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8.5.8 Enterovirus infections 787 8.5.8 Enterovirus infections Philip Minor and Ulrich Desselberger ESSENTIALS Enteroviruses are single-stranded, positive-sense RNA viruses com- prising poliomyelitis viruses (3 types), coxsackie A viruses (23 types), coxsackie B viruses (6 types), and echoviruses (33 types). The human enteroviruses are classified into four species (A–D) on the basis of sequence comparisons. Transmission is by the faeco-oral route, with marked seasonal peaks of infection in areas of temperate cli- mate, but infections occurring all year round in tropical regions. Pathogenesis—following transmission, enteroviruses undergo a first round of replication in cells of the mucosal surfaces of the gastrointestinal tract and in gut-associated lymphoid cells, followed by viraemia, which leads to infection of distant organs (brain, spinal cord, meninges, myocardium, muscle, skin, and so on), where lesions might be produced. Shedding of virus occurs from throat and faeces for many weeks. Clinical manifestations and diagnosis Most enterovirus infections are silent or only produce minor illness, but severe major illness can develop in a few of the infected. Infection with poliovirus is normally inapparent, but a few of the infected (1% or less) develop neurological symptoms comprising aseptic meningitis, or paralytic poliomyelitis. Five to ten days after a mild upper respiratory tract infection, the disease presents with flaccid paralysis resulting from motor neuron destruction; this can affect the limbs (spinal form) or muscles supplied by the medulla oblongata or bulb (bulbar form), with potentially life-threatening re- spiratory muscle involvement. Treatment is supportive; mortality is 2–5% in children and 15–30% in adults, and there is residual paralysis in 90% of survivors. Other clinical syndromes include: (1) aseptic meningitis, the most frequent clinical presentation of enterovirus infection, caused by coxsackie viruses and echoviruses; (2) encephalitis, a rare event, possibly following aseptic meningitis; (3) pleurodynia (Bornholm disease), presenting abruptly with fever and chest pain and usually caused by coxsackie B viruses; (4) myopericarditis; (5) herpangina; (6) exanthema, rubella-like or hand-foot-and-mouth disease; and (7) conjunctivitis. Diagnosis is by virus isolation in cell culture or by viral genome detection using RT-PCR. Prevention Paralytic poliomyelitis has been eradicated in most countries of the world following universal mass vaccination with formaldehyde- inactivated poliovirus (Salk vaccine) and/or live-attenuated viral vaccine (Sabin vaccine). However, it persists in a few countries (e.g. Pakistan, Afghanistan, and Nigeria) from which it has been exported to

otherwise polio-free states. For example, historically strains from Nigeria have caused disease in other countries of Western Africa, Indian strains were repeatedly isolated in Angola, there was a major outbreak in Tajikistan caused by strains from Northern India, and in 2011 there were at least 18 cases in China caused by strains from Pakistan. The incident in China was particularly unexpected as the immunization programme has been well executed and was effective for many years. As long as there are pockets of infection, the world remains at risk of re-emergence of the disease. At present there are three countries that have never eradicated polio; namely Pakistan, Afghanistan and Nigeria. India was declared polio-free in 2014. Nigeria was on the verge of being declared polio free in 2015 after three years without a case caused by wild type virus when one was reported. No case attributable to a naturally occurring wild type 2 virus has been reported anywhere in the world since October 1999 and there has been no case attributed to a wild type 3 virus since November 2012. Progress is striking but as long as there are pockets of infection, the world remains at risk of re-emergence of the disease. Introduction Enteroviruses are a major group of viruses causing systemic infec- tion in humans. They form two genera of the Picornaviridae family (the Enterovirus and Parechovirus genera) and occur in at least 66 serotypes in humans. They infect via the gastrointestinal tract and are mostly clinically inapparent. However, viraemia can be fol- lowed by infection of organs distant from the site of entry with often devastating effects in the form of meningitis, encephalitis, paralysis, myopericarditis, and also rashes and conjunctivitis. The viruses Viruses of the Picornaviridae are nonenveloped icosahedral particles of 27 to 30 nm in diameter and contain single-stranded RNAs of posi- tive polarity and 7.2- to 8.4-kb size as their genome. The nucleic acid is polyadenylated at the 3′ end and carries a small protein, VPg, co- valently linked at its 5′ end. The enteroviruses and parechoviruses form two of the current genera of the Picornaviridae family. In 2016 there were a further 27 genera based on the sequence of the genomes. By late 2018 there were 40 and the reader is referred to the website of the International Committee on Virus Taxonomy (ICTV) for the current score. In turn, the Picornaviridae are one of five families of the new order Picornavirales. Three serotypes of poliomyelitis virus (poliovirus), 23 types of coxsackie A virus, six types of coxsackie B virus, and 33 types of enteric cytopathic human orphan (echo) vir- uses are recognized within the Enterovirus genus. The parechoviruses comprise echoviruses 22 and 23, and 14 other human viruses, and were established as a separate genus on the basis of the highly diver- gent sequence of their genomes. Other classic features of the entero- viruses, such as their stability at acid pH (in contrast to rhinoviruses or aphthoviruses), their buoyant density in caesium chloride gradi- ents, and the nature of their broad clinical effects and persistence in the environment are also shared by the parechoviruses. The three-dimensional structure of the poliovirus particle has been elucidated by crystallographic analysis (Fig. 8.5.8.1). The viral capsid consists of 60 protein subunits, each containing the four unglycosylated viral proteins VP1 to VP4. The capsid proteins are arranged in such a way that VP1 molecules form the apices at the fivefold symmetry axis of the icosahedron, whereas two other proteins VP2 and VP3 are arranged in the centre of the triangular face near the threefold axis of symmetry; VP4 is an internal pro- tein. All proteins interact with each other. The N-terminus of VP4 is myristoylated.

