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Human Parechoviruses, New Players in the Pathogenesis of Viral Meningitis

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1. Introduction

Human parechoviruses (HPeVs) belong to the family of *Picornaviridae* and have been recognized as a separate group on the basis of distinct molecular and biological properties since 1999. The identification of HPeV3 in 2004 and its association with neonatal sepsis and meningitis made it particularly clear that HPeVs are related to severe disease in infants. Molecular techniques are increasingly being used for the identification of HPeVs and with the increase in epidemiological and clinical data, HPeVs are now considered to be the second predominant cause of viral meningitis, following enteroviruses (EVs). This review focuses on the role of HPeVs, with particular notice to HPeV3, as a causative agent in viral meningitis as well as neonatal sepsis and encephalitis. Data on epidemiology, clinical manifestations, immunology and molecular and cellular biology, underlying the greater pathogenicity of this novel group of viruses as well as the diagnosis, management and treatment options will be discussed.

2. Classification and biology of HPeV

Human parechoviruses (HPeVs) belong to the large *Picornaviridae* family, comprising small non-enveloped, single-stranded positive-sense RNA viruses infecting both humans and animals. The *Picornaviridae* family currently consists of 12 established genera as of 2011: *Enterovirus*, *Parechovirus*, *Hepatovirus*, *Kobuvirus*, *Aphthovirus*, *Erbovirus*, *Teschovirus*, *Cardiovirus*, *Tremovirus*, *Sapelovirus*, *Avihepatovirus* and *Senecavirus*. Many new genera such as the *Cosavirus*, *Klassevirus* and *Aquamovirus* have recently been proposed, and are awaiting genus/type classification (<http://www.picornastudygroup.com/>).

HPeVs were first discovered in 1956 during a summer diarrhea outbreak in the USA by Wigand & Sabin et al. (1961) and originally classified within the *Enterovirus* genus as echovirus 22 and 23. This was based on their biology in cell culture, exhibiting a similar cytopathogenic effect (CPE) as enteroviruses (EVs), their clinical presentation and non-pathogenicity in both mice and monkeys. Despite these similarities, subtle differences such as a slow progression of CPE of infected cells in cell culture and their more common

association with mild gastrointestinal disease, in comparison to the more severe symptoms reported for other enterovirus types, led to their description as atypical enteroviruses. Later, evident differences in genome organization and structure, divergence of encoded proteins and other biological properties (Hyypia et al., 1992; Stanway et al., 2000), prompted their reclassification in 1999 (Stanway et al., 1994; Stanway & Hyypia, 1999) as HPeV types 1 and 2 within the genus *Parechovirus*. Almost half a century after the discovery of HPeV1 and 2, a third HPeV type was discovered in Japan (Ito et al., 2004) and since then the number of HPeV types increased rapidly with the development of more state-of-the-art molecular techniques. Up to date there are 16 HPeV types known (Table, Figure 1). The *Parechovirus* genus also comprises a second group of viruses isolated from rodents; the Ljungan viruses (Niklasson et al., 1999; Stanway et al., 2005).

Type	Strain	Origin	Reference
HPeV1A	Harris	Ohio, USA	Hyypia et al., 1992
HPeV1B	BNI-788 St	Bonn, Germany	Baumgarte et al., 2008
HPeV 2	Williamson	Ohio, USA	Ghazi et al., 1998
HPeV 3	A308/99	Aichi, Japan	Ito et al., 2004
HPeV 4	K251176-02	Amsterdam, the Netherlands	Benschop et al., 2006b
HPeV 5	CT86-6760	Connecticut, USA	Oberste et al., 1998
HPeV 6	NII561-2000	Niigata, Japan	Watanabe et al., 2007
HPeV 7	PAK5045	Badin, Pakistan	Li et al., 2009
HPeV 8	BR/217/2006	Salvador, Brazil	Drexler et al., 2009
HPeV 9	BAN2004-10902	Bangkok, Thailand	Oberste et al., unpub.
HPeV 10	BAN2004-10903	Bangkok, Thailand	Oberste et al., unpub.
HPeV 11	BAN2004-10905	Bangkok, Thailand	Oberste et al., unpub.
HPeV 12	BAN2004-10904	Bangkok, Thailand	Oberste et al., unpub.
HPeV 13	BAN2004-10901	Bangkok, Thailand	Oberste et al., unpub.
HPeV 14	451564	Amsterdam, the Netherlands	Benschop et al., 2008c
HPeV 15	BAN-11614	Bangkok, Thailand	Oberste et al., unpub.
HPeV 16	BAN-11615	Bangkok, Thailand	Oberste et al., unpub.

