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REVIEW

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Hand, foot and mouth disease and herpangina caused by enterovirus A71 infections: a review of enterovirus A71 molecular epidemiology, pathogenesis, and current vaccine development

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ABSTRACT

Enterovirus A71 (EV-A71) infections are one of the main etiological agents of hand, foot and mouth disease (HFMD) and herpangina worldwide. EV-A71 infection is a life-threatening communicable disease and there is an urgent global need for the development of vaccines for its prevention and control. The morbidity rate of EV-A71 infection differs between countries. The pathogen's genetic lineages are undergoing rapid evolutionary changes. An association between the occurrence of EV-A71 infection and the circulation of different genetic strains of EV-A71 virus has been identified around the world. In this review, we present and discuss the molecular epidemiology and pathogenesis of the human disease caused by EV-A71 infection, as well as current prospects for the development of an EV-A71 vaccine.

KEYWORDS: Hand, foot and mouth disease. Herpangina. Enterovirus A71. Vaccine.

INTRODUCTION

Enterovirus A71 (EV-A71) is an important cause of hand, foot and mouth disease (HFMD) and herpangina[1]. EV-A71 infects infants and young children and most cases of infection (>70%) are symptomatic[2,3]. The predominant clinical presentation of HFMD is characterized by a febrile illness accompanied by a maculopapular rash or blisters on the hands, soles of feet and buttocks[1,2]. It is usually a self-limiting infection, but it is highly contagious through fecal-oral transmission of oropharyngeal secretions[2]. The most common involved area in severe or fatal cases of EV-A71 is the brain stem[2,3].

EV-A71 is an RNA virus that belongs to the human *enterovirus A* (EV-A) species, genus *Enterovirus*, family *Picornaviridae*[1]. Viral particles have an icosahedral shape, are not enveloped and contain a single-strand, positive-sense RNA genome. The most common method for enterovirus genotyping targets the 1D gene encoding the VP1 capsid protein containing the major neutralizing epitopes[4]. Human-infecting enteroviruses species include enterovirus A (EV-A), EV-B, EV-C, and EV-D. The classification of human enteroviruses is based on each strain's homology within the RNA region coding for the VP1 capsid protein[1].

Although the EV-A71 virus has been isolated in many countries, epidemics of EV-A71 infection are predominantly found in the Asia-Pacific region[2,3,5-9]. The morbidity associated with EV-A71 infection varies from country to country[1]. Seasonal epidemic patterns of EV-A71 infection have been observed in several countries. In Asia, a higher incidence has been observed during the summer months[10-12]. Several studies have also shown variations in the peak season between different years[13,14]. Most cases

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of EV-A71 infection occur in children five years of age and younger, and boys have a higher risk of EV-A71 infection than girls[2,5]. Because the virus has the potential to cause high morbidity and mortality among children, it is critical to understand the mechanisms of and control measures for EV-A71 infections. In this review, we present and discuss the molecular epidemiology and pathogenesis of EV-A71 infections, as well the prospects for the development of an EV-A71 vaccine.

Data sources

All manuscripts used in this review were published between January 1965 and December 2017; these reports related to EV-A71 infections were extracted by searching Medline (National Library of Medicine, Bethesda, Maryland, USA) and PubMed using the phrases "*enterovirus-A71*" and "molecular epidemiology" or the key words "pathogenesis" or "vaccine." The results were limited to manuscripts available in English.

Molecular epidemiology

In 1969, EV-A71 was first isolated from a child with encephalitis in the USA[14]. Subsequently, several EV-A71 epidemics were reported in the 1970s around the world, including the Americas, Europe, Australia and Asia[15-31].

The EV-A71 virus is enclosed by the capsid proteins VP1, VP2, VP3, and VP4[1]. VP1 shows the major antigenicity and has been defined as the neutralization determinant[1]. Based on VP1 nucleotide sequence analysis, EV-A71 can be divided into three distinct genogroups (e.g., A, B, and C)[1,25]. Genogroup A includes the prototype EV-A71 strain (BrCr-CA-70); this strain was first isolated in 1969 in the USA[14], although it was not identified until 2008, in China[9]. Genogroup B can be divided into subgenogroups B1-B5, and genogroup C can be further divided into subgenogroups C1-C5[16]. Recently, the C4 genogroup was once more subdivided into the C4a and C4b lineages. Genogroup D was initially identified in India[4], and genogroups E and F were initially identified in Africa[4].

