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Role of the environment in the transmission of JC virus

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JC virus is etiologically associated with a fatal demyelinating disease known as PML. JCV produces persistent infections in the kidney and is excreted in the urine of healthy individuals and in the urine of PML patients. The characteristics of the JCV excreted in the environment have been studied by analyzing sewage samples from divergent geographical areas. The intergenic region of JCV strains detected in the sewage of Barcelona (Spain), Umeå (Sweden), Nancy (France), Pretoria (South Africa), Patras (Greece), Cairo (Egypt), Washington, D.C. (USA), and diverse areas of Northern India has been sequenced, and the phylogenetic analysis showed their relationships with JCV strains previously described in urine or clinical samples in these geographic areas. The JCV regulatory region of the JCV DNA detected in sewage presented archetypal or archetypal-like regulatory regions with the exception of one of the twenty clones obtained from a sewage sample of the area of Washington, D.C. that presented a tandem repeated structure. Infectivity studies showed that archetypal JCV present in the urine of a pregnant woman productively infected SVG cells. Also JC viral particles showed considerable stability in sewage at 20°C and in front of treatments with acidic pH and trypsin. The high prevalence of JCV in urine and in sewage and the stability of the viral particles observed suggests that contaminated water, food, and fomites could be the vehicles of JCV transmission through the oral route. Virions partially degraded or noninfectious could be a source of JCV DNA and may represent an additional mechanism of entry of viral genes into cells. *Journal of NeuroVirology* (2003) **9(suppl. 1),** 54–58.

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Introduction

JC virus (JCV) is etiologically associated with a fatal demyelinating disease known as progressive multifocal leukoencephalopathy (PML), a frequent complication of acquired immunodeficiency syndrome (AIDS) in human immunodeficiency virus (HIV)-infected individuals (Berger *et al*, 1987). JCV produces persistent infections in the kidney (Shah, 1995). Most of the primary infections with JCV occur early in childhood and are asymptomatic. The virus persists in the kidney and is excreted in the urine of healthy individuals and in the urine of PML patients (Kitamura *et al*, 1990; Agostini *et al*, 1996). The infections established by these viruses persist indefinitely in infected individuals. More than 80% of the adult human population presents antibodies against JCV (Padget and Walker, 1973).

Although JCV has not been related to any urogenital disorder, the prevalence of JCV in the kidney is relatively high, despite high variability. A prevalence that ranges between 10% (Arthur *et al*, 1989; Jin *et al*, 1993) and 50% (Kitamura *et al*, 1990; Shah *et al*, 1997; Markowitz *et al*, 1993) has been reported. The frequency of excretion of JCV in urine depends on the ethnic group (Agostini *et al*, 1997) and on the age (Kitamura *et al*, 1994). The level of excretion of JCV in immunocompetent population is higher than those with BK virus (BKV) (Shah *et al*, 1997). The shedding of both viruses occur independently, although both excretions increase with aging (Kitamura *et al*, 1990).

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Human infections with JCV seem to be population-associated; the JCV genotype excreted by individuals of defined ethnicities is determined by the geographical origin of the ethnic group rather than the JCV genotypes that are prevalent in their current location (Agostini *et al*, 1997). The viruses excreted in urine present an archetypal regulatory region (RR) characterized by presenting no duplications and 23-bp and 66-bp insertions. JCV detected in the brain and cerebrospinal fluid of PML patients commonly present tandem repeated structures in the RR. Human primary fetal glial cell cultures efficiently support the growth of JCV presenting tandem repeated structures in the RR (Padget and Walker, 1971).

JCV has been detected in various cell types (reviewed in Major *et al*, 1992), including cells of the immune system (reviewed in Gallia *et al*, 1997).

