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JC virus and glial cells: Viral strategies for cell cycle deregulation in progressive multifocal leukoencephalopathy

A. Ariza

Dept. of Pathology, University Hospital Germans Trias j Pujol, Autonomous University of Barcelona, Badalona, Spain.

Progressive multifocal leukoenCephalopathy (PML), a demyelinating opportunistic infection of the central nervous system that was extremely rare before the advent of AIDS, has become a relatively familiar condition in the AIDS era. The disease, caused by infection with the JO virus (JCV), is associated with inclusion bearing, large-sized oligodendrocytes and strikingly bizarre astrocytes. In fact, the atypical changes of astrocytes are of such magnitude that they have occasionally prompted an erroneous diagnosis of high-grade glioma. The conspicuousness of these histological changes renders PML an inviting scenario for the study of viral interactions with host glial cells.

When pondering over the mechanisms of JCV onslaught on glial cells, interplay with p53 emerges as a plausible viral strategy. Specifically, since some JOy T-proteins are known to bind cellular p53 *in vitro,* it seems reasonable to assume that p53 protein also interacts with a JOV product in the setting of PML. This binding, by stabilizing p53 and prolonging its half-life, would allow its immunohistochemical detection.

In agreement with this, JOy-infected oligodendrocytes and bizarre-looking astrocytes strongly immunostain for p53. Moreover, dual (p53/DNA) flow cytometry analysis of PML frozen tissue samples reveals that their p53 content is above that of isotypic controls. Thus, p53 build-up in JOy-harboring glial cells suggests a connection between the JOy-induced stabilization and inactivation of p53 and the striking tumor-like changes shown by these cells. In other words, the anomalous glial phenotypes seen in PML may well be linked to a loss of normal p53 function, allowing unrestrained entrance into the DNA synthesis phase of the cell cycle.

The question then arises as to whether p53 protein accumulation in PML is really the result of its sequestration by JCV or whether it is the outcome of a p53 gene mutation which would also prolong p53 protein half life. A DNA sequencing study has demonstrated that the p53 gene harbors no mutations in PML and, therefore, that the p53 protein build-up in JOV-infected cells is not the consequence of a mutagenic interaction between JOV and the cell genome. Thus, it can be proposed instead that p53 accumulation results from its binding and stabilization by JOV T-protein.

Taking into account that stabilization and inactivation of p53 is associated with the development of genomic instability, abnormal cell DNA contents are to be expected in JOy-infected cells. Accordingly, image analysis of DNA content performed on PML tissue sections treated with the Feulgen technique has shown that inclusion-bearing oligodendrocytes exhibit near tetraploid DNA indices, whereas atypical astrocytes are often polyploid. These disparate DNA contents can be construed as a consequence of the greater JOy permissiveness shown by oligodendrocytes, whose early lysis would prevent their reaching ploidy levels as high as those of the far more permissive astrocytes.

The aforesaid evidence of DNA amplification in PML glial cells is congruent with the occurrence of a functional abolition of p53 protein in association with JOV infection, since p53 roles include those of keeper of diploid status and guardian of genomic stability. Additionally, the overexpression of proliferating cell nuclear antigen (PONA) and Ki-67 also observed in JOy-infected cells would reflect the proliferative status to be expected in cells undergoing viral sequestration and inactivation of their p53 protein.

How this JOy-induced cell cycle deregulation influences cyclin expression poses another intriguing question. As is often the case in JOy research, the answer can be anticipated from the known effects of the much better studied simian virus 40 (5V40), another papovavirus that also makes use of its large T-antigen to subvert the host cell replicative machinery in order to serve its own reproductive needs.

It is known that 5V40 large T-antigen is able to induce the expression of cyclins A, Bi and E (but not of cylin DI) in transfected diploid cells. In accordance with this, JOV infection has recently been shown to induce immunohistochemical positivity for cyclins A and Bi in inclusion-bearing oligodendrocytes and pleomorphic astrocytes. This recapitulation of 5V40 T-antigen-associated cyclin fluctuations suggests that JOV T-antigen shares some of the previously described capabilities of 5V40 T-antigen to alter cyclin expression for the sake of viral replication.

In summary, a picture of PML glial cell pathobiology is gradually emerging in which the central motif seems to be cellular p53 inactivation caused by binding with JCV large T-antigen. Aneuploidy and overexpression of cyclins A and Bi and of cell proliferation antigens such as PCNA and Ki-67 are other salient features of glial cell reaction to JCV infection. Finally, formal demonstration of p53 T-protein complex formation by means of coimmunoprecipitation studies will lend further support to the above-delineated pathogenetic construct of JCV strategies for glial cell cycle deregulation.

