture is destroyed. In the N_J culture, free of antibody and producing infectious virus, the cytopathology of dying cells was consistently around 40%. Superinfection of the N_J cells produced a steady 40% destruction even 96 h post infection. By contrast, the antigen-positive cell clones (nonproducers of infectious virus) had no detectable cytopathology with superinfection at 24 h and a very minimal amount, roughly 10%, at 96 h, a time when control neuroblastoma cells would be totally destroyed. Three clones were also found to be antigen negative. These cells were similar in all other ways to the antigen positive cells. These clones also resisted superinfection by 0.1 PFU/cell of JHM virus in the same fashion as the antigen positive clones.

To determine the nature of the viral antigens, two clones, designated S-1 (antigen positive) and S-3 (antigen negative), were radiolabeled with ^{3}H-amino acids and analyzed by polyacrylamide gel electrophoresis (PAGE) [7]. PAGE patterns indicated no differences between the S-1, S-3, or uninfected neuroblastoma cells. Using this technique following infection of uninfected neuroblastoma cells with JHM virus, one can distinguish at least three structural proteins as early as 10 h post infection. Cytoplasmic extracts tested by complement fixation tests using anti-JHM virus and polyvalent anti-mouse hepatitis virus antisera (Microbiological Associates, Rockville, Maryland) were also negative. Membranes derived from virus-infected cells have been effective immunogens in eliciting antiviral antibody responses in other viral systems [6]. However, neither membranes from S-1 nor S-3 cells when used as immunogens in the A/J mice elicited any anti-JHM virus antibody. The sera were negative when tested by microneutralization, immunofluorescence, and complement fixation tests. In addition, the antiserum which produced the positive immunofluorescence in the S-1 clone did not react by complement fixation with the membrane antigens from the S-1 clone. These studies suggest that the antigen-positive cells did not have sufficient viral protein expressed to be differentiated from the host cell proteins by PAGE or serological studies.

The S-1 antigen-positive clone and S-3 antigen-positive clone were resistant to superinfection by the JHM viruses previously described. This was initially the only evidence that the S-3 clone might be infected, since over 100 single cell clones of uninfected neuroblastoma were all found to be susceptible to JHM virus. When the S-1 and S-3 cell clones were fused to DBT and 17 CL-1 cells, both permissive for JHM virus, infectious virus was recovered. The fusing agent was polyethylene glycol [4]. Virus was recovered from S-1 clones in five of seven separate attempts using either DBT or 17 CL-1 as the indicator cell. In all fused cultures destined to yield infectious virus, cytopathology was first evident 48 h post fusion and by 96 h involved all of the permissive cells in the culture, while sparing all of the latently infected neuroblastoma cells. To our surprise, virus was also rescued in three of six attempts from the S-3 antigen-negative cells, two times using DBT cells as the permissive cell and once using 17 CL-1 cells. No virus was recovered following fusion of DBT to DBT, DBT to 17 CL-1, or 17 CL-1 to 17 CL-1 cells.

The yields of infectious virus in all the cultures was approximately 10^6 PFU/ml when titered on monolayers of DBT cells. The clones were also fused with UV-inac-

tived Sendai virus to DBT, 17 CL-1, and NCTC 1469 cells, but no infectious virus was detectable in three attempts [3]. It is of importance that the first evidence of CPE was seen at about 48 h post fusion, and by 96 h all the permissive cells were involved. This would suggest that the viral genome needed 24–48 h to generate viral proteins, to assemble infectious virus, and to produce cytopathic effect. This is in contrast to other systems, particularly SSPE, where almost immediately after fusion infectious virus can be found. The latter instance is consistent with an assembly defect. The time lapse in the JHM virus system suggests that the defect in viral maturation is more consistent with a translational problem. The fusion could have derepressed the viral genome. The virus could not be rescued by other techniques [1, 9]. Cells derived from the clones were also cocultivated with approximately equal numbers or tenfold excess of indicator cells at 32°, 37°, and 39° C without the release of infectious virus. The nature of the viruses designated S-1 JHM and S-3 JHM was of interest in that studies indicated no evidence of restricted growth at 37° or at 39°; however, the virus rescued after all the fusions of either S-1 or S-3 clones were restricted at low temperatures, namely 32° C. We have tentatively characterized this as a cold-sensitive mutant of JHM virus (Stohlman, Sakaguchi, and Weiner, unpublished data).

Neuroblastoma cells have been shown to express neurospecific functions, such as neurite formation, following an increase of intracellular cyclic adenosine-3′5′monophosphate (cAMP). This differentiation of neuroblastoma cells is also characterized by a decrease in DNA synthesis, a failure to divide, and the presence of increased protein synthesis, particularly the production of specific neuroenzymes related to neurotransmitters [5]. In addition, elevation of intracellular cAMP can cause an increase of viral antigen production and causes accelerated death in a measles-infected Vero cells (Katz and Weiner, unpublished data).

When S-1 and S-3 clones were treated with 0.5 mM of cAMP and 0.5 mM of theophylline, a phosphodiesterase inhibitor, both clones died within 96 h of treatment. No expression of differentiation, as defined by neurite formation, was seen, nor was there evidence of either increased viral antigen or production of infectious virus. Control cultures of uninfected neuroblastoma cells responded to the drugs by the expression of a differentiated morphology in approximately 40% of the cells, and cells remained in culture for 1 week or more. In the carrier culture producing infectious virus (N_J), the response to cAMP is also inhibited, but the dramatic effect of cell death seen following treatment of S-1 and S-3 clones does not occur.

The importance of these experiments to the study of MS is that a virus which is known to produce demyelination in its natural host can be induced by the presence of low levels of antibody to become increasingly defective. The defectivity is such that viral antigens are undetectable by conventional techniques, even in the presence of complete viral genome. Although there are numerous examples of the rescue of DNA and RNA viruses from antigen-negative cells lacking virus-induced cytopathic effect, such reports with RNA viruses whose replication does not require a DNA intermediate are rare. The JHM virus is a positive-stranded RNA virus [2] and latency with such viruses may indeed be unique. Of further importance to work on MS is that the cells harboring the viral genome will function normally until they are asked to perform a specific task. In the case of the neuroblastoma cells, the specific function we have analyzed is the expression of neurites. We have not yet mea-

sured the specific neuroproteins or neurospecific enzymes in the neuroblastoma cells to see what effect the viral genome has on synthesis, but work is in progress. It is of further importance that the effect on cellular function and the eventual cell death are not dependent on the production of either viral antigen or infectious virus. Although the mechanism is not clear, the latent virus genome might interfere with differentiation by affecting cellular gene expression. One could further postulate that if such a system were to exist in an oligodendroglia, a latent viral genome could produce cell death at times when there is high metabolic requirements demanded of the oligodendrocyte or stress due to other extrinsic factors.

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