JCV has been reported to transform cells in culture and to have oncogenic properties in experimental animals; a role for JCV in brain tumors has been recently suggested (reviewed in Del Valle *et al*, 2001). The presence of JCV DNA in the human upper and lower gastrointestinal tract (Riccardiello *et al*, 2000) and in tonsils (Monaco *et al*, 1998) has been described and JCV has been also related to human colorectal cancer (Laghi *et al*, 1999). The route of infection of JCV has not yet been defined. We could hypothesize that oral transmission, probably including both tonsil and gastrointestinal tract, could be a route of entry of JCV into the organism.

In this issue, we have reviewed data on the characteristics of the JCV excreted and present in the environment that could have implications in the transmission of JCV throughout the human population.

Presence of JCV in the environment

Because JCV is excreted in urine, we decided to conduct a study on its presence and behavior in the environment by testing sewage samples, searching for JCV and the other polyomaviruses BKV and simian virus 40 (SV40). We have applied a methodology previously developed by our research group (Puig *et al*, 1994), which is based on the concentration of the viral particles present in sewage followed by a nucleic acid extraction and nested polymerase chain reaction (PCR) amplification.

We analyzed sewage samples from widely divergent geographical areas: Barcelona (Spain), Umeå (Sweden), Nancy (France), Pretoria (South Africa) (Bofill-Mas *et al*, 2000), and also from Patras (Greece), Cairo (Egypt), and Washington DC (USA) (Bofill-Mas *et al*, 2001).

From a total of 52 samples, we detected JCV DNA in 98% of them, the mean concentration of JCV estimated to be between 10^2 and 10^3 viral particles per milliliter. Ninety percent of the samples tested were positive for BKV (10^1 to 10^2 viral particles per milliliter) and none of them were positive for SV40. We also have found JCV in 7/23 polluted water samples collected in various areas from North India.

We analyzed the presence of JCV in 10 samples of oysters and mussels after homogenization of the digestive divercula of 10 to 12 animals in glycine buffer, pH 10, and concentration of viral particles (Pina *et al*, 1998). Shellfish are used as biosensors because they filter larger volumes of water and concentrate the viruses present in water. Five samples were positive for JCV at concentrations ranging from 1 to 10 viral particles per gram of shellfish digestive tract. The shellfish samples tested were negative for BKV and SV40.

In accordance with what has been reported by other authors, the data obtained clearly indicate that JCV is more frequently excreted in urine and it is detected in the environment at higher concentration than BKV.

Some of the JCV strains detected in sewage samples were further sequenced and showed archetypal regulatory regions. The sequences observed for the intergenic region clearly correlated with the geographic origin of the samples collected. The variability observed in the intergenic region of the JCV analyzed argued against laboratory contamination. The intergenic region of JCV has been used for tracing human migrations. We constructed a phylogenetic tree that includes reference strains of JCV and the strains that we have detected in sewage samples from all the different areas studied (Figure 1).

The JCV regulatory region of the viral DNA concentrated from two sewage samples, one from Barcelona and one from Washington, were amplified and 20 of the clones obtained from each sample were sequenced. Seventeen out of the twenty clones from Barcelona presented an archetypal structure, whereas three of them presented little variations of this structure. It is known that the archetypal configuration is highly conserved, although little modifications represented by point mutations and small deletions or duplications have been detected in urine; some of these changes can be related to immunodeficiency (Agostini *et al*, 1995; Kitamura *et al*, 1994). Twelve out of the twenty clones from a sewage sample collected in the area of Washington were identical to the archetypal strains, seven presented little variations, and one of them presented a tandem repeated structure similar, but not identical, to Mad-4 strain. Rearranged JCV, those who have been described to efficiently infect primary human fetal glial cells, are under some circumstances shed in urine and, as a consequence, they may be present at low concentrations in the environment.

Before these studies were carried out, there were no data reporting the presence of polyomaviruses in the environment. The high level of excretion of JCV supports the idea that urine is the most likely source of JCV infections in humans.