 Viruses initiate replication by attachment to their cellular receptors, and some of these have been characterized. The poliovirus receptor (PVR or CD155) is a member of the immunoglobulin superfamily. Transgenic mice expressing the human PVR become susceptible to poliovirus infection with a subsequent pathology similar to that of infected primates. Tests using these animals have been incorporated into regulatory requirements as supplements or

replacements for pri- mates for vaccine testing (see next). Other enterovirus receptors are the decay accelerating factors (DAF; receptor for various echovirus types, coxsackie B virus types, and coxsackievirus A21), implicated in the complement pathway, and the integrin VLA-2 (receptor for echovirus types 1 and 8). Other cell surface molecules might be in- volved as coreceptors in the virus–cell receptor interactions of many enteroviruses, as the expression of a single identified receptor is not always sufficient to make a previously resistant cell line susceptible to productive infection. It is also of interest that some strains of polio- virus, mainly of serotype 2, are able to paralyse mice after infection. The receptor involved in mice has not been identified. The positive-sense RNA genome acts as a messenger molecule. All enterovirus RNAs have a genome linked protein (VPg) at the 5'end of an untranslated region (UTR) of approximately 750 nu- cleotides in length. The region is highly structured and contains an internal ribosomal entry site. This is important for binding of the RNA to ribosomes and subsequent translation of the RNA into pro- tein. Downstream of the 5′ UTR is a large single open reading frame containing three parts: P1, coding for structural proteins VP1 to VP4; P2, coding for proteins 2A, 2B, and 2C; and P3, coding for proteins 3A to 3D. Proteins 2A and 3C are viral proteases and pro- tein 3D is the RNA-dependent RNA polymerase (RdRp). P2 and P3 proteins (with the exception of VPg = 3B) are only found in in-fected cells. The P1 to P3 proteins are synthesized as one large pre- cursor from which the individual proteins are produced by complex autocleavage and cleavage cascades. RNA replicates via double- stranded replicative intermediates. The ratio of positive-stranded to negative-stranded RNA molecules in infected cells is approximately 100:1. During replication, RNA recombination occurs frequently. Replication of and translation from the same RNA cannot occur at the same time. Poliovirus-infected cells undergo a shut off of cel- lular mRNA synthesis and cellular protein translation. Naked en- terovirus RNA is infectious on transfection (poliovirus was the first RNA virus rescued this way) and can be transcribed and recovered from full-length cDNA clones which are also infectious upon trans- fection. This technique (reverse genetics) permits biochemical ma-nipulation and structure–function studies at the molecular level. The extensive antigenic variation of enterovirus capsid proteins allows typing into polioviruses, coxsackieviruses, and echoviruses (b) VP1 VP1 Canyon 5x 3x VP3 VP2 VP2 VP3 (a) (d) (c) Fig. 8.5.8.1 Structural features of picornaviruses. (a) Electron micrograph of negatively stained poliovirus. Magnification ×270 000. (b) Diagram of the picornavirus capsid, showing the packing arrangements of VP1, VP2, and VP3, and the interspersed canyon. VP4 is located on the interior of the capsid. The biological protomer (grey) is different from the icosahedral subunit (triangle shown at right). (c) Model of poliovirus type 1, Mahoney, based on X-ray crystallographic structure determined at 2.9 Å. The fivefold axis of symmetry is marked (5×). Surrounding the fivefold axis are canyons, the receptor-binding site. (d) Model of poliovirus type 1, Mahoney, produced by image reconstruction from cryoelectron microscopy data obtained at 20 Å resolution. From Racaniello VR (2007). Picornaviridae: the viruses and their replication. In: Knipe DM, et al. (eds) Fields virology, 5th edition, pp. 795–838. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia. With permission of author and publisher.

 using type-specific neutralizing antisera, but there is some cross-reactivity. The main antigenic sites are located on all three major virion proteins (VP1–VP3), and some involve sequences from more than one protein. The molecular mechanisms for the high genomic diversity of picornaviruses are thought to be based on misincorporations of nucleotides during chain elongation (due to the high error rate of the viral RdRp) and to frequent RNA recom-bination events, which also occur in natural infections. Comparison of complete RNA genome

