In the Literature

**Cycloviruses as a Possible Cause of Neurological Disease**


Cyclovirus is a proposed third genus in the Family Circoviridae, which has been composed of the circoviruses and gyroviruses. The Circoviridae are nonenveloped, spherical viruses with a single-stranded circular DNA genome of 1.8–3.8 kb. Two groups, one in Malawi and one in Vietnam, have detected novel cycloviruses in patients with neurological disease. Once again, however, the search for novel viral pathogens has led to more questions than answers.

Smits and colleagues analyzed cerebrospinal fluid (CSF) and serum samples of patients with paraplegia of unknown etiology in Malawi for the presence of viral sequences using random polymerase chain reaction (PCR) amplification followed by next-generation sequencing. They detected evidence of a novel cyclovirus in 15% of serum and 10% of CSF samples obtained from 58 enrolled adults. No controls were, however, examined.

Separately, Tan and colleagues used a combination of amplified fragment length polymorphism-based virus discovery and next-generation sequencing to examine the CSF of adults and civilians in Vietnam with acute central nervous system (CNS) infection of unknown etiology for the presence of viral sequences. Samples were collected from 1999 to 2009. The investigators initially identified a novel cyclovirus in the CSF of 2 Vietnamese patients with central nervous system infections of unknown etiology. Using a sensitive and specific PCR, they subsequently detected the same virus in 26 of 642 (4%) randomly selected CSF samples from Vietnamese patients with suspected CNS infection, but in none of 122 control patients with noninfectious neurological diseases. The virus was detected in patients from 7 provinces of central and southern Vietnam. Whole-genome sequencing confirmed that the virus, which they called cyclovirus-Vietnam (CyCV-VN) novel.

The ages of the 26 cyclovirus-positive patients ranged from infancy to >60 years. The virus was detected in 10 of 273 (3.7%) CSF specimens from patients with CNS infection of unknown etiology and in 16 of 369 (4.3%) samples from patients who had a demonstrated etiology of their infection. The latter group included 4 of 141 (2.9%) with viral encephalitis (Japanese encephalitis and dengue), 7 of 108 (6.5%) with bacterial meningitis, and 5 of 121 (4.1%) with confirmed or suspected tuberculous meningitis. The overall mortality of patients in whom cyclovirus was detected was 4%. Among patients with positive CSF results, cyclovirus sequences were also detected in 1 of 5 rectal swabs, 1 of 4 pharyngeal swabs, and 0 of 3 serum samples.

CyCV-VN DNA was also detected in 8 of 188 (4.2%) fecal specimens from healthy children as well as in samples from 58% of poultry and pigs from the home province of the index case. The virus detected in these animals was of the same species (>97% sequence similarity) as the human isolates.

These findings do not represent the first detection of a novel cyclovirus in a patient with neurological disease. The presence of a novel cyclovirus in the feces of a child with nonpolio flaccid paralysis (NFP) was reported in 2009. Victoria and colleagues, using pyrosequencing of viral nucleic acid from stools of 35 South Asian children with NFP identified a mean of 2.6 viruses per sample (samples from 6 healthy contacts also frequently yielded viral sequences) [1]. Among the patient samples, one yielded a novel circovirus-like virus most closely related to porcine circovirus (55% amino acid identity), which would now be considered a cyclovirus. The investigators, however, pointed out that they could not determine whether this was a human virus or whether its presence in stool resulted from ingestion of infected meat—a critical caveat. In fact, cycloviruses of possibly human origin have been found in 7% to 17% of non-U.S. human stools and 3% to 55% of non-U.S. meat sample [2]. They have also been detected in a wide variety of farm animals, as well as in chimpanzees, bats, cockroaches, and dragonflies.

Thus, the detection of novel cycloviruses in humans with neurological diseases does not necessarily indicate an etiologic role—especially since, in the Vietnam study, 16 of the 26 people who carried the cyclovirus also had a laboratory-confirmed infection with another pathogen. These included human immunodeficiency virus (HIV) and hepatitis B virus—each found in 2 patients. The search for new...
viruses using sequence-independent molecular methods has been very productive, but the unequivocal demonstration that they cause human disease lags well behind.

References

A Novel Virus in Patients With Seronegative Hepatitis

Xu and colleagues used Solex deep sequencing for viral discovery using serum samples of 92 patients with non-A-E-hepatitis in Chongqing, China, seen between 1999 and 2007. Dividing the samples into 10 serum pools, they identified in each pool a 3780-bp contig whose sequence was closest to parvoviruses. The contig contained 3 major open reading frames (ORF). ORF1 encoded a protein with homology to the replication-associated protein (Rep) of bat circovirus, ORF2 encoded a capsid protein (CP) homologous to the parvovirus coat protein of porcine parvovirus and goose parvovirus, and ORF3 encoded a small protein without identified homologies. The investigators provisionally designated the virus as NIH-CQ. Deep sequencing demonstrated that NIH-CQ exhibited great intrapatient genetic heterogeneity indicating the presence of quasi-species.

Using a quantitative polymerase chain reaction (PCR) for the rep region of NIH-CQ with a sensitivity <10 copies, they detected sequences of the virus in 63 of 90 (70%) patient samples, with a mean viral load of 1.05 e4 copies/μL, and with no significant quantitative difference between those with acute or chronic hepatitis. In contrast, NIH-CQ sequences were detected in none of 45 healthy controls. Immunoblotting using the ORF-encoded CP found that 76 of 90 (84%) patients had immunoglobulin G antibodies to NIH-CQ, as did 35 of 45 (78%) controls. However, 28 (31%) with seronegative hepatitis had immunoglobulin M antibodies to the virus, whereas no control did. Although the NIH-CQ CP antigen used had homology with paroviruses, there was no serological cross-reactivity with known major paroviruses.

Although NIH-CQ had no homology at the nucleic acid level between the detected 3789-bp contig and known viruses in GenBank, its Rep and CP proteins did have limited homologies with circoviruses and parvoviruses, respectively. Phylogenetic analysis indicates that NIH-CQ is a hybrid virus at the interface of the Parvoviridae and the cycloviruses and is likely the result of genetic recombination. Porcine circovirus-2 causes postweaning multisystemic wasting syndrome in pigs with hepatitis being part of the disease. Parvovirus B19 infection has been associated with acute hepatitis in humans. The overall genomic organization of NIH-CQ has characteristics of the Parvoviridae.

The excess incidence of IgM antibody to NIH-CQ antigen suggests acute infection with the virus, although a concern regarding falsely positive IgM antibody tests must be maintained. Whether NIH-CQ is etologic in patients with hepatitis remains to be determined.

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