Table. HPeV prototype strains (<http://www.picornastudygroup.com/>)

Like other picornaviruses, the HPeV genome is small containing approximately 7300 basepairs encoding for a single polyprotein flanked by 5′ and 3′ untranslated regions (UTRs) and a poly A tail at the 3′end (Figure 2) (Stanway & Hyypia, 1999).

Following virus entry, the RNA is directly translated into a long polyprotein, which is subsequently cleaved by the viral protease (3C) into three structural proteins (VP0, VP1 and VP3) forming the capsid structure and seven non-structural proteins (2A–2C and 3A–3D) that are needed for replication. In contrast to other picornaviruses, VP0 is not further cleaved into VP4 and VP2. Additionally, while VP0 is not antigenic in any other genera, the predominant antigenic sites of HPeV have been mapped to the N-terminal region of the VP0 protein (Joki-Korpela et al., 2000). For HPeV1, additional antigenicity eliciting the production of neutralizing antibodies is found to be located at the C-terminal end of the capsid protein VP1, where the receptor binding motif arginine-glycine-aspartic acid (RGD) is located.

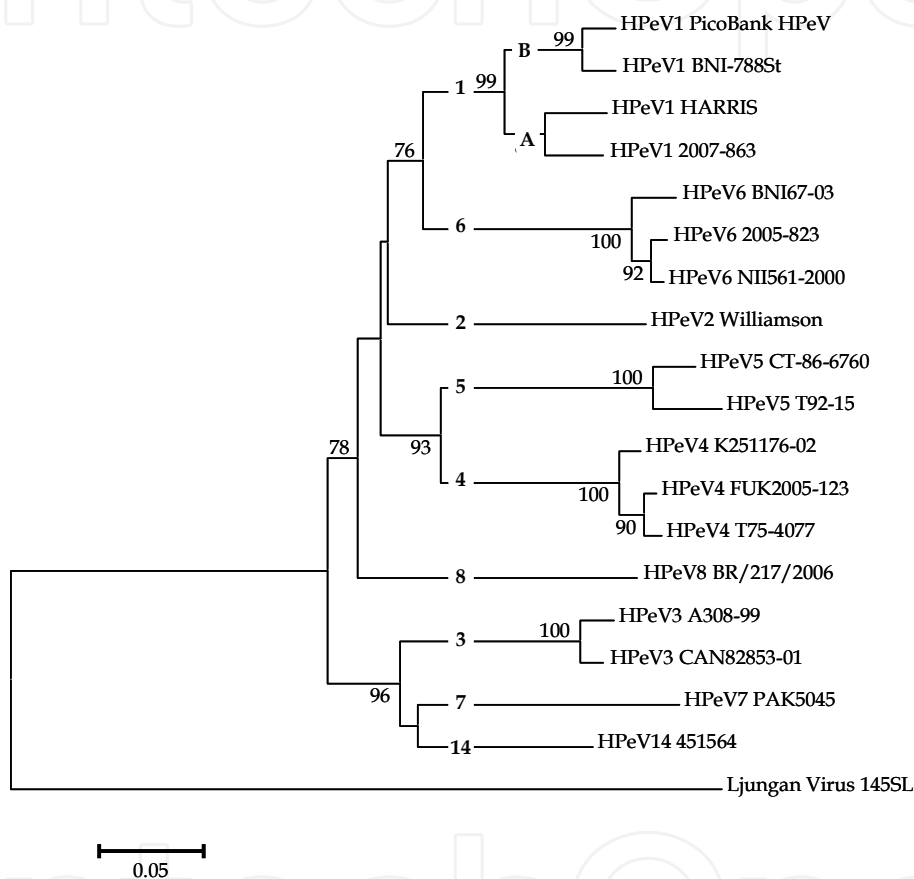


Fig. 1. Neighbour joining tree based on the complete VP1 gene of HPeV types 1-8 and 14 (amino acid p-distance). Complete VP1 sequences for HPeV9-13 and 15-16 were unavailable at the time of publication.

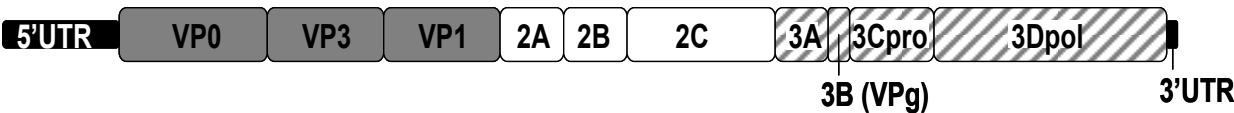


Fig. 2. HPeV genome.

To allow efficient translation, most picornaviruses enable the majority of host cellular mRNA translation to be shut off by the non-structural protein 2A. However, there is little evidence that HPeVs elicit the same shut-off (Stanway et al., 1994).