sequences of many en- teroviruses shows a very close relationship between some entero- virus and rhinovirus sequences. Within the echoviruses, however, there is great diversity. A subdivision of human enteroviruses into four species according to genomic relatedness has been proposed: 1 Human enterovirus A: coxsackieviruses A2 to A8, A10, A12, A14, and A16, and human enteroviruses EV71, EV 76, EV89–91, and EV114 2 Human enterovirus B: coxsackieviruses B1 to B6, A9, all echo- viruses except types 22 and 23 (now in the Parechovirus genus), and human enteroviruses EV69, EV73 to 75, EV77–88, EV97, EV100–101, EV106, and EV107 3 Human enterovirus C: poliovirus types 1 to 3, coxsackieviruses A1, A11, A13, A17, A19 to A22, and A24, and human entero- viruses EV96, EV99, EV102, EV104–105, EV109, and EV116–118 4 Human enterovirus D: human enterovirus enteroviruses EV68, EV70, EV94, and EV111 Hepatitis A virus has previously been designated EV72, but is now the type species in a separate genus, Hepatovirus. There are an add- itional five enterovirus species which encompass the nonhuman enteroviruses and three rhinovirus species making a total of 12 species in the enterovirus genus on the basis of sequence similarity. Enteroviruses are sometimes designated by a letter indicating their species and a number indicating their identity; for example entero- virus 71 is of species A and is sometimes referred to as A71; entero- virus 68 is of species D and is sometimes referred to as D68. Pathogenesis The most widely accepted pathway of enterovirus pathogenesis is based on that developed by Bodian for poliovirus, in which the virus infects the host via the gastrointestinal tract and undergoes primary replication in lymphoid cells lining the alimentary tract (oropha- ryngeal, intestinal). A viraemic phase follows, allowing infection of distant target organs: spinal cord and brain, meninges, myocardium, skeletal muscles, skin, and mucous membranes. Other tissues (e.g. lymph nodes and brown fat tissue), can also become infected. Intensive multiplication in the central nervous system (CNS) leads to the destruction of motor neurons and results in paralysis. A slightly different and more subtle model of poliovirus pathogen- esis was proposed by Sabin, in which the virus infects the mucosal surface, thus accounting for the fact that virus can be shed in faeces long after it has become undetectable in lymphoid tissues and when neutralizing antibody is detectable in the blood. The primary repli- cation creates a viraemia which seeds distant, still unknown, sites and virus replication there results in a second viraemia, which can be detected about 1 week postinfection and can lead to systemic in- fection including CNS involvement. There are very many unknowns in polio pathogenesis. For instance, it is still not clear where the virus that is shed in the faeces is produced, nor precisely from which cells. It can be inferred that they are few in number and of limited func- tion because of the level of virus excreted and the fact that infection is almost always silent if confined to the gut. Shedding of virus occurs from the throat and faeces for many weeks and even months after infection and thus ensures transmis- sion (see next). Virus replication in sites distant from the port of entry normally terminates with the appearance of neutralizing antibody, first IgM at 1 to 2 weeks after infection and then IgG and secretory IgA. Individuals with B-cell immunodeficiencies might develop persistent infections. Most enterovirus infections are silent or produce a ‘minor illness’ with the symptoms of a mild upper respiratory tract infection, with or without a fever. In a minority of infections (1% or less) one of the following systemic ‘major diseases’ may develop: • Paralytic poliomyelitis, aseptic meningitis (polioviruses) • Aseptic meningitis, herpangina, conjunctivitis, hand-foot-and- mouth disease (coxsackie A viruses) • Aseptic meningitis, myopericarditis, encephalitis, pleurodynia (coxsackie B viruses; EV71) • Aseptic meningitis, rashes, conjunctivitis (echoviruses) • Polio-like illness, aseptic meningitis, hand-foot-and-mouth dis- ease, epidemic conjunctivitis (EV68–71) Symptoms of clinical illness caused by enteroviruses are summar- ized in Table 8.5.8.1 and are discussed in more detail next. Clinical

symptoms Central nervous system infections (See Chapter 24.11.2.) Poliomyelitis Evidence of poliomyelitis as an ancient human disease is revealed on a funerary stele from Middle Kingdom Egypt, about 1300 bc, but there is little documentation of its occurrence until nearly the end of the 19th century when it appeared in epidemics in children (hence the alternative name ‘infantile paralysis’). The appearance of poliomyelitis coincided with the improvement in standards of public hygiene and is explained by the consequent exposure of infants to infection at a later age. Maternal antibody is capable of confining infection to the gut, where the virus can persist until the immune response develops to eliminate it. In contrast, when maternal antibody has declined in older infants, the virus can spread to sites outside the intestine, causing paralysis. Even under modern conditions of hygiene, infection with all three poliovirus types is normally inapparent and illness with neuro- logical symptoms results in about 1% of infections or less. This can present as aseptic meningitis with neck stiffness, usually followed by recovery after 10 days (abortive or nonparalytic poliomyelitis). Meningitis is also caused by several other enteroviruses (see next). The more serious presentation is paralytic poliomyelitis, appearing 5–10 days after a mild upper respiratory tract infection (‘minor illness’) and progressing to flaccid paralysis resulting from motor

 neuron destruction (‘major illness’). This might be accompanied by spasms and lack of coordination of nonparalysed muscles. Various forms of the ‘major illness’ reflect infection of

different parts of the CNS. Paralysis of limbs (Fig. 8.5.8.2) results from destruction of motor neurons in the lower part of the spinal cord (‘spinal form’), while the more life-threatening bulbar poliomyelitis (‘bulbar form’) involves infections of the medulla oblongata or bulb. Respiratory function can be affected in both the spinal

and bulbar forms of the disease; encephalitis is rare. In children under 5 years old, paralysis of one leg is most common; in children 5–15 years of age, weakness of one limb or paraplegia are frequent; quadriplegia is most common in adults and is often accompanied by urinary bladder and respiratory muscle

dysfunction. Muscular function in limbs might return slowly, but there is residual paralysis in 90% of survivors. Of paralytic cases, 10–25% have bulbar symptoms with hypertension, shock, and dysphonia. Complications are nosocomial pneumonias (by staphylococci or Gram-negative bacteria), urinary tract infec- tions, and

emotional problems. The mortality from paralytic polio is 2–5% among children and 15–30% among adults. Muscle weakness can develop many years after the initial polio disease (postpolio syn- drome or postpolio neuromuscular atrophy). A persistent poliovirus infection as cause of this has been assumed, based on the presence of

viral RNA in cerebrospinal fluid and neural tissue. However, such RNA has also been found in patients with other neurological and nonneurological diseases and is, therefore, less likely to be related to the postpolio syndrome. The alternative view is that the postpolio syndrome is anatomical in origin, such that the initial attack of polio destroys

motor neurons and reduces the backup available as the patient ages. Aseptic meningitis Aseptic meningitis is the most frequent clinical presentation of en- terovirus infection and can be caused by coxsackieviruses of both groups A and B, and echoviruses, mainly of types 4, 6, 11, 14, 16, 25, 30, and 31 (see Table 8.5.8.1). The

disease starts with fever, headache, neck stiffness, and photophobia. Sensory or motor deficits are un- usual, but confusion is common. The symptoms can persist for 4 to 7 days. The cerebrospinal fluid usually shows pleocytosis consisting of 10 to 500 leucocytes/μl, mainly lymphocytes. Polymorphonuclear cells may predominate at the

onset, but bacterial infection and pos- sibly abscesses should be considered if they persist. The protein concentration in cerebrospinal fluid may be normal or slightly in- creased; the glucose level is normal. Complete recovery is the usual outcome of aseptic meningitis.