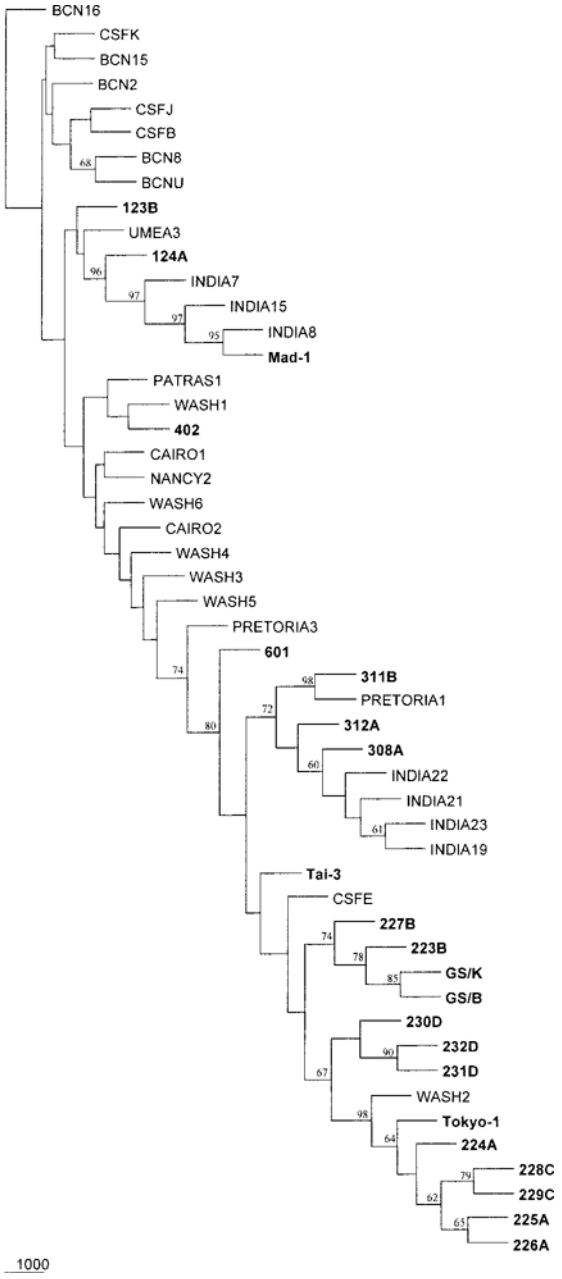

Figure 1 Phylogenetic reconstruction of the JCV strains analyzed in the study. Reference strains (represented in bold) 123B, 124A, Mad-1, and 402 belong to European Types 1 and 4. 601 belongs to Type 6 typical from West Africa. Strains 311B, 312A, and 308A belong to Afro-Asiatic Type 3. 227B, 223B, GS/K, and GS/B belong to Type 2B typical from Eurasia. Strains 230D, 231D, and 232D belong to the South Indian Type 2D. Tokyo-1, 224A, 228C, 229C, 225A, and 226A belong to Afro-Asiatic Type 2 characteristic from Japan and Native Americans. Tai-3 belongs to Afro-Asiatic Type 7 characteristic from South Asia. JCV strains CSFJ, CSFB, CSFK, CSFE are isolated from cerebrospinal fluid of PML patients in Barcelona. All other strains represented correspond to JCV strains isolated from sewage samples. The neighbor joining (NJ) tree has been constructed using 461 nucleotides (nt 2177 to nt 2637) of the intergenic region of JCV genomes. The bootstrap confidence levels obtained for 1000 replicates are shown (only significant values are indicated). Genbank accession numbers for the JCV strains isolated in India are AY148414–AY148420. (BCN, Barcelona; WASH, Washington).

JCV stability in the environment

Polyomaviruses have been described as being relatively resistant to heat and to survive up to 1 h in water at 55°C (Atwood, 2001).