Replication is driven by the RNA-dependent RNA polymerase (3Dpol) and involves RNA structures present on the 5'UTR (Nateri et al., 2000, 2002) and VP0 (*cis*-acting replication element (CRE)) (Al-Sunaidi et al., 2007) as well as the 2C protein (NTPase activity and RNA binding) (Samuilova et al., 2006). Following binding of the protein VPg (3B) at the 5' end, the positive stranded RNA is copied to produce negative stranded RNA, which in turn can be replicated back to positive stranded RNA needed for assembly and additional translation activity to produce virus particles.

Finally, these particles are released from the cells. In most picornaviruses, virus release is achieved through capsid maturation by the cleavage of VP0 into VP2 and VP4. However, the cleavage of VP0 into VP2 and VP4 does not appear in parechoviruses and the manner of how maturation and virus release is achieved for HPeVs remains largely unknown (Stanway et al., 1994). Nonetheless, the external appearance of HPeV particles has proved consistent with the external appearance of other picornaviruses, most closely resembling Foot and Mouth Disease Virus (FMDV) in the *Aphthovirus* genus as has recently been shown by cryoelectron microscopy and image reconstruction (Seitsonen et al., 2010).

3. HPeV infections, from mild to severe disease

Clinical symptoms of HPeV infections are generally similar to those found in EV infections ranging from mild respiratory and gastrointestinal disease to more severe disease like meningitis and sepsis-like illness. In earlier decades when only HPeV1 and 2 were known, HPeV infections were considered of little clinical importance. Even though occasionally, severe disease was reported for HPeV such as acute flaccid paralysis, myocarditis, meningitis, encephalitis and encephalomyelitis (Benschop et al., 2006a; Figueroa et al., 1989; Koskiniemi et al., 1989; Legay et al., 2002; Maller et al., 1967) the majority of HPeV1 infections caused mild gastrointestinal and/or respiratory symptoms.

This all changed with the discovery of HPeV3 (Ito et al., 2004). By comparing clinical data from children infected with HPeV1 and children infected with HPeV3, we showed the increased clinical relevance of this type. HPeV3 infections were predominantly associated with neonatal sepsis and CNS infections while children infected with HPeV1 had milder symptoms (Benschop et al., 2006a). These findings were later confirmed by others, increasingly detecting this type in the CSF of children with CNS disease, such as meningitis and encephalitis, as well as neonatal sepsis (Harvala et al., 2009; Levorson et al., 2009; van der Sanden et al., 2008; Verboon-Macielek et al., 2008a; Wolthers et al., 2008).

Children infected with HPeV3 often present with symptoms of fever and irritability as a sign of CNS infection, either meningitis or encephalitis. The majority (54-80%) of the children also show sepsis-like illness, defined as fever or hypothermia with signs of circulatory and/or respiratory dysfunction defined by tachycardia or bradycardia, low blood pressure and/or decreased saturation (Benschop et al., 2006a; Harvala et al., 2009; Selvarangan et al., 2011; Wolthers et al., 2008). Additional clinical symptoms frequently seen in HPeV3 infections are maculopapular rash, gastrointestinal symptoms and respiratory symptoms (Benschop et al., 2006a; Selvarangan et al., 2011; Verboon-Macielek et al., 2008a; Wolthers et al., 2008).

Severe clinical manifestations are mainly described in relation to HPeV3 infections, although occasionally other HPeV genotypes have been associated with serious illness (Schnurr et al.,

1996; Watanabe et al., 2007). However, similarly to HPeV1 and 2, newer types have mainly been associated with mild gastro-intestinal and respiratory symptoms in children and are often found in children with an underlying illness (Pajkrt et al., 2009).

4. Epidemiology of HPeV in relation to CNS infections

Because HPeVs are reported as being transmitted through the fecal-oral route, epidemiological studies are often performed on stool samples and provide an accurate estimation of the prevalence of the different types. HPeV1 is considered as the predominant strain mainly affecting young children (Baumgarte et al., 2008; Benschop et al., 2006a, 2008c, 2010a; Harvala et al., 2008; Joki-Korpela & Hyypia, 1998; Stanway et al., 2000; Tapia et al., 2008; Watanabe et al., 2007). Epidemiological studies dating back over 30 year ago, showed HPeV1 to be widely spread (Ehrnst & Eriksson, 1993; Grist et al., 1978; Joki-Korpela & Hyypia, 1998; Khetsuriani et al., 2006b). In recent studies, involving the detection of the newer HPeV types, the new HPeV type 3 is identified as the second predominant strain (Benschop et al., 2006a, 2008c, 2010a; Ito et al., 2010; Tapia et al., 2008; Watanabe et al., 2007). HPeV3 comprised 22 to 26% of the HPeV types identified in HPeV positive stool samples collected in the Amsterdam region, while the majority of the HPeV positive stool samples comprised HPeV1 (65-71%)(Benschop et al., 2006a, 2008c, 2010a). Similar data were found in studies on stool samples in other European countries (Baumgarte et al., 2008; Tapia et al., 2008), and Asia (Ito et al., 2010; Pham et al., 2010, 2011a; Watanabe et al., 2007) where HPeV1 was identified as the most prevalent type, followed by HPeV3. HPeV4 is frequently found in stools as well (Benschop et al., 2008c, 2010a; Boros et al., 2010; Pham et al., 2011b; Zhong et al., 2011), while HPeV6 seems to prevail as a secondary respiratory pathogen (Harvala et al., 2008). Infections with HPeV2 and 5 are reported sporadically (Benschop et al., 2010a; Ehrnst & Eriksson, 1996; van der Sanden et al., 2008). Circulation patterns of the newly reported HPeV types 7-16 are yet to be determined (Benschop et al., 2008c, 2010a; van der Sanden et al., 2008).