Table 8.5.8.1 Clinical symptoms and their possible

enteroviral causes Clinical symptom (phenotype) Causative viruses PV1–3 CVA1–24 CBV1–6 EV1–33 EV68–116 Paralysis + + (+) (+) (+) Meningitis + + + +1 Encephalitis + + +2 Febrile illness + + + Neonatal syst. inf. + +3 Herpangina +4 (+) Exanthema + + +5 Conjunctivitis +6 (+) +7 +8 HFMD +9 +10 Pneumonia +

Pleurodynia (+) + (+) Myocarditis + + Hepatitis + Diarrhoea + +11 PV, poliovirus; CVA, coxsackie A virus; CBV, coxsackie B virus; EV, enterovirus; HFMD, hand-foot-and-mouth disease; 1, types EV4, 6, 11, 14, 16, 25, 30, 31; 2, type EV71; 3, types CVA6, 7, 13; 4, types CVA1–6, 8, 10, 22; 5, types EV4, 9, 16; 6, type CVA24; 7, types EV7, 11; 8, type EV70; 9, types CVA10, 16; 10, type EV71.
Fig. 8.5.8.2 Acute monoplegia in a Thai child in 1979 caused by poliomyelitis. Copyright D. A. Warrell.

8.5.8 Enterovirus infections 791 Encephalitis Enterovirus encephalitis is rare but might follow aseptic meningitis. Enterovirus infection in patients with hypogammaglobulinaemia or agammaglobulinaemia can persist for years with chronic men- ingitis or encephalitis and a high mortality rate as sequelae. Enterovirus 71 infection, which is normally associated with hand- foot-and-mouth disease, has been found to cause severe meningo- encephalitis (with brain stem involvement), polio-like acute flaccid paralysis, and a high case fatality rate in children during several recent outbreaks in Bulgaria, Taiwan, and Malaysia. In some of the fatal cases there might have been coinfections with a species B adenovirus. Enterovirus 71 occurs in three genotypes and is rapidly evolving; it is most closely related to coxsackievirus A16 which also causes hand-foot-and-mouth disease. Vaccines against EV71 have been licensed in China where EV71 of the C4 genotype has caused fatalities. Neonatal infections Neonatal infection followed by severe generalized disease can be caused by coxsackie B viruses and echoviruses, mainly of types 6, 7, and 11. These viruses seem to be transmitted late in pregnancy, perinatally, or postnatally by the mother or other virus-infected infants in neo- natal wards or special care baby units. The infants develop either heart failure due to a severe myocarditis or a meningoencephalitis; hepatitis and adrenalitis might also occur. The mortality is high. Viruses can be recovered from brain, spinal cord, myocardium, and liver at autopsy. Bornholm disease (epidemic pleurodynia) This is usually caused by coxsackie B viruses but can also be caused by echoviruses of types 1, 6, 9, 16, and 19 and by coxsackie A vir- uses of types 4, 6, 9, and 10. The disease can strike families in small outbreaks. It typically starts abruptly with fever and chest pain due to the involvement of the intercostal muscles or abdominal pain re- sulting from involvement of muscles of the abdomen. There might be severe frontal headache. The symptoms last 3 to 14 days and are followed by complete recovery. Myopericarditis Enterovirus-induced myocarditis is mostly due to infection with cox- sackie B viruses in the young. The onset of disease is usually acute, very severe, and may be fatal in neonates; however, in adolescents and adults it is normally mild. The virus may persist after the initial infection and cause dilated cardiomyopathy. In fatal cases (usually neonates 2–11 days after onset of disease) there is cardiac dilatation, myocyte necrosis, and an inflammatory reaction. The diagnosis is often difficult, particularly in older patients, as pericarditis, coronary artery occlusion, or heart failure may have been diagnosed initially. Typical clinical findings are often tachycardia, arrhythmias, murmurs, rubs, and cardiomegaly. Besides causing acute myocarditis, chronic enterovirus infection can lead to chronic myocarditis and dilated cardiomyopathy, possibly due to immunopathological mechanisms. In chronic disease, neither infec- tious virus nor viral antigens are normally detected in heart biopsies; however, viral RNA is regularly found in cardiac muscle suggesting that the viral genome persists. The true significance

of the presence of the viral genome in such cases is still under discussion since viral RNA is also found in cardiac muscle of apparently healthy controls. The disease can be produced with coxsackie B viruses in mice. In this animal model there is also initial viraemia and replication in myocytes, but this is followed by disappearance of infectious virus and destruction of myocytes, possibly by autoimmune mechanisms. Herpangina This is caused by coxsackieviruses of types A1 to A6, A8, A10, and A22. Children and young adults between 2 and 20 years of age are mainly affected. The disease presents with acute onset of fever, sore throat, and pain on swallowing, as well as vomiting and abdominal symptoms. Small vesicular lesions or white papules surrounded by a red halo can be seen on the fauces, pharynx, palate, uvula, and tonsils (Fig. 8.5.8.3). The disease is mild and self-limiting. Exanthemas Rubella-like rashes can be produced by echoviruses of types 4, 9, and 16, but also coxsackieviruses A9, A16, and B5 (Fig. 8.5.8.4). They usually occur in the summer and might be accompanied by fever, malaise, cervical lymphadenopathy, and aseptic meningitis. Fig. 8.5.8.3 Herpangina due to coxsackievirus A6 infection. Courtesy of the late Dr B. E. Juel-Jensen. Fig. 8.5.8.4 Exanthema due to coxsackievirus infection. Courtesy of the late Dr B. E. Juel-Jensen.