Data on the behavior of JCV in the environment could contribute to evaluate its mechanism of transmission. In order to determine whether or not the JCV detected in sewage samples could be a source of infection for humans, we decided to study the stability of the virions in sewage samples through time. When evaluating the stability of JCV in sewage at 20°C, we estimated a t_{90} (time required for the degradation of the 90% of viral particles) of 26.7 days and a t_{99} (time required for the degradation of the 99% of viral particles) of 61.5 days. Structured JCV viral particles were detected after 72 days in sewage at 20°C (Bofill-Mas *et al*, 2001). JCV is then relatively resistant in sewage samples, providing the possibility of being transmitted by contact with contaminated food or water.

Infectivity of the excreted virions

It has been extensively reported that human primary fetal glial cells efficiently support the growth of JCV presenting tandem repeated structures in their regulatory region (Padget and Walker, 1971); however, there is only one study reporting the growth of archetypal JCV after infection of COS-7 cells with urine (Hara *et al*, 1998).

We have detected productive infection of archetypal JCV in SVG cells by concentrating the viral particles present in the urine of a pregnant woman before the infection (Bofill-Mas *et al*, in press).

We have also studied the effect of acidic pH and 10 μg/ml of trypsin, both important factors affecting the entry of infectious viruses through the gastrointestinal environment, on JCV Mad-4 viral particles and on its infectivity in SVG cells. JCV intact viral particles were obtained after been exposed to pH 3 for 30 min, whereas free JCV DNA was detected after a treatment with pH 1 for 30 min (Bofill-Mas *et al*, 2001). We have also observed that trypsin has no effect in the infectivity of Mad-4.

A treatment with pH 3 for 1 h did not inhibit the infection of Mad-4 in SVG, although there was an important decrease in the progeny titer (Bofill-Mas *et al*, in press).

Urine appears then to be the principal source of infection for humans and contain principally archetypal strains that could be potentially infectious after oral transmission.

Model of transmission in the population

A model of transmission for JCV may imply an oral entry of the virus into the gastrointestinal tract, then

undergoing a primary replication in the lymphoid tissue associated with the pharynx and the gut. A low level of viremia may occur, leading to a secondary systemic spread and establishment of persistent infections in the kidney and probably latent infections in other cell types.

We have reported that archetypal strains have the capability to infect SVG cells; however, the natural cells that support JCV replication, proving a route of transmission of the virus, still remain unknown.

The high prevalence of JCV in urine and sewage samples suggests that contaminated water, food, and fomites could be the source of infection and could then be vehicles of JCV transmission. We hypothesize that JCV could be orally ingested and may infect persons through the gut mucosa as it appears to be relatively stable to acidic environment and proteinase exposure present in the gastrointestinal tract. The viruses probably will arrive to lymphocytes through M cells in Peyer's patches and, in addition, there is also the possibility that JCV or JCV DNA could enter into the enterocytes by diverse mechanisms, including processes of pinocytosis. Tonsils have also been suggested as a possible site of entry of the virus (Monaco *et al*, 1998) and, as in the case of other viruses, replication of JCV may occur in the lymphoid tissues in the tonsils and in the gut. Gastrointestinal tissues seem to present JCV DNA (Riccardiello *et al*, 2000). It is important to consider that foreign DNA is not completely degraded when ingested with food, and it has been proven in animals that it can reach peripheral leukocytes and some other organs (Schubbert *et al*, 1997). Virions partially degradated or noninfectious could be a source of JCV DNA and may represent an additional mechanism of entry of viral genes into cells or even of JCV infection, because polyomaviral DNA has shown to be infectious when transfected into permissive cells.

Archetypal strains have been the most common strains detected in sewage. Tandem repeated strains have been suggested to have evolved from an initial infection of archetypal strains and to have a higher level of pathogenesis due to an extended cell tropism (Yogo *et al*, 2001). More information is also needed regarding the effect of primary infection, coinfection, or reinfection with tandem repeated strains and its potential effect on the pathogenesis of JCV infection.

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