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Human herpesvirus 8 and its diseases

G. Cathomas

Dept. of Pathology ZLirich University Hospital, Switzerland,

Based on the epidemiology and the clinical course of disease, Kaposi's sarcoma has long been suspected to be either caused by, or associated with, an infectious agent. A variety of viruses and bacteria have been implicated, including cytomegalovirus, the human immunodeficiency virus (HIV) and mycoplasma. In 1994, Chang and coworkers reported the presence of a herpesvirus-like DNA, detected by representational difference analysis in tissue of Kaposi's sarcoma (1). Subsequent work confirmed these findings and a new human herpesvirus, referred to as Kaposi's sarcoma-associated herpesvirus (KSHV), or human herpesvirus 8 (HHV8), was detected.

The virus

HHV8 belongs to the family of the herpesviruses and, based on the nucleotide sequence, is most closely related to herpesvirus saimiri, an animal virus causing lymphoproliferative disorders in new-world primates, and to the Epstein-Barr virus. HHV8 is the first representative of the subfamily of the ~y2-herpesvirgenus *Rhadinovirus* (2). The linear viral genome is approximately 165 kb and consists of a 140.5 kb coding region flanked by terminal repeats (3). Within the genome, two types of genes have been described: conserved gene regions similar to other herpesviruses that code for structural and replication-associated proteins. In addition, the HHV8 genome reveals regions unique to this virus, coding for a variety of gene homologues to human genes, including viral homologues for interleukin-6, *bcl-2,* a G-protein-coupled receptor, cyclin D, and macrophage inflammatory proteins. *In vitro* analysis revealed that many of these viral analogs show functional activity similar to that seen in the natural counterpart. In addition, virus-derived gene products, as well as the infection of primary human endothelial cells, have been associated with transforming activity. Most of these data, however, have been performed in permanently HHV8 infected lymphoma cell lines and little is known about the primary infection of endothelial cells.

Detection of human heroesvirus 8

In the original description of the herpesviruses-like DNA sequences of HHV8, a polymerase chain reaction (PCR)-based method, the representational difference analysis, has been used to detect the viral sequences. The vast majority of subsequent analyses of clinical material used POR to detect fragments of the viral DNA, either in fresh tissue samples or in formalin-fixed biopsy material (1, 4). In addition, to increase the sensitivity of the test, a nested PCR, i.e., two consecutive PCRs with an inner and an outer primer pair, have been performed. Although the tests can be performed by using appropriate controls, these assays are prone to produce false positive results. However, so far no other test achieves equal sensitivity. Although successful cultivation of the virus in cell cultures, using fresh Kaposis sarcoma tissue, has been reported, this technique is restricted to an experimental design.

PCR *in situ* hybridization has shown that HHV8 DNA is present in the spindle and vascular cells of Kaposi's sarcoma (5, 6). In addition, using the same technique, HHV8 DNA has been reported to be present in the epidermal cells overlying Kaposi's sarcoma tissue as well as in the pneumocytes of patients with the tumor. Using isotopic and nonradioisotopic colorimetric *in situ* hybridization with probes directed against the open reading frame (ORE) K12/T0.7 and the cyclin D viral homolog, a restricted viral gene expression in most tumor cells of Kaposi's sarcoma have been reported (7-9). Conflicting results have further been reported with respect to the presence of HHV8 RNA expression in various epithelial cells.

kann~i'~c ~IL,JIII0 ~1HU WWV$1

Since the original description of Kaposi's sarcoma in 1872, a variety of different clinical forms of this lesion have been described. The classic, or endemic form of Kaposi's sarcoma, originally found in older men of Eastern European or Mediterranean origin, usually shows an indolent clinical course. In contrast, the more recently described forms associated with prolonged immunosuppression, as seen in solid organ transplant recipients and especially in patients infected with HIV, show a more aggressive clinical behavior often disseminating and involving inner organs such as the lung or the gastrointestinal tract. Furthermore, additional variants of Kaposi's sarcoma in Africa have been described.

Histologically, all clinical forms of Kaposi's sarcoma show similar features: in the early or patchy stage, jagged and dilated vascular spaces with interstitial inflammatory cells and extravasated red blood cells can be seen. In the more characteristic plaque and finally nodular stage, the tumor is made up of plump spindle cells with irregular slit-like vascular spaces, aligned by a recognizable endothelium and filled with erythrocytes. Using PCR, usually in the nested form, HHV8 has been detected in 90-100% of Kaposi's sarcoma, including all clinical forms mentioned above. It can therefore be concluded that HHV8 DNA is present in all variants of the tumor and that the detection of HHV8 DNA can be used as a diagnostic test for this disease. Failure to detect HHV8 in a given lesion (and a positive internal control to confirm the presence of appropriate DNA) should shed doubts on the primary diagnosis. The detection of HHV8 can further be used as a diagnostic tool in clinical sped-