Clinical and molecular insights into human parechovirus infection

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General discussion
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The identification of HPeV3 in 2004 and its association with neonatal sepsis made it particularly clear that HPeVs can be related to severe disease in young infants. New parechoviruses continue to be identified. However, little is known about the clinical and molecular characteristics of this expanding group of viruses. In our studies, we show that distinct clinical and molecular differences exist between HPeV3 and other HPeV types. Furthermore, we demonstrate the clinical relevance of HPeVs and the need for specific diagnostic methods. The studies presented here have contributed to knowledge on this, as yet, small group of viruses, have changed the view regarding their clinical relevance and have aided in the development of ongoing and future studies regarding the pathogenesis and treatment of HPeV.

EPIDEMIOLOGY

HPeVs are highly prevalent viruses as is illustrated by high seroprevalence rates found in different parts of the world (24,32,37,38,40). HPeV1, previously classified as an enterovirus, was reported as 1 of the 6 most prevalent enteroviruses in Finland (21). More than 99% of the Finish adults are seropositive for HPeV1 by the age of 1 year indicating HPeV1 infection is common in young children (3,18,19,39, and chapters 3, and 5). Identified as the second predominant strain, at least in Europe (39, and chapters 3, and 5), the seroprevalence data of HPeV3 would be quite high but lower in comparison to HPeV1. In Japan, the seroprevalence data for HPeV3 was found to be 78% among adults, even though HPeV3 was first identified in Japan in 1999 (23) which would mean that the strain already circulated long before it was first identified. In the Netherlands, we were able to find the strain to already circulate in 1994 (chapter 10) at similar frequencies reported in Japan (48)) and nowadays in Europe, suggesting that seroprevalence data within Europe would be of similar proportions as in Japan.

The high seroprevalence seen for HPeV1 suggests that the majority of infants should be protected from HPeV1 infection early in life due to maternal antibodies. However, Ehrnst and Eriksson suggested that the presence of maternal HPeV1 antibodies did not always protect against infection (10). Nevertheless, the children infected with HPeV1 were older than 6 months (1,42, and chapter 2), suggesting that neonates indeed were protected
against infection with HPeV1. In contrast, children infected with HPeV3 generally were not older than 1 month (1,42,48, and chapter 2), despite the high seroprevalence in adults, which may indicate a lack of protection from maternal antibodies against HPeV3. One might consider that previous infections by HPeV3 are serologically distinct from current infections, failing to protect neonates from recent infections. However the low divergence between HPeV3 strains collected in recent years and more than 15 years ago (chapter 10), thus not support a genetic drift towards these serologically distinct variants, which would be expected based on the 2 year cycle seen for HPeV3 (18,42, and chapters 4, 5, and 7) as seen for several enteroviruses (31,34). However if indeed this HPeV3 protection is failing, the virus should be commonly found among adults as well, as a result of re-infection. However this is not the case; several studies that include samples obtained from adults show that HPeV is almost exclusively found in young children (3,18,19,38,39, and chapter 7). So why are children more susceptible than adults? A possible clue may lie in the biological differences between the different HPeV types or between children and adults.

HPeVs, FROM MILD TO SEVERE

Infections with HPeVs were previously found to be relatively mild (10,11,24,38). More so than seen for enteroviruses as shown by the study of Grist et al (14), which have predominantly been associated with severe central nervous system (CNS) associated symptoms (14,25,38). With the identification of HPeV3, the view that HPeV are mild infections changed. Although infection with HPeV1 had occasionally been associated with paralysis (13) and encephalitis (27), infection with HPeV3 seems more frequently associated with more severe symptoms (5, 18,42-44, and chapter 2). Moreover, this type was found to be related to severe morbidity and sequelae of infection ranging from seizures to learning disabilities at a later age (43). Recent data showed HPeV3 to be the predominant HPeV type detected in CSF (18,43). Based on the high percentage of HPeV found in CSF of children with CNS associated diseases and also neonatal sepsis, HPeVs can be considered as another major viral cause of these diseases (chapter 4).

Remarkably, despite the detection of HPeV in CSF of children with such severe disease symptoms that indicate infection of the CNS, the CSF cell counts and protein levels were not significantly increased (44). This has also been found for enteroviruses (8,36,44,45). In children with detectable HPeV in the CSF, symptoms of sepsis-like illness were more frequently observed.
than CNS-associated disease such as meningitis or encephalitis (chapters 2 and 4). It could therefore be that the presence of HPeV in the CSF was due to a “leaky” blood-brain-barrier in neonates, without overt infection of the brain or meninges and therefore no cell reaction in the CSF. Unfortunately, data on the viral load in plasma are lacking to support this notion. Notably, pleocytosis was also not observed in the children that did exhibit signs of CNS infections. An interesting hypothesis was proposed by Volpe (47), who suggested that HPeV3 infections could result in intracellular binding of toll like receptor (TLR) 8, previously found to be involved in the host immune response against HPeV1 (41), that can lead to the release of reactive oxygen and nitrogen and pro-inflammatory cytokines that are toxic to neural cells. This is an interesting theory that could potentially explain why we do not observe a cell reaction within CSF. Remarkably, TLR8 is specifically distributed in axonal perturbations and only in the developing nervous system. This implies that HPeV infection within the CNS at a very young age, leading to neural cell death, can be detrimental for the development of the child, as seen by Verboon-Macieleck et al. (43), but also explains why we specifically observe the severe HPeV3 infections in very young infants.