The distribution of HPeV types is significantly different when only CNS infections are taken into account. Screening of CSF samples show HPeV3 to be the dominant type found in children, in particular neonates (Benschop et al., 2008c; Harvala et al., 2009, 2011; Pineiro et al., 2010; Renaud et al., 2011; Selvarangan et al., 2011; Verboon-Macielek et al., 2008a, 2008b; Watanabe et al., 2007; Wolthers et al., 2008). These infections account for approximately 3-17% of CNS infections reported as meningitis or encephalitis (Harvala et al., 2009; Pineiro et al., 2010; Watanabe et al., 2007; Wolthers et al., 2008; Yamamoto et al., 2009), far exceeding the percentage of herpes simplex virus infections. In contrast, EV in CSF is still most frequently detected in these samples, with 14-19% positive cases each year in the summer (Harvala et al., 2011; Pineiro et al., 2010; Wolthers et al., 2008). This ranks HPeV as the second dominant pathogen of viral meningitis and encephalitis. HPeV1 infections are rarely described in CSF (Harvala et al., 2011); the other HPeV types have never been described in CSF.

Of interest is that HPeV infections of the CNS circulate intermittently every 2-3 years, and always in the summer months. However, the circulation patterns seem to be geographically distinct. HPeV3 detected in both CSF and stool in Northern Europe is described most commonly every 2 years, in the summer period of the even years (Benschop et al., 2006a, 2008c, 2010a; Ehrnst & Eriksson, 1993; Harvala et al., 2009; van der Sanden et al., 2008;

Wolthers et al., 2008). In contrast, HPeV3 infections in the USA occur most frequently in the uneven years every 2 years (Renaud et al., 2011; Selvarangan et al., 2011). Pineiro et al. (2010) described HPeV3 detection rates in Spain to peak in 2006 and 2009, suggesting a longer cycle of perhaps every 3 years. However more data are needed to confirm this circulation pattern. In Asia, where HPeV3 was first identified, HPeV3 epidemics are more dispersed over the years (Watanabe et al., 2007). This intermittent circulation is also described for EV types, e.g. echovirus 9 and 30 (Antona et al., 2007; Khetsuriani et al., 2006b), and is associated with the emergence of antigenic diverse genetic lineages or novel recombinants for which the community presumably is not immune protected (McWilliam Leitch et al., 2010). However, based upon the lower evolution rate and diversity of circulating HPeV3 strains (Benschop et al., 2010b; Calvert et al., 2010), diversification of HPeV into new lineages that are antigenically distinct is unlikely. The reason for the intermittent circulation of HPeV3 thus remains unclear.

In contrast to HPeV3, HPeV1 circulates every year in high numbers in the fall and winter months in Europe and Asia (Benschop et al., 2006a, 2008c, 2010a; Ehrnst & Eriksson, 1993; Tauriainen et al., 2007; Watanabe et al., 2007). The same is described for HPeV4 and 6 (Benschop et al., 2008c, 2010a; Harvala et al., 2008; Pajkrt et al., 2009). Again, not only in clinical sense, but also from an epidemiological point of view, HPeV3 behaves different from other HPeV types.

5. Risk groups for HPeV infections

While EVs generally affect individuals of all ages, more than 90% of HPeV infections have been described in children younger than 5 years of age, (Abed & Boivin, 2006; Baumgarte et al., 2008; Benschop et al., 2008c, 2010a; Chen et al., 2009; Grist et al., 1978; Khetsuriani et al., 2006a, 2006b; Pham et al., 2010; Tapia et al., 2008). WHO data from 1967-1974 reported that 94% of the HPeV1 infections were found in children < 4 years of age, of which 60% were < 1 year of age. Only 2,6% were isolated from adults (Grist et al., 1978). A survey in Sweden (1966-1990) reported similar findings, where 92% of the 109 patients with an HPeV1 infection were < 2 years old and only 1,8% were adults (Ehrnst & Eriksson, 1993). A more recent survey performed in the USA spanning over the years 1970 and 2005 reported approximately 95% of HPeV1 infection (n=880) to be from children <5 years of age. Children aged < 1 year with an HPeV1 infection were reported in 73% of the 880 infections (Khetsuriani et al., 2006b).