Table 1 shows the phylogenetic origins of the EV-A71 strains that have been recently circulating in the Asia-Pacific region[2,5,8,9,15-20]. Genogroup A was not identified in the Asia-Pacific region until 2008[9]. In contrast, genogroups B and C have been responsible for several large-scale outbreaks in the Asia region since 1997[16]. Four distinct subgenogroups (B3, B4, C1 and C2) were found to have been cocirculating in the Asia-Pacific region from 1990-2016. Subgenogroup C4, particularly the C4a lineage, has also emerged in the Asia-Pacific region. The evolutionary branch C4a has crucial nucleotides and amino acid mutations relative to the branch C4b, and these changes may be the primary reason for its increased neurovirulence, causing outbreaks in China[18,19]. Through genetic and antigenic analysis, EV-A71 subgenogroup C4a has been confirmed to have spread from China to Vietnam; a subsequent large-scale outbreak occurred in Ho Chi Minh City and Southern Vietnam in 2011[20].

The phylogenetic origins of EV-A71 strains circulating outside the Asia-Pacific region are presented in Table 2[21-31]. From 1963 to 1986, strains of EV-A71 belonging to subgenogoup B0, B1 and B2 were isolated in the Netherlands[27]. In contrast, after 1987, genogroup B was replaced by genogroup C, lineages C1 and C2[23-30]. The emergence of C2 was associated with a peak in hospitalizations in 2007. According to an epidemiological study, the EV-A71 subgenogroups B1, B2, C1 and C2 circulated simultaneously in Europe, Australia and the USA[21-29]. B3-B5, C4and C5, which have caused large

Table 1 - A summary of human enterovirus A71 genotypes circulating in Asia-Pacific countries, 1960-2016[2-9,15-20].

Countries	Years					
	1960-1969	1970-1979	1980-1989	1990-1999	2000-2009	2010-2016
Singapore	--	--	--	B3, B4	B4, B5, C1	--
Malaysia	--	--	--	B3, B4	B4, B5, C1,	--
Australia	--	--	--	B3, C2	C1	--
Japan	--	--	--	B3, B4, C2	B4, B5, C2, C4a	C2
Korea	--	--	--	B4, C2	C2, C3, C4a, C4b	C4a
Taiwan	--	--	--	B4, C2	B4, B5, C4, C5	B5, C4
China	--	--	--	--	C4	C4, C4a
Cambodia	--	--	--	--	--	C4
Viet Nam	--	--	--	--	--	C4, B5

-- indicates no data available.

Table 2 - A summary of human enterovirus A71 genotypes circulating in countries outside the Asia-Pacific region, 1960-2016[21-31].

Countries	Years					
	1960-1969	1970-1979	1980-1989	1990-1999	2000-2009	2010-2016
France	--	--	--	--	C1, C2, C4	C4
UK	--	--	--	C1	C1, C2	--
Germany	--	--	--	--	C1, C2	--
Austria	--	--	--	--	C1, C4	-
Norway	--	--	--	--	C1	--
Netherlands	B0	B1	B2	C1	C1, C2	--
Hungary	--	B1	--	--	C1, C4	--
Bulgaria	--	B1	--	--	--	--
USA	A	B1	B2	C1, C2	C2	--
Canada	--	--	--	--	--	--
Peru	--	--	--	--	C1	--

-- indicates no data available.

epidemics in the Asia-Pacific region since 1997, have not been observed outside the Asia-Pacific region (Table 2).

Comparing the epidemics that have occurred over the past four decades, the epidemiologic features of EV-A71 infections appear to be changing. EV-A71 infection has emerged as an important public health problem around the world. No association has been established between genotype and disease severity[32]. From 1900 to 2016, infections were successively caused by viruses of subgenogroup B0, B1and B2, followed by a shift in this predominance to those belonging to subgenogroups C1, C2, C3, C4a and C4b. A molecular epidemiological study suggested that the evolution of the EV-A71 virus has global characteristics. Global herd immunity against C1 and C2 viruses could possibly explain why epidemics caused by subgenogroups B4 and C4 are restricted to the Asia-Pacific region[27]. Subgenogroup B5 has been reported to be antigenically distinct from B1, B4, C2 and C4[17] and could therefore pose a potential risk for epidemic spread outside the Asia region.