792 section 8 Infectious diseases Hand-foot-and-mouth disease A typical distribution of vesicular lesions in hands, feet, and mouth (but also buttocks and genitalia) is produced by infection with coxsackievirus type A16 and enterovirus 71, and less frequently with coxsackieviruses A4, A5, A9 and A10, B2, and B5 (Fig. 8.5.8.5a, b). Enterovirus 71 can produce more severe clinical symptoms (see earlier). Foot-and-mouth disease The aphthovirus causing foot-and-mouth disease in cloven-hoofed ani- mals is endemic in Africa, Asia, and South America. Virus is secreted before blisters on the mouth and feet appear in animals. The zoonosis in humans is very rare, with about 37 recorded cases. Human infection occurs from virus entering through broken skin, drinking unpasteur- ized milk, or by inhalation of droplets. A 2- to 6-day incubation period is followed by blisters of hands, feet, and mouth, fever, and sore throat; complete recovery ensues. No person-to-person spread is recorded. Conjunctivitis Several enterovirus types cause conjunctivitis, often affecting large numbers of people epidemically. Most notable causes are echovirus types 7 and 11, coxsackievirus A24 and B2, and enterovirus 70 that often produces a haemorrhagic conjunctivitis. Diabetes and pancreatitis Insulin-dependent diabetes mellitus (IDDM, or type 1 diabetes) is likely to be an autoimmune disorder in which the insulin-secreting pancreatic islet cells (β cells) are destroyed. The human disease has long been thought to be caused by infectious agents, particularly since association between enterovirus infection and the development of IDDM has been shown in animal model studies (infection of mice with coxsackie B3–B5 viruses). However, there is also a strong gen- etic component in the development of IDDM. Gastroenteritis and diarrhoea Although enteroviruses infect via the gastrointestinal tract and readily replicate there, they very rarely cause diarrhoea. Outbreaks of diarrhoea with echovirus type 11 have been reported. In Japan, the Aichi virus, the type species of the Kobuvirus genus of the Picornaviridae family, has been identified as the cause of multiple outbreaks of gastroenteritis in humans, mostly associated with the consumption of raw oysters. This virus seems to circulate widely in populations of Japan and other south-east Asian countries, with subclinical infections likely to be common (see Chapter 8.5.9). Chronic fatigue syndrome Chronic fatigue syndrome, also previously known under the names of myalgic encephalomyelitis (ME), Royal Free disease, Iceland dis- ease, postviral fatigue syndrome, and neuromyasthenia, can occur both sporadically and epidemically. The main clinical feature is excess fatigability of skeletal muscle, accompanied by pain. Other symptoms include headaches, inability to concentrate, paraesthesia, and impair- ment of short-term memory. A major problem in

diagnosis is a clear definition of the clinical entity. Several virus infections have seemed to precede the development of chronic fatigue syndrome; they are mainly enterovirus infections, chronic Epstein–Barr virus infection, and also infections with Toxoplasma and Leptospira spp. The stringency of the association of chronic enterovirus infection with the appearance of chronic fatigue syndrome is very controversial. A re- port of a joint working group of the Royal Colleges of Physicians, Psychiatrists, and General Practitioners has concluded that persist- ence of enteroviruses is unlikely to play a role in the development of chronic fatigue syndrome. Similar conclusions have been drawn for the possibility of a causal link between chronic Epstein–Barr virus infection and chronic fatigue syndrome (see Chapter 8.5.3). Laboratory diagnosis of enterovirus infections Virus isolation Virus isolation is an excellent procedure to diagnose enterovirus infections although its use is declining in favour of molecular methods. Virus is shed for weeks, and sometimes months, from the primary infection sites (cells lining the gut, see earlier). Starting from a few days after infection, virus can be found in concentra- tions of 105 to 106 tissue culture infectious doses 50%/g (TCID50/g) of faeces. Throat swabs are also a good source for virus, particularly early in infection and when there are respiratory symptoms. In cases of meningitis, enteroviruses can be propagated in cell culture from the cerebrospinal fluid, but the method is much less sensitive than genome detection (see next). Viruses are readily isolated in secondary cultures of monkey kidney cells, or in cultures of per- manent cell lines derived from human embryonic kidney, human amnion, or human fetal lung. The cytopathic effect produced by enteroviruses is nonspecific. Typing of a cytopathic agent is carried out using antiserum pools (see next) or in multistep procedures. Most coxsackie A viruses (with the exception of coxsackievirus A9) do not grow well in cell culture but can be readily isolated by (b) (a) Fig. 8.5.8.5 (a, b) Hand-foot-and-mouth disease due to coxsackievirus infection. Courtesy of the late Dr B. E. Juel-Jensen.

 intracerebral, intraperitoneal, or subcutaneous infection of mice, causing flaccid paralysis and death. In contrast, coxsackie B viruses cause spastic paralysis. Polioviruses or echoviruses do not usually grow in mice although polioviruses will replicate in transgenic ani- mals that have appropriate receptors (see earlier). Serology Neutralization assays were the method of choice for typing entero- viruses for many years. Due to the large number of enterovirus types, these tests are labour intensive and not apt for rapid diagnosis. Pools of type-specific antisera (prepared by Drs Lim, Melnick, and Benyesch, and termed LMB pools) have greatly helped to establish the epidemiology of enterovirus infections worldwide. Recurrent enterovirus infections during a lifetime often result in elevated serum antibody titres which obscure diagnostic changes. Significant antibody rises are, therefore, rarely observed in paired sera (taken at the onset of and during convalescence from disease). A coxsackie B virus-specific IgM test (using an IgM antibody cap- ture technique) has been developed for rapid diagnosis. However, there is cross-reactivity between the IgM responses to different en- teroviruses, including different genera of the picornaviruses, and so this test is not very specific. Prolonged presence of enterovirus- specific IgM has also been observed. In summary, the usefulness of serology for the diagnosis of enterovirus infection is limited. Genome detection The reverse transcription–polymerase chain reaction (RT-PCR) technique is widely applied to test for the presence of enterovirus genomes and is the method of choice for identifying virus infection in many countries. This technique is very sensitive and specific, par- ticularly in diagnosing CNS infections from cerebrospinal fluid spe- cimens, and has become the ‘gold standard’ of diagnosis, surpassing viral culture. Enterovirus RNAs have also been detected in myocar- dial biopsies from patients with myocarditis and dilated cardiomy- opathy, in muscle of people with inflammatory muscle disease and chronic fatigue syndrome, and in brain