In 2006, we demonstrated in an observational pediatric study that the median age of children infected with HPeV3 was 1.3 months. This was significantly lower than the median age of children infected with HPeV1 (6.6 months) (Benschop et al., 2006a). Since then, the majority of HPeV3 infections have been found in children under the age of 2 months, most frequently in neonates (Benschop et al., 2008a, 2008c; Harvala et al., 2009, 2011; Pineiro et al., 2010; Renaud et al., 2011; Selvarangan et al., 2011; Verboon-Maciolek et al., 2008a; Watanabe et al., 2007; Yamamoto et al., 2009). This age difference in relation to the disease severity between the two HPeV types suggests that neonates in comparison to older children might be less protected against HPeV3 infection. Seroprevalence data showed that 95-99% of neonates had antibodies against HPeV1 which is most likely to be maternal (Joki-Korpela & Hyypia, 1998; Nakao et al., 1970; Stanway et al., 2000; Takao et al., 2001; Tauriainen et al., 2007). The high HPeV1 seroprevalence thus suggests that the majority of infants should be

protected from HPeV1 infection early in life via maternal protection. However, this may not always be the case as suggested by Ehrnst et al. (1993). The seroprevalence decreased after 6 months of age, only to rapidly increase among children older than 1-3 year of age to 95%. The low seropositivity from 6 months to 1-3 years is marked by an increase in infection frequencies among children in this age group, (Benschop et al., 2006a; Joki-Korpela & Hyypia, 1998; Stanway et al., 2000; Takao et al., 2001; Tauriainen et al., 2007). Seroprevalence rates among adults remained stable at 97-99% (Joki-Korpela & Hyypia, 1998; Stanway et al., 2000; Tauriainen et al., 2007).

For HPeV3, seroprevalence is approximately 70% among adults (Ito et al., 2004). Data on seroprevalence in neonates are not available, but the seroprevalence rate for children between 7-12 months is 15% which steadily increases to 91% in adolescent adults only to decline again to 56-87% in adults. This is in contrast to what is seen for HPeV1 where over 97% of adults still have antibodies against HPeV1 (Joki-Korpela & Hyypia, 1998; Stanway et al., 2000; Tauriainen et al., 2007). Interestingly, seroprevalence among adults in the child-bearing age (20-39 years) is the lowest (57-74%) (Ito et al., 2004). This may indicate that children are less protected through maternal antibodies specific for HPeV3, explaining the young age and increased disease severity of HPeV3 infected children in comparison to HPeV1 infected children. The reason for the lower seroprevalence of HPeV3 is presumably to be related to its proposed recent emergence in the late eighties, which suggests that HPeV3 circulation is not widespread enough to provide sufficient protection in the neonatal period through maternal antibodies (Calvert et al., 2010; Harvala et al., 2011).

Despite the lower seroprevalence of HPeV3, infection with this type has not been reported in adults or older children (>5 years). HPeV1 infections in adults have been documented but only rarely, and usually in patients with an underlying immune deficiency (Wolthers et al., manuscript in preparation). This confirms that antibodies against HPeV1 protect the adult immune competent population. In this respect, with 20-30% of adults seronegative for HPeV3 antibodies, infection with this type in adults should be described as well. A possible explanation will be discussed in paragraph 7.

6. Laboratory findings and diagnosis of HPeV infection

Diagnosis of viral meningitis typically involves analysis of CSF and the establishment of an etiological agent. CSF analysis of HPeV infected children can show a mildly elevated leukocyte count, but in the majority of the children (especially neonates) pleiocytosis is not detected. Normal values for leukocyte cell count vary with age, and in general a higher value is accepted as normal in younger children. In a recent study normal values for leukocyte counts (under 95th percentile) in children younger than 56 days were redefined as <19/ μ l for infants aged 0-28 days and <9/ μ l for infants aged 29-56 days (Kestenbaum et al., 2010). Furthermore, CSF glucose levels in children infected with HPeV are usually normal, while CSF protein levels can be normal or elevated (Pineiro et al., 2010; Selvarangan et al., 2011; Verboon-Macielek et al., 2008a; Wolthers et al., 2008); thus normal CSF findings therefore do not rule out an HPeV meningitis or encephalitis.

Laboratory evaluation of blood from HPeV infected children typically shows a normal leukocyte blood count, although leukopenia is also reported in 33% of the children (Selvarangan et al., 2011). C-reactive protein remains low or slightly elevated (Pineiro et al.,

2010; Verboon-Maciolek et al., 2008b; own observations). In some cases liver enzymes can be elevated, sometimes leading to hepatitis and liver necrosis (Levorson et al., 2009; Selvarangan et al., 2011; Verboon-Maciolek et al., 2008b).