Pathogenesis

The clinical spectrum of EV-A71 infection presentations is quite wide, including skin eruptions, internal organs and neurological manifestations and even death[1,2].

EV-A71 is a highly neurotropic virus[33]. The most common internal organ involved in EV-A71 infections is the brain stem[33]. According to data derived from a study developed in mice, the strong neurotropism of EV-A71 and its retrograde axonal transport in neurons could underlie its major transmission route[34]. In previous studies[34,35] in which mice were infected via oral and parenteral routes with a murine-adapted virus strain that originated from a fatal human case, the EV-A71 virus entered the CNS via peripheral motor nerves after skeletal muscle infection and continued to spread within the CNS through motor and other neuronal pathways. Inflammation was the most profound in the spinal cord gray matter, brainstem, hypothalamus, subthalamic and dentate nuclei in autopsy samples investigated in Malaysia[35]. Previous studies[36,37] have found that the EV-A71 virus mechanism of infection is primarily focused on the respiratory tract epithelium, from which it is subsequently able to enter a pre-existing population of dendritic cells at the infection site; these cells could potentially transmit the virus from local sites to other organs through the blood stream during the infectious process.

In a previous study[38], a total of 46 children with EV-A71-related brainstem encephalitis (EBE) were enrolled and subsequently underwent 1.5 Tesla magnetic resonance (MR) examinations of the brain. Among these 46 children, 35 had MR images evidencing dorsal medulla oblongata involvement, 32 had evidence of pons involvement, 10 had evidence of midbrain involvement and 7 had evidence of dentate nucleus involvement. Patients with dorsal medulla oblongata involvement or multiple areas involvement were significantly more likely to have poorer outcomes than patients without these features.

Additional noteworthy findings include clinical manifestations of viremia. Viremia reportedly occurs more frequently in children one year of age or younger[17,39]. Most patients with viremia do not show severe clinical manifestations of EV-A71 infection. In addition, the occurrence of CNS involvement is not reportedly different between patients with and without viremia[39].

What might cause severe or fatal clinical presentations of EV-A71 infection? Based on the results of one autopsy, EV-A71 infection can lead to severe or fatal disease due to pulmonary edema. The mechanism of pulmonary edema among patients with severe EV-A71 infection is currently unclear. Evidences[40] suggest that the brainstem involvement characteristic of EV-A71 infection may be an important etiological mechanism of neurogenic pulmonary edema. Virus-host interactions significantly influence viral replication, virulence and pathogenicity during the viral life cycle[41]. However, tissue-specific virulence is still not well understood in both, cell-based assays and animal models, therefore further studies are needed.

Vaccine development

Close person-to-person contact is considered the most common route of EV-A71 transmission. In areas where EV-A71 circulation is present, especially because the clinical manifestations of the majority of EV-A71 infections are mild or asymptomatic, public health interventions, such as the promotion of hand washing, are necessary for the effective prevention of EV-A71 infection. Because effective treatment may not always be available, the best way to control and eradicate EV-A71 infection is to develop an effective vaccine. Several EV-A71 vaccines have been produced[1], including a subunit vaccine, a virus-like particle vaccine, DNA vaccines, a live-attenuated vaccine and an inactivated virus vaccine. The potential vaccines targeting EV-A71 are discussed in greater detail below.

Subunit vaccine

Currently, there are no effective antiviral drugs or vaccines for prevention of EV-A71 infection. A previous study has suggested the potential of the VP1 protein as a candidate antigen for an EV-A71 vaccine[42,43]. A recombinant VP1 protein of the EV-A71 virus has been produced in *Escherichia coli*, yeast and the baculovirus system[42-44]. Vaccination with a VP1 protein vaccine can induce neutralizing antibodies to protect against EV-A71 infection[42-44]. However, another study showed that vaccination with a recombinant VP1 induced a lower titer of EV-A71-specific IgG antibodies than inoculation with the inactivated virus[42,43]. Although recombinant VP1 vaccine can elicit similar levels of neutralizing antibodies, it provided effective protection only at a low challenge dose of EV-A71[42].