biopsies. The significance of these findings is not clear, as infectious virus can rarely be isolated and viral antigen cannot be detected. Highly conserved sequences of the 5′ end of enterovirus genomes have allowed the design of PCR primers detecting most enterovirus RNAs. As the EV22 genome is very different from that of the other enteroviruses (see earlier), tailor-made primers have to be added in a multiplex RT-PCR to in- clude detection of these viruses, which cause infections particularly in neonates and infants. A modified RT-PCR procedure can differ- entiate between wild type and vaccine-derived poliovirus infections. Modern sequencing techniques (Deep, Massive parallel or Next Generation sequencing) offer the possibility of sequencing without either cell culture isolation, PCR or knowledge of the sequence to be sought and are having a major impact on diagnosis of enterovirus and other infections, and environmental surveillance. Epidemiology of enterovirus infections Enteroviruses are mainly transmitted by the faeco-oral route, due to the fact that viruses are shed in faeces for weeks or months after infection. Spread is particularly intense within families, usually starting from the primary infection of young children. In temperate climates, there are seasonal peaks (July–September in the northern hemisphere and December–February in the southern hemisphere), whereas in subtropical and tropical climates enterovirus infections occur all the year round. Most primary human enterovirus infec- tions occur during the first decade of life. Type-specific surveillance in several geographical regions has shown that coxsackieviruses A9, A16, P and B4 and echovirus types 6, 9, 11, 19, 22, and 30 are most frequently found. Prevention of enterovirus infections As there are only three poliovirus types and no significant animal reservoir, it has been possible to develop very successful poliovirus vaccines. In 1954, a formalin-inactivated poliovirus vaccine (IPV) was introduced by Dr Jonas Salk in the United States of America, and in 1962 Dr Albert Sabin introduced a vaccine consisting of live-attenuated strains of the three poliovirus types which could be given orally (OPV). Protection by the live-attenuated vaccine is effected mainly at the site of entry by eliciting locally virus-specific IgAs and IgGs. Inactivated vaccine mainly elicits serum IgGs which prevent infection of the CNS and other sites distant of the port of entry by neutralization of viraemic virus. The main characteristics of IPV and OPV are summarized in Table 8.5.8.2. Inactivated poliovirus vaccine The early IPVs developed by Salk were of relatively low potency. High potency vaccines, based on large-scale cell culture followed by virus purification and concentration but using the same inacti- vation procedures, were developed in the Netherlands in the 1980s and form the basis of IPVs used today. Much of the developed world including Europe and the United States of America now uses only IPVs, having previously used the live-attenuated vaccines. Other countries including Mexico and Russia have changed from using OPV to IPV, and middle income countries such as Argentina and Uruguay are proposing to do so when poliomyelitis is eradicated, since OPV was thought to be better able to eradicate poliomyelitis and was proven to be able to break epidemic transmission. However, Scandinavian countries and the Netherlands had eliminated the disease with IPVs. IPV is given by injection and is more expensive per dose than the oral vaccine, but has advantages as outlined next, mainly the avoidance of vaccine-associated paralysis. IPV is the vaccine of choice in cases of immunodeficiency. The World Health Organization has recommended the introduction of a single dose of IPV in all countries in preparation for the cessation of OPV usage (see next). Live-attenuated poliovirus vaccine This vaccine has several advantages compared to the inactivated vac- cine (Table 8.5.8.2) as it: • parallels the natural infection; • stimulates both local secretory IgA in the pharynx and alimentary tract, and systemic circulating virus-specific IgG antibody; • is easy to administer as an oral vaccine; • is more cost effective; and • is proven to be capable of interrupting virus circulation and epidemics.

