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Studies of the Antigenic Relationships of the New Human Papovaviruses by Electron Microscopy Agglutination

JOHN B. PENNEY, JR., AND OPENDRA NARAYAN

The Departments of Neurology and Laboratory Animal Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

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Virus-antibody interactions observed by electron microscopy show that new human papovavirus isolates are antigenically distinct, but share common antigens.

Several papovaviruses of the simian virus 40 (SV40)-polyoma subgroup have been isolated from humans in the past 2 years. Most have been from the brains of patients with progressive, multifocal leukoencephalopathy (PML), a rare demyelinating disease. The JC virus, isolated by Padgett et al. (5) was originally reported as immunologically distinct from known papovaviruses, but it has subsequently been shown that some SV40 immune sera have small amounts of neutralizing and hemagglutination-inhibiting activity against JC virus (4). Weiner et al. (8, 9) isolated papovaviruses from two patients with PML. These viruses appeared to be immunologically identical to SV40, but the DNA of one of these agents has been shown to have minor differences from prototype SV40 (7). Subsequent papovaviruses isolated from brains of patients with PML have resembled the JC virus (10; and D. L. Walker, *In* W. Zeman and E. Lennette (ed.), *Slow virus diseases*, Williams and Wilkins, Baltimore, in press). Another papovavirus (BK) was isolated by Gardner et al. (3) from the urine of a patient without neurological disease after renal transplantation. Hemagglutination inhibition and electron microscopy agglutination (EMA) studies showed minor cross-reactions between BK virus and SV40. Although JC and BK viruses share many properties, such as morphological identity, hemagglutinating properties, a common host, and common antigenic properties with SV40 (2-5), there is no report of a direct serological comparison between these agents.

This study was undertaken to compare the antigenic relationships of these new human agents by using the sensitive EMA technique in which reactions of antibody molecules and virions can be observed directly (1).

Viruses used included SV40 and SV40-PML viruses (cases 1 and 2) grown in BSC_1 cells; BK virus grown in Vero cells (provided by K. Shah); JC virus grown in human, fetal glial cells (provided by B. Padgett); and M-PML virus, a recently isolated virus serologically indistinguishable from JC virus (10), and extracted directly from brain tissue by methods previously described (6). Each virus was tested with acute and hyperimmune rabbit sera prepared against the other viruses. Acute sera were obtained 10 days after a single intravenous injection of 10^7 to 10^8 virus particles; hyperimmune sera were obtained 10 days after six weekly intravenous inoculations of the same material. Polyoma virus, grown in mouse embryo fibroblasts, and hyperimmune antiserum to polyoma virus (Microbiological Associates) were used for controls. All virus suspensions were treated with Freon-113, and serum-virus mixtures for EMA were prepared as previously described (6).

A positive virus-antibody interaction occurred when virions were aggregated into clumps in which antibody molecules could be seen cross-linking the virions. The percentage of the virions agglutinated decreased if antiserum was diluted before reacting it with virus. The background level of nonspecific particle aggregation for Freon-treated virus suspensions was found to be approximately 10 to 20 virions per 500 counted. A standard virus preparation (approximately 10^6 to 10^7 virions) was reacted with fivefold dilutions of sera, and the titer is reported as the highest dilution of antiserum at which 50% of the virions were agglutinated by visible antibody. At least 30 particles were counted in each preparation.

The results of the reactions are shown in Table 1. Reactions with acute sera demonstrate

TABLE 1. *Electron microscopy agglutination reactions*[a]

Viruses	Acute rabbit antiserum against				Hyperimmune rabbit antiserum against				
	SV40 virus	SV40-PML_1 virus	BK virus	M-PML virus	SV40 virus	SV40-PML_1 virus	BK virus	M-PML virus	Polyoma virus
SV40	+++	+++	±	−	+++	+++	+	+	−
SV40-PML_1	+++	+++	±	−	+++	++++	+	+	−
SV40-PML_2	++	+++	±	−	+++	+++	++	+	−
BK	−	−	++++	−	+	±	++++	+	−
JC	−	−	−	++	+	++	±	++++	−
M-PML	−	−	−	++	+	+	+	++++	−
Polyoma					−	−	−	−	++++

[a] Symbols for reactions: ++++, 50% agglutination of virions at serum dilutions of 1:2500 or greater; +++, 1:500; ++, 1:100; +, 1:20; ±, definite agglutination of less than 50% of virions at initial 1:20 dilution; and −, no agglutination.

serological identity of JC virus with M-PML virus and SV40 with SV40-PML 1 and 2. They also show that JC and BK viruses are antigenically distinct, establishing three antigenic types which react significantly only with homologous type-specific acute sera. Hyperimmune sera also reacted most significantly with their homologous antigenic type of virus. A smaller heterologous reaction of each serum with members of the other two antigenic types indicates shared capsid antigens among these human papovaviruses. A consistent negative reaction with polyoma virus shows that this is not a nonspecific phenomenon. Papovaviruses have previously been regarded as antigenically distinct; however, these results indicate antigenic relationships between all known members of the SV40-polyoma subgroup of papovaviruses of primate origin.

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