In another study[45], antiserum was produced in mice against overlapping synthetic peptides elicited by the VP1 capsid protein of EV-A71. Peptides SP55 (amino acids 163-177 of VP1) and SP70 (amino acids 208-222 of VP1) could elicit neutralizing antibodies against EV-A71 *in vitro*. SP70 was identified as particularly potent in eliciting a neutralizing antibody titer compared to that obtained with whole virion-immune serum. The amino acid residues of epitope SP70 are more conserved than the VP1 sequences of various subgenogroups of EV-A71. However, obtaining superior synthetic antigens requires the use of more effective adjuvants. Although the yeast-expressed VP1 protein provides good immunogenicity, VP1 subunit vaccines require further refinement to contribute significantly to an effective vaccine strategy[43].

Virus-like particle vaccine

Virus-like particles (VLPs) for EV-A71 are similar in morphology to the natural viral capsid structure and have been developed as potential vaccines[46]. Vaccination with an EV-A71 VLP vaccine showed significant potency against lethal challenge in newborn mice. However, an ideal enterovirus vaccine should be effective against both EV-A71 and Coxsackie virus-A16 (CV-A16) infections. Consequently, a bivalent enterovirus vaccine based on chimeric EV-A71 virus-like particles (ChiEV-A71 VLPs) has been proposed[47]. In chimeric EV-A71 VLPs, the neutralizing epitope SP70 within the capsid protein VP1 of EV-A71 was replaced by CV-A16. Chimeric VLPs are able to elicit protective neutralizing antibodies against EV-A71 and CV-A16 in mice[47]. Although studies have demonstrated that VLP vaccines can induce protective neutralizing antibodies and show cross-protective efficacy against different EV-A71 subtypes not present in other experimental vaccines, these vaccines have a lower efficacy than inactivated vaccines. They must also be further studied.

DNA vaccines

DNA vaccines and recombinant vector DNA vaccines have been studied in EV-A71 vaccine development. Several research groups have designed and constructed a DNA vaccine against EV-A71 infection using its viral capsid protein (VP1) gene in order to verify the functionality of this vaccine *in vitro* and/or *in vivo*[48-50]. One study showed that vaccination with an EV-A71 DNA vaccine could elicit VP1-specific IgG titers and neutralizing antibodies against EV-A71, although it resulted only in low levels of antigenicity[48]. Another study by Chiu *et al.*[49] investigated the potential use of attenuated *Salmonella enterica* serovar Typhimurium strains to express and deliver VP1 of EV-A71 as a vaccine to prevent EV-A71 infections in mice. They showed that offspring born to female mice immunized

with *Salmonella*-based VP1 vaccine had higher survival rates (50-60%) than offspring of unvaccinated control mice (0%)[49]. Another study indicated that recombinant adenoviruses, expressing EV-A71 P1 and 3CD genes, could elicit production of neutralizing antibodies and protect against EV-A71 infection, both useful characteristics for the prevention of EV-A71 infections[50]. Although promising preliminar results have been shown, further investigation on the immunogenicity of this potential EV-A71 DNA vaccine is needed.

Live-attenuated virus vaccines

Cynomolgus monkeys inoculated with an attenuated EV-A71 vaccine showed mild neurological symptoms but survived lethal challenge by virulent EV-A71 (BrCr-TR) without exacerbation of symptoms[51]. Although this study indicated that the monkeys' immunization with attenuated EV-A71 vaccine had the potential to produce significant titers of neutralized antibodies against different genogroups of EV-A71, including A, B1, B4, C2 and C4[51], several safety issues concerning live-attenuated virus vaccines need to be overcome. For example, immunization with the attenuated strain might cause mild neurological symptoms when inoculated via the intravenous route. However, a high-fidelity variant of EV-A71 exhibited an attenuated phenotype and showed an intriguing potential as a live-attenuated EV-A71 vaccine[52].

Inactivated virus vaccines

Compared to the various vaccine candidates, inactivated EV-A71 vaccines are more capable of fulfilling the demand for prevention of EV-A71 infections. Inspired by previous developments of inactivated vaccines, the development of inactivated EV-A71 vaccines has progressed rapidly in recent decades.