794 section 8 Infectious diseases The disadvantage is that in a few cases the attenuated vaccine strains have reverted to virulence in vaccine recipients or their contacts. Since the early 1980s, all cases of polio in the United States of America and Europe were found to be vaccine-related, occurring either in vac- cine recipients or their close contacts who became infected by them, or were imported from endemic countries and were not indigenous original wild type strains. The risk of vaccine-associated poliomyelitis is between 0.5 and 3.4 cases/million of susceptible children immun- ized. Vaccine-related polio is mostly caused by type 2 or type 3 viruses, probably due to the fact that the number of point mutations in type 1 vaccine virus compared to wild type virus is much higher than in type 2 and type 3 vaccine viruses. However, as the disease becomes in- creasingly rare in the countries concerned and the world at large, indi- genous cases or importation of virus are increasingly rare, and the risks of oral vaccination begin to outweigh its benefits. The major current disadvantage, however, is that in addition to poliomyelitis in vaccine recipients and their immediate contacts, the re-introduction of polio by the use of oral vaccine has now been frequently documented in many regions where immunization programmes are imperfect and the virus is able to regain the ability to transmit from person to person and cause outbreaks. This poses a risk to the whole eradication programme. A further issue is the chronic infection of hypogammaglobulinaemic patients unable to mount a humoral response. Such individuals, if given the live vaccine, can go on to excrete virus for many years, al- though this is not common. These risks are not associated with the use of IPV, which has become the vaccine of choice in many countries. Polio eradication and surveillance For many years it was thought that the Sabin oral poliovirus vac- cines were ineffective in tropical countries, being unable to the con- trol the disease, much less eradicate it. While many reasons were put forward, the lack of impact of polio vaccination programmes was probably due to loss of vaccine potency through failure to maintain storage at cool temperatures (‘cold chain’), and also the epidemiology of poliovirus infection. In temperate countries, poliomyelitis is sea- sonal with infections peaking in the summer months. A strategy of vaccination based on immunization of young children at a set age (usually a few months) is, therefore, able to build up a highly im- mune population resistant to infection in the winter so that trans- mission of the wild type virus becomes more difficult; thus, the virus and the disease are eradicated. In tropical countries, where exposure is year round, it is a matter of chance whether a child will first be naturally infected or immunized, and virus circulation can continue. This was recognized by Sabin in 1960, but not acted upon until some 20 years later when the strategy of National Immunization Days was developed in South America. This approach involves immunizing all children below a certain age in a country within a very short period, so that all susceptible children’s intestinal tracts are occupied by vac- cine virus and are, therefore, resistant to infection by the wild type virus. Transmission of wild type virus is therefore broken, and the virus dies out. The World Health Organization (WHO) pronounced the inten- tion of eliminating poliomyelitis due to wild type virus in 1988, with a target date of 2000 for completion. While the target date was clearly missed, the Americas have been free of polio since 1992. In 2000, the Western Pacific Region was declared polio-free by the WHO, and the European region in 2002. The enormous progress achieved between 1988 and 2004 is shown in Fig. 8.5.8.6. The scale of the undertaking is colossal, and the progress towards eradication is extraordinary. For example, in 1992 in China, all children aged 5 or less were immunized during a 1-week period. This amounted to one-quarter of the world’s children. However, eradication of polio has proved to be very difficult in some regions. At the time of writing, virus is still known to be Table 8.5.8.2 Characteristics of poliovirus vaccines Characteristic Live-attenuated poliovirus vaccine (OPV) Inactivated poliovirus vaccine (IPV) Virus source Attenuated virus (Sabin strains) Virulent virus strains Primary course 3

doses at monthly intervals starting at age of 2 months (temperate climates; more doses in tropics) Three doses at 2-month intervals Administration route Oral Parenteral (injection) Immunity produced—systemic IgA, IgM, IgG IgM, IgG, (IgA) —local IgA (IgA, minimal) Booster doses required

1. at school entry Yes (every 3–5 years or when exposed)
2. between 15 and 19 years
3. in adult life when exposed (last dose 10 years or more ago) Efficacy Good in temperate climates, variable in tropics Good Spread to contacts Yes No Vaccine-associated paralysis 0.5–3.4 cases/million first doses in susceptible children No Production cost per dose .07 .7 Requirement on personnel Not highly trained Trained and skilled Requirement of 'cold chain' Yes Less than OPV Combination with other vaccines No Possible Use in immunodeficient children No Possible

8.5.8 Enterovirus infections 795 endemic in only Pakistan and Afghanistan, although some cases have been reported in Nigeria, where full access to at-risk areas is difficult (Fig. 8.5.8.7). Eradication before long is a real possibility although it is not a trivial matter. It was only in 2011 that India re- corded no cases at all. It was certified to be polio-free in 2014. The virus has been reintroduced repeatedly into countries where it had been eradicated, particularly into Angola from India and into the Democratic Republic of Congo from Angola. It has also been intro- duced into Tajikistan (European Region) from India, and into China from Pakistan. The effect of importation from one country to others was graphically illustrated in 2004 when immunization stopped in Nigeria after it had been suggested that the vaccine contained oestro- gens to render recipients sterile. The result was the re-emergence and re-introduction of polio into much of Central Africa, where it had been previously considered eradicated, and outbreaks occurred in Yemen and Indonesia; it is surmised that pilgrims returning from Mecca were infected by coreligionists from Nigeria and reintroduced the virus. The situation was only brought under control by massive coordination of immunization activities throughout the region. Lingering on of wild type polio up until 2010 in India was in part due to vaccine refusals similar to those in Nigeria. The last case of polio in India was in 2011. In Pakistan the numbers increased by 62% in 2010, due to civilian turmoil, flood catastrophes, and lack of political will to eradicate. In 2014 a poliovirus strain from Pakistan was found to be circulating silently in Israel. Israel has used only IPV for nearly ten years and was initially reluctant to re-introduce OPV to break transmission. The in- cident showed that under certain circumstances IPV will not prevent circulation, although in other circumstances such as in Scandinavia and the Netherlands it has been very effective at eradicating the virus as well as disease. Two poliovirus isolates of another Pakistan-derived virus were made in Egypt, again without any cases but no further isolations of this strain have been reported, possibly because of the continued routine use of OPV in Egypt. Finally, an outbreak of cases caused by a Pakistan virus occurred in Syria where there has been armed conflict, and immunization is very difficult. The outbreak was brought under control. Similar concerns arise in Africa, where Nigeria remained a po- tential source of polio virus for many years and there have been out- breaks in Chad, Kenya, and other countries. The Central African Republic has no cases at the time of writing, but is a particular focus of attention because of its poor domestic circumstances and the occurrence of cases in the surrounding countries. The eradication of poliomyelitis is not trivial. Nonetheless there has been no case of poliomyelitis attributable to a naturally occurring type 2 strain of poliovirus since October 1999 and at the time of writing there had been no case of poliomyelitis caused by a wild type 3 virus since November 2012. Moreover, Nigeria has had few reported cases since 2012, and the two