In children with signs of encephalitis cranial ultrasonography and MRI are useful for the detection of cerebral abnormalities. Cranial ultrasonography showed extensive periventricular echogenicity in one study of neonates with HPeV encephalitis (Verboon-Maciolek et al., 2008a). MRI can demonstrate white matter abnormalities (Gupta et al., 2010; Verboon-Maciolek et al., 2008a). The severity of the imaging abnormalities correlated with neurodevelopmental problems later in age (Verboon-Maciolek et al., 2008a).

Classically, HPeVs, like EVs, can be diagnosed through cell culture isolation, usually involving monkey kidney cells and human fibroblasts (Benschop et al., 2010a; Schnurr et al., 1996). Other cell lines, such as the HT29 cell line (human colon adenocarcinoma cells), A549 (human lung carcinoma cell line), RD (Rhabdomyosarcoma cells) can be used for culturing HPeV isolates as well (Abed & Boivin, 2006; Al-Sunaidi et al., 2007; Benschop et al., 2010a; Watanabe et al., 2007). However, cell culture has its limitations. As shown for culturing EVs, different HPeV types display difference in cell growth in various cell lines, and panels of at least three cell lines may be required to efficiently isolate the subsequent types from clinical material (Abed & Boivin, 2006; Benschop et al., 2010a; Watanabe et al., 2007). The colon carcinoma cell line HT29 was found to efficiently support the growth of HPeV1, 2, and 4-6. However, for the isolation of HPeV3, only a few specific cell lines (e.g. Vero and LLCMK2 monkey kidney cells) were found to support the growth, albeit slow, of this type (Abed & Boivin, 2006; Benschop et al., 2010a; Watanabe et al., 2007). CPE produced by HPeV3 on these cells was scarce as well.

In general, CPE produced by HPeVs is not significantly different from the CPE elicited by EVs and HPeVs can mistakenly be identified as EVs in the event specific serotyping is not routinely performed (Benschop et al., 2006a). This also explains the original classification of HPeVs as EVs (Wigand & Sabin, 1961). The neutralization EV-panel of specific antibodies only includes antisera against HPeV1 and 2 (Kapsenberg, 1988). Other HPeV types cannot be serotyped because antibodies are not available (HPeV3-6), or because they cannot be cultured at all (HPeV7-14).

To isolate viruses from CSF, cell culture is proven to be less sensitive, as the load in patients with meningitis or encephalitis is fairly low ranging from 10 to 1000 TCID₅₀ infectious virus per milliliter CSF (Rotbart, 2000). Therefore, PCR is the preferred method for detection of viruses in this material (Espy et al., 2006; Read et al., 1997; Romero 1999; Rotbart HA & Romero JR, 1995; van Doornum et al., 2007). But while with cell culture, both EVs and HPeVs can be diagnosed, PCR specific for EVs will fail to detect HPeVs because the targeted 5'UTR is too diverse between HPeVs and EVs (Beld et al., 2004; Benschop et al., 2006a; Hyypia, 1989; Hyypia et al., 1989; Oberste et al., 1999). Therefore a separate RT-PCR specifically targeting the 5'UTR of HPeVs is required. Real-time RT-PCRs have been developed and validated for HPeV detection, which are faster, less laborious and with a lower contamination risk than conventional endpoint PCRs (Baumgarte et al., 2008; Benschop et al., 2008b; Corless et al., 2002; Nix et al., 2008; Noordhoek et al., 2008; Tapia et al., 2008). By genotyping methods targeting the variable capsid region VP1, positive CSF samples from infants with CNS associated disease, can be characterized directly from the

clinical material (Harvala et al., 2009; Verboon-Maciolek et al., 2008a). Direct genotyping from clinical material avoids secondary cell culture isolation of EV/HPeV from CSF, a material for which virus culture is already an insensitive method of detection (Romero, 1999; Rotbart & Romero, 1995).

Pathogen detection in CSF is the strongest indicator of the pathogen's association to the disease, but it is not uncommon to diagnose an EV or HPeV infection by detection of the virus in other clinical samples while the patient presents neurological symptoms (Benschop et al., 2006a). Several real-time PCRs for HPeV have been validated and tested on stool samples, and/or throat swabs (Baumgarte et al., 2008; Benschop et al., 2008c; Nix et al., 2008; Noordhoek et al., 2008; Tapia et al., 2008). The high viral loads in these samples make them suitable for diagnosis by cell culture as well. However, it should be taken into account that both HPeVs and EVs can be shed in high amount in stool and respiratory material, even after clearance of symptoms (Chung et al., 2001; Harvala et al., 2008).