It has been demonstrated in mice that immunization with a formalin-inactivated EV-A71 vaccine can elicit high titers of EV-A71 virus-specific neutralizing antibodies, conferring protection against an EV-A71 lethal challenge[53]. After successful preclinical experiments, phase I and phase II clinical trials were performed to determine the efficacy of inactivated EV-A71 candidate vaccines. Immunization with inactivated EV-A71 vaccine elicited high titers of neutralizing antibody and induced specific T-cell reactions in the study population, with no significant inflammatory reaction reported[54]. In addition, another study indicated that inactivated EV-A71 vaccine candidates can elicit cross-neutralizing antibody responses against EV-A71 subgenogroups B1, B4, B5 and C4A[55]. Due to their stability, the research and development of inactivated EV-A71 vaccines has progressed more rapidly compared to other types of vaccines. In recent years, at least five inactivated EV-A71 vaccine candidates have been developed and advanced to clinical trials. All EV-A71 vaccines developed in China are inactivated whole-virus alum-adjuvant vaccines[1]. All of them use the C4 subgenogroup virus as the vaccine strain[1]. Randomized, double-blind, placebo-controlled trials of EV-A71 vaccines have been completed[56]. More than 30,000 infants and children have been involved in Phase III clinical trials of inactivated EV-A71 C4 vaccines in China[56]. These studies have shown that the EV-A71 vaccines can prevent more than 90% of EV-A71-associated HFMD or herpangina and 80% of other EV-A71-associated disease symptoms. It has been reported that the seroconversion rate is 100% after two vaccinations, and all C4-based vaccines prevented EV-A71-associated hospitalizations. Furthermore, preexisting antibodies due to undetected subclinical infections in young children did not interfere with vaccine efficacy against different EV-A71 genogroups[57]. However, the inactivated EV-A71 vaccines did not protect against CV-A16[1,57].

Indurations, erythema, and pain at the injection site have been the most common side effects of EV-A71 vaccination; high fever has also occasionally been observed[58]. The rate of rare serious adverse events (SAEs) in vaccinated groups was not significantly different from that observed in control groups, and SAEs were not causally related to vaccination[58].

An estimation of the cost-effectiveness of a national EV-A71 vaccination has been performed in China[59]. It was shown that the vaccination would be cost-saving or cost-effective due to prevention of EV-A71-related morbidity, mortality and use of health services among children aged five or younger, compared to anticipated costs with no vaccination. This finding bodes well for the introduction of a safe and effective EV-A71 vaccine in the near future. In December 2015, the Food and Drug Administration (FDA) of China approved an EV-A71-targeting vaccine[59]. In China, inactivated EV-A71 vaccines are now commercially available products.

An inactivated EV-A71 vaccine still faces one major challenge[60]. Although both, C4-based and B4-based antibodies cross-neutralize the current circulating EV-A71 isolates[2], the B4 vaccine poorly neutralizes an atypical C2 strain. In addition, no formalin-inactivated EV-A71 vaccines developed to date protect against CV-A16, which is a primary cause of annual HFMD outbreaks. Additionally, the humoral immunity associated with protection initially observed, appears to wane after the first 6 months of vaccination. However, inactivated EV-A71 vaccines have significant safety advantages over live-attenuated ones because of their inability to replicate.

CONCLUSIONS

EV-A71 is currently an important, threatening infectious agent in the world. It can cause severe neurological disorders and death in infected young children. Due to the lack of effective control measures, the development of effective vaccines is urgently needed for prevention and control of EV-A71 infections. Currently, several candidates for an EV-A71 vaccine exist, including a formalin-inactivated whole virus vaccine, DNA vaccines, VLP and recombinant protein vaccines. Monovalent EV-A71 vaccines are expected to be marketed soon in mainland China, if the process of production goes well. It has been estimated that EV-A71 vaccines will be effective and cost-saving in most developing countries[59]. Several promising inactivated EV-A71 vaccines have been developed in the last few years; however, they have certain limitations. Currently, inactivated EV-A71 vaccines can protect against EV-A71 but not against CV-A16 infections. All of these EV-A71 vaccines remain in the initial stages of development. Stability, purity and cost of production are the primary future challenges for these vaccines.

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