remaining endemic countries Pakistan and Afghanistan had fewer than 60 cases in 2015. It is very possible that poliomyelitis will be eradicated in the near future. As long as pockets of infection persist, the world will remain at risk of the re-emergence of polio. Thus, part of the challenge is to demon- strate that the virus has in fact been eliminated, and this depends on rigorous effective surveillance. One approach is to obtain data on cases of acute flaccid paralysis of whatever cause, including the Guillain– Barré syndrome. All cases should be investigated to see whether they are due to poliovirus infection or not. It is considered that the back- ground rate of paralysis in the absence of poliomyelitis should be one case per 100 000 members of the population, providing a control for the adequacy of the surveillance scheme. All poliovirus isolates iden- tified in paralysis surveillance are examined to establish whether they are derived from vaccine or represent wild type strains. Surveillance of environmental samples including sewage for poliovirus also plays an increasing role in global polio eradication, and isolates have been obtained in areas where there are no recorded cases of disease as afore- mentioned in Israel and Egypt and at one stage in India. Many coun- tries perform routine environmental studies of this kind. There are possible concerns over the adequacy of either approach. Once wild type poliovirus has been eradicated, the only sources of the virus will be manufacturers of vaccines, laboratories holding stocks or potentially infected samples, and recipients of live- attenuated vaccine. While manufacturers and laboratory workers can be required to work under high containment level conditions to avoid escape of virulent virus, vaccinees pose a particular problem. The oral vaccine works by establishing an infection in the recipient, and there are numerous instances of outbreaks caused by vaccine-derived polioviruses that have recovered the ability to circulate (cir- culating vaccine-derived viruses or cVDPVs). This has been observed in Haiti, Egypt, the Philippines, and Madagascar among many others, and might be relatively common, particularly where vaccination con- tinues with the live vaccine for a long time with poor coverage, so that vaccinated and unvaccinated individuals mix, providing the ideal conditions for the selection of transmissible virus. However, the Polio incidence: 1988 Polio incidence: 2004 Equator Equator Known or probable wild poliovirus circulation Fig. 8.5.8.6 World maps depicting the circulation of wild type poliomyelitis virus for 1988 and 2004, as reported by the World Health Organization. From Palllansch M, Roos R (2007) Enteroviruses: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses. In: Knipe DM, et al. (eds) Fields virology, 5th edition, pp. 839–93. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia. With permission of author and publisher.

 vaccine virus seems to be poorly transmissible compared to the wild type. In countries such as Cuba where it has been given only in the early part of the year as a matter of policy, virus is not detectable after 6 months. Thus, it might be possible to stop vaccinating with no fur- ther precautions, and deal with the re-emergence of polio as cVDPVs on a case-by-case basis. The Global Action Plan (third draft) or GAP3 provides instructions of the containment of poliovirus in manufac- ture, diagnosis, research, and any other usage. Natural wild type 2 poliovirus has not been implicated in polio- myelitis since 1999. The only cases occurring now are due to the vac- cine directly in vaccinees or their contacts or indirectly in the form of cVDPVs. Immunization with type 2 OPV should therefore cease, but the strategies to be followed are complex, requiring that within a country or region all type 2 usage should stop together, prepar- ations should be made for possible type 2 outbreaks, particularly of cVDPVs and to protect the population from disease by the use of IPV. The withdrawal of type 2 OPV, was implemented in 2016, and will provide a model for the cessation of all OPV use and the final eradication of polio. The final stages and containment of polioviruses are the subject of the WHO document on the final strategic plan for eradication, which includes timings of the responses required and GAP3, both of

which are available from the WHO website. A further concern relates to people with B-cell immunodeficiency who can become chronically infected but be apparently healthy, sometimes for decades as described earlier. During this time the virus might adapt to an extent that neurotropism is regained, and an unvaccinated population will again be highly susceptible. The numbers and geographical distribution of such long-term excretors are unknown but most exposed individuals do not excrete virus for very long periods, and even those that do usually cease excreting virus spontaneously, albeit after a period of a few years. On the other hand, an individual is known to have excreted type 2 polio for at least 28 years. Long-term secretors of poliovirus could initiate a new outbreak of polio in a population in which poliovirus circulation has been considered to be eliminated. It would be unwise to stop polio vaccination with IPV until such individuals are known to no longer excrete virus. Stocks of OPV, preferably monovalent type 1, 2, and 3 poliovirus vaccines, should be kept in reserve in case there is an outbreak. New approaches to vaccine development are under con- sideration; for example, producing live attenuated strains that are considered unable to revert to virulence, or new strains of virus to be used for production of IPV that are hyperattenuated and genetically very stable and therefore safe or expression of stable empty capsids which are non infectious. These developments are ongoing. The de novo synthesis of infectious particles from poliovirus RNA transcribed from synthetic cDNA in a cell-free HeLa cell extract has created a huge debate on the scientific value of such an experiment, concerns about poliovirus eradication, issues of national security and freedom of virological/biological research. Since then, other viruses (ΦX174, the 1918 H1N1 influenza virus a.o.) have been synthesized de novo, permitting the effect of drastic changes in viral sequence on viral phenotypes to be examined in more detail than is possible by established methods of genetic manipulation. The use- fulness of the method to carry out broad-based research into ques- tions of viral pathogenesis and attenuation for vaccine production (e.g. by changing codon usage) is becoming appreciated but it has also led to considerable efforts by the scientific community and in- dustry to define and assess possible misuse of this technology. Case or outbreak following importation Endemic countries Wild virus type 1 Wild virus type 3 Wild virus type 1 & 3 Excludes viruses detected from environmental surveillance and vaccine derived polioviruses. Data in WHO/HQ as of 22 Apr 2008 Fig. 8.5.8.7 Cases of wild type polio in 2011. From www.polioeradication.org, with permission from WHO.

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