In summary, HPeV3, which is the most pathogenic HPeV type, and the most prevalent type detected in CNS disease, is difficult to grow in cell culture and is not routinely serotyped by the available antibody pools. Data on prevalence of HPeV types that rely on virus isolation by cell culture alone (Abed & Boivin, 2006; de Vries et al., 2008; van der Sanden et al., 2008; Watanabe et al., 2007) will be biased by the cell panel used for virus culture, the difference in growth characteristics between the HPeV types, and the inability of the newer HPeV types 7-14 to grow in cell culture. Therefore, detection of HPeV for clinical or epidemiological purposes should rely on real-time PCR and subsequent genotyping based on VP1 or VP1/VP3.

7. Pathogenesis

It is not known why HPeV3 is more associated with severe neonatal sepsis and CNS disease in infants in comparison to other HPeV types. But several serological as well as biological and genomic features of HPeV3 in conjunction with preliminary experimental data may provide clues as to why this type has a neurovirulent phenotype and is predominantly identified among neonates.

7.1 Host immune response to HPeV

The defense mechanism against HPeV meningitis/encephalitis or HPeV infections in general is largely unknown. Most of what we know from picornavirus immunity is distilled from immunological studies with EV infections. An efficient host response against EVs is most likely dependent on a proper humoral immune response with release of neutralizing antibodies. This is underlined by the increased incidence of severe EV infections in patients with primary antibody deficiency (PID), such as X-linked agammaglobulinemia (XLA), in which chronic enteroviral meningoencephalitis (CEMA) is one of the most severe complications (Moin et al., 2004; Plebani et al., 2002; Wildenbeest et al., 2011). In a recent survey in the USA, a mortality rate of 35% was reported in patients with PID due to chronic or disseminated EV infections and 40% of the survivors of the initial illness had long-term neurological symptoms (Halliday et al., 2003). In this large survey echovirus 11 was the most common EV type identified and the CNS was nearly always involved. Interestingly, changes in CSF parameters, such as elevated CSF protein levels did not correlate with

changes in neurological signs and symptoms. Successful treatment with therapeutic immunoglobulin therapy (e.g. intravenous immunoglobulins (IVIG)) in PID patients with an EV meningitis/encephalitis provides additional evidence for an important role of neutralizing antibodies for an adequate host immune response in EV meningitis/encephalitis. In neonates, characterized by an impaired humoral immune response, lack of specific maternal EV antibodies is shown to be a risk factor for the development of severe illness (Abzug, 2004).

Knowledge of the host immune response to HPeV is in comparison to EV even more limited. In contrast to the evidence as described in the section above, there are no data available on the protective role of neutralizing antibodies in HPeV infections. The lower seroprevalence of HPeV3 in adults might suggest a lack of maternal protection in the early months of life. To study the protective role of these antibodies against both HPeV1 and 3 infections, our group has set up a case-control study of neonates and children < 1 year that is currently ongoing.

Only recently, toll like receptors (TLR) 7 and 8 were identified as important key players in the innate immune defense against HPeV1 (Triantafilou et al., 2005). TLRs are transmembrane proteins that play an important role in immune responses against microbial pathogens, by inducing inflammatory cytokines in response to bacteria and viruses. Although never published, one can assume that these TLRs are equally important in the defense against HPeV3 infections. Volpe (2008) suggested that HPeV3 infections could result in intracellular binding of TLR8, leading to the release of reactive oxygen and nitrogen and pro-inflammatory cytokines from microglia and as a result neural cell death. Interestingly, TLR8 is specifically distributed in axonal perturbations and only in the developing nervous system which could explain why we specifically observe the severe HPeV3 infections in infants rather than among older children and adults.

7.2 Cell tropism & receptor interactions

The specific detection of HPeV3 in CSF might direct towards a difference in cell tropism between HPeV types. This is already reflected by the slow growth of HPeV3 on a limited number of cell lines and its poor production of CPE (Abed & Boivin, 2006; Benschop et al., 2010a; Watanabe et al., 2007). That HPeV3 infects other cell lines than for example HPeV1, is underlined by recombination studies done in our laboratory. HPeV3 recombination is highly limited, while other HPeV types frequently recombine with each other (Benschop et al., 2008d, 2010b; Calvert et al., 2010). This indicates that HPeV3 rarely gets into contact with other types in the same cell where recombinants are produced during replication of the infected viruses.