**HPeV3, THE ODD VIRUS OUT**

The severe clinical manifestations of HPeV3 infection in comparison to other types points out to a difference in cell tropism between the types. In vitro data have already implied a difference in cell tropism to exist (1,48, and chapter 7). Thus, one of the most important questions in parechovirus research is what cells HPeV3 specifically may target and how the virus enters the cells, and whether this contributes to its more severe pathogenicity in comparison to other HPeV types. In this respect, the absence of the (Arginine-Glycine-Aspartic Acid) RGD motif in HPeV3 is intriguing. The RGD motif is known to bind to integrins (12,22) and was found to be crucial for HPeV1 receptor binding (6). Its absence in HPeV3 would thus indicate a different receptor usage that is RGD independent. The RGD motif has been shown to play a role in the pathogenesis of Coxsackie A Virus (CAV) 9 and echovirus 9 infections (7,15,33,51,54) and it was shown that both viruses can enter a specific cell via an RGD-independent mechanism. But in contrast to what we observe for HPeV3, the absence of the RGD motif was not found to be related to severe disease. In fact, the direct opposite was observed. In vivo, the RGD-containing echovirus 9 strain (Barty) was found to be pathogenic for newborns.
mice, while the RGD-negative echovirus 9 strain (Hill) was not (9,53-55). Also CAV9 mutants without an RGD motif were found to be less pathogenic in these mice (16,17). A possible clue for the difference between these 2 viruses and HPeV can be found in in vitro studies which show CAV9 RGD-negative mutants to infect different cells than HPeV3 (1,20,48, and chapter 7). It thus seems probable that the viruses use different RGD independent pathways.

Interestingly, studies on echovirus 9 (9), using both newborn mice and older mice depicted a differential pathogenicity between these mice. While the Barty strains could induce a paralytic response in newborn mice, this response remained absent in older mice, despite a significant increase in viral titres within the affected tissues. This differential pathogenicity was also seen for CAV9 (15,17).

An interesting notion was made by Harvala et al (15) that could explain this differential pathogenicity; it was found that the alpha(v)beta(3) integrin, a host cell receptor used by both virus was down regulated during mice development (4,30). If translated to a human infection, where various receptors can also be down or upregulated during development, a shift of specific cell receptor expression necessary for HPeV3 entry would support the observation why HPeV3 infections are rarely or never seen in older children and adults even when the humoral protection against HPeV3 might be lacking.

**CONCLUDING REMARKS: HPEV tropism unravelled, a step toward specific antiviral therapy against picornaviruses**

Picornaviruses cause more than 6 billion infections per year and have a significant clinical impact on global health care. As a major viral cause of CNS-associated disease and neonatal sepsis in children, HEV and HPeV infections lead to severe morbidity in children. With the exception of poliovirus, effective anti-viral therapy or vaccines are still lacking for these viruses.

Antiviral agents based on steps in the picornavirus life cycle may be considered. As the initial event in the replication cycle of picornavirus is attachment of the viral capsid protein to a cell surface molecule or receptor, a major target for antiviral therapy could be the inhibition of the viral capsid function. The experimental drug pleconaril was developed to bind to the viral capsid protein VP1 and inhibits viral adsorption. Experience with pleconaril in infants with severe HEV infection is limited and virological and clinical
efficacy of this drug were not always demonstrated (2,17,26,33,35,50,54). Although the drug should be effective against 98% of the most commonly circulating types, an explanation for the efficacy differences of the drug could be that the composition of the pocket within the capsid protein VP1, where the pleconaril compound should bind, may differ between types and type specific entry inhibitors may be more beneficial.

Unravelling specific viral factors involved in the infectious cycle, that can influence pathogenesis, are pivotal in the development of antiviral drugs. A few pathogenesis studies have implicated different viral factors to influence pathogenesis (28,49). Studies on CAV9 and echovirus 9 (29,46,52) found the capsid protein to play a major role in influencing cell tropism and pathogenesis, whereas studies on enterovirus 71 showed the replication capacity and also evolutionary factors such as recombination to define pathogenesis. However, the occurrence of over 100 non polio-HEV serotypes with a large diversity of clinical syndromes makes it difficult to study viral factors that may play a role in defining pathogenesis of one type in comparison to another. Up till now, no correlation could be found between receptor usage of different types in relation to cell tropism, and specific disease entities.

HPeVs form a clinically well defined group with distinct differences in biological characteristics. These factors render this group of viruses ideal to study cell tropism in relation to disease severity, which could be a key in investigating cell entry inhibitors as antiviral agents, and to learn more about picornavirus pathogenesis.

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