The difference in tropism can be explained by a difference in receptor usage. HPeV types 1, 2, and 4-6 contain an RGD motif in the C-terminus of VP1 that is utilized by several other viruses, such as FMDV, Coxsackie A virus 9 and echovirus 9, for their attachment to cell surface integrins. The RGD motif is essential for HPeV1 infection (Boonyakiat et al., 2001; Joki-Korpela et al., 2001; Pulli et al., 1997; Stanway et al., 2000). However, HPeV3 does not contain an RGD motif and thus it is assumed that infection of HPeV3 is established via a different receptor that is RGD independent, but is as yet unknown. Based on receptor studies of different EV types we also know that the cell surface expression of receptors

during organ development plays a major role in defining tropism (Harvala et al., 2005; Scassa et al., 2011; Yamayoshi et al., 2009). This shift of specific cell receptor expression during development would support the observation that HPeV3 infections are rarely or never seen in older children and adults and could partly explain why neonates are more prone for HPeV3 infections.

8. Treatment

There is no antiviral treatment against HPeVs currently available (Wildenbeest et al., 2010). Therefore, supportive treatment and administration of IVIG are the only available options. IVIG is sometimes given to neonates to reduce disease burden from EV infection, although its efficacy has not been proven. Neutralizing EV antibody (nAb) titers in IVIG vary between batches and geographic regions (Cao et al., 2010; Galama et al., 1997; Planitzer et al., 2011). For therapeutic purposes, high nAb titers against the specific serotype might be needed (Abzug et al., 1995). The seroprevalence rates of HPeV1 and HPeV3 found in adults would suggest IVIG to contain moderate to high titers of nAbs against these HPeV types. A case-report of a twin with neonatal sepsis and hepatitis infected with HPeV3 showed one child to have recovered after having received IVIG (Al Maamari et al., 2009). However, data on IVIG titers against HPeV3 was not given in this study. We found high HPeV1-specific nAb in two batches of IVIG, but low nAb titers against HPeV3 in these IVIG batches (Westerhuis et al., manuscript in preparation; Wildenbeest et al., 2011). Therefore, IVIG might be beneficial in the treatment of HPeV1 infections and less effective in the treatment of HPeV3, although more data are needed. Moreover, the dispersed circulation of HPeV3 could have effect on the nAb titers in IVIG batches collected in different years and should be taken into account.

Recently, a case report was published on the use of pleconaril in a patient with HPeV-associated enteropathy (van de Ven et al., 2011). Pleconaril inhibits viral replication by integration into the hydrophobic pocket inside the viral capsid. As a result, uncoating and binding of the virus to the host cell are interrupted (Pevear et al., 1999). The hydrophobic pocket is relatively well preserved among EVs and HRVs, resulting in a broad-spectrum anti-enteroviral and -rhinoviral activity of pleconaril. However, the capsid of HPeVs is different (Stanway et al., 2000; Seitsonen et al., 2010), indicating that the hydrophobic pocket differs from that of EVs. Indeed data from our laboratory show that HPeV1 and HPeV3 are resistant against pleconaril *in vitro* (Wildenbeest et al., 2010). In agreement, pleconaril did not have any effect on replication of an unspecified HPeV found in the patient with enteropathy, nor did ribavirin (van de Ven et al., 2011).

In summary, no systematic data are available on HPeV treatment, and clinical experience with severity and treatment of HPeV infections is just beginning to build up. Treatment options for HPeV and the related EV are urgently needed. In the meantime, administration of IVIG is the only option.

9. Concluding remarks: HPeV3 infection of the CNS

Before the turn of the century, HPeVs infections were described as mild infections in children. At that time only two types were known, and severe symptoms were occasionally reported. With the discovery of a third HPeV type (HeV3), the view on HPeVs as relevant pathogens in viral CNS infections such as meningitis and encephalitis has changed

drastically. While various EV types are known to be commonly involved in meningitis, (echovirus 9 and 30, EV71 and CBV5), of the 16 HPeV types currently described, HPeV3 is the primary type to cause CNS disease in children.

The introduction of rapid PCR detection for both EVs and HPeVs in CSF demonstrate that both viruses play an important role in the pathogenesis of meningitis/encephalitis. Increased routine screening of HPeVs will lead to an increase in the identification of HPeV infected children and a decrease in the use of antibiotics. Differences in maternal protection and its genetic make up in relation to its biological characteristics should provide clues in the near future on how HPeV3 type specifically infects the CNS of particularly newborns. This will aid in the development of new treatment options that will allow effective care of these children, possibly preventing neurological sequelae in these children.

10. References

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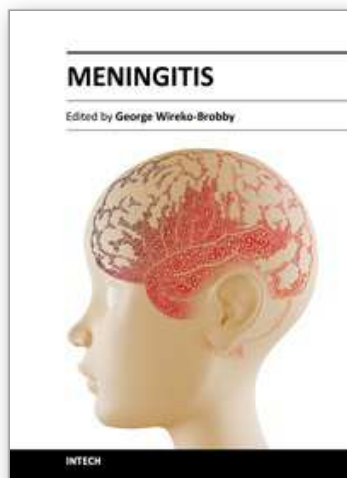
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