Institut Pasteur
Microbes and Infection 17 (2015) 155–162
www.elsevier.com/locate/micinf

Towards broadly protective polyvalent vaccines against hand, foot and mouth disease

Qingwei Liu [a,1], Xin Tong [b,1], Zhong Huang [a,*]

[a] Vaccine Research Center, Key Laboratory of Molecular Virology & Immunology, Institut Pasteur of Shanghai, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yueyang Road, Shanghai 200031, China
[b] Shanghai Zerun Biotechnology Co., Ltd., Building 9, 1690 Zhangheng Rd, Zhangjiang, Pudong New District, Shanghai 201203, China

Received 13 November 2014; accepted 21 November 2014
Available online 29 November 2014

Abstract

Hand, foot, and mouth disease (HFMD) caused by multiple enterovirus infections is a serious health threat to children in the Asia–Pacific region. This article reviews progresses in the development of vaccines for HFMD and discusses the need for polyvalent HFMD vaccines for conferring broad-spectrum protection.

Keywords: Hand, foot, and mouth disease; Enterovirus 71; Coxsackievirus A16; Polyvalent vaccine

1. Introduction

Hand, foot, and mouth disease (HFMD) is a highly contagious disease that mostly affects children under the age of 5 [1,2]. HFMD patients often present with mild fever and rash or blister on the surface of hand, foot and mouth [1]. A proportion of them may develop severe neurological and cardiopulmonary complications, including aseptic meningitis, brainstem encephalitis, poliomyelitis, encephalomyelitis and pulmonary edema, and ultimately death [1,3]. In addition, convalescent patients are at the risk of long-term neurological sequelae and cognitive impairment [1,4].

HFMD was first reported in New Zealand in 1957 [5]. However, a large outbreak has not taken place until 1997 when HFMD was epidemic in Malaysia [6]. Since then, HFMD epidemics hit many countries in the Asia–Pacific region, including Singapore, Malaysia, Taiwan, Japan, Korea, Vietnam, Cambodia, Thailand and China. In China alone, millions of HFMD cases and hundreds of HFMD-related deaths occurred annually in the last five years. According to China CDC, as of October 7, there were a total of 2,385,764 HFMD cases and 459 deaths reported in 2014 (http://www.chinacdc.cn). Besides Southeastern Asia, sporadic HFMD epidemics also occurred in other regions, including North America, Oceania, Europe, and even Africa [7,8]. It is likely that HFMD will soon become a global health problem and thus the development of HFMD vaccines is of significant importance for worldwide control of this highly contagious disease.

A number of the human enterovirus species A (HEV-A) within the *Picornaviridae* family are the major causative agents of HFMD, including enterovirus 71 (EV71), coxsackievirus A16 (CA16), coxsackievirus A6 (CA6), and coxsackievirus A10 (CA10) [9,10,1,11]. These are non-enveloped viruses with a single-stranded, positive sense RNA genome of ~7.4 kb encapsidated within an icosahedral protein shell made of 60 copies each of VP1, VP2, VP3 and VP4 subunit proteins. Several receptors for EV71 have been identified [12]. Existing surveillance data indicate that these viruses either circulate in alternative years or co-circulate in same years with one of them being the predominant causative agent [9,10,13]. Co-

* Corresponding author. Tel.: +86 21 54923067.
E-mail address: huangzhong@ips.ac.cn (Z. Huang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.micinf.2014.11.004
1286-4579/

infections with two viruses were frequently reported [14,15] and they are likely to have contributed to the emergence of the recombinant viral strains that caused recent HFMD outbreaks [16,17].

The magnitude and severity of the recent HFMD endemics has drawn the attention of the governments and vaccine makers in the Asia–Pacific regions. In particular, China has declared the HFMD vaccine development a national priority [18]. Consequently, a few vaccine candidates have rapidly progressed into clinical trials through a "green channel" mechanism [19], and many more vaccine candidates are at the preclinical development stage. However, concerns are also raised with regard to the breadth of protection conferred by these candidate vaccines [20,21]. Below we summarize the recent achievements and challenges in the field of HFMD vaccine development.

2. Vaccines targeting EV71

Among all HFMD pathogens, EV71 is more often associated with severe HFMD cases with neurological complications and even death [9,22]. Therefore, it has been the only target for developing HFMD vaccines for a very long time [23,24]. A number of approaches have been taken to develop vaccines targeting EV71; those at advanced developmental stages are listed in Table 1.

2.1. EV71 inactivated vaccine

Because of the past success of inactivated poliovirus vaccine (IPV), it was naturally a first choice to develop inactivated EV71 vaccines. In fact, the first inactivated EV71 vaccine candidate was developed in 1975 when EV71 was epidemic in Bulgaria, but it was discontinued for evaluation due to no further outbreaks in Bulgaria after 1976 [23]. Recent HFMD outbreaks in the Asia–Pacific region have renewed interest in developing EV71 vaccines and have driven active research and development programs in both public and private sectors. A large body of preclinical studies of inactivated whole virus EV71 vaccines has been published [23,24,18], and the protective efficacy of this vaccine approach was validated in animal models – thus providing supporting evidence for clinical trials of some of the leading vaccine candidates. So far, five inactivated EV71 vaccines developed by different companies/organizations in Taiwan, Singapore and Mainland China have entered the clinical trial stage (Table 1). In Taiwan, National Health Research Institute (NHRI) selected a local isolate E59 (B4 subgenotype) as the vaccine strain and used formalin for inactivation. The immunogenicity of the formalin-inactivated vaccine was validated in different animal models [25]. Based on the preclinical results, a phase I clinical trial with the inactivated EV71 vaccine candidate at 5 μg and 10 μg doses was launched in 2010 and completed in 2012 [26]. The trial not only showed that the neutralizing antibody titers against the B4 vaccine strain increased by more than 4 fold even after a single immunization [26], but also revealed the presence of cross-neutralizing antibodies against subgenotypes B1, B5 and C4a, but not C2 [27]. A Singapore-based company, Inviragen, initiated phase I clinical trial with 0.6 μg and 3 μg per dose of inactivated EV71 vaccine (subgenotype B3) [28], the results of which are pending. In mainland China, three vaccine makers, including Beijing Vigoo, Sinovac and Chinese Academy of Medical Science (CAMS),

Table 1
Selected EV71 vaccine candidates currently at advanced development stage.

Vaccine type	EV71 strain (subgenotype)	Cell substrate	Immunogenicity/protective efficacy	Clinical trial	Developer	Reference
Inactivated virus	E59 (B4)	Vero	Induce neutralizing antibodies in humans	Phase 1 completed	NHRI (Taiwan)	[26]
Inactivated virus	INV21 (B)	Vero	Not reported	Phase 1 completed	Inviragen (Singapore)	www.inviragen.com
Inactivated virus	H07 (C4)	Vero	94.8% efficacy against EV71-associated HFMD or herpangina and 88.0% against EV71-associated diseases in humans	Phase 3 completed	Sinovac (China)	[30]
Inactivated virus	FY7VP5/AH/CHN/2008 (C4)	Vero	90.0% efficacy against EV71-associated HFMD and 80.4% against EV71-associated disease in humans	Phase 3 completed	Vigoo (China)	[29]
Inactivated virus	FY2008 (C4)	KMB_{17}	97.4% efficacy against EV71-associated HFMD in humans	Phase 3 completed	CAMS (China)	[31]
VLP	Neu (C2)	Sf9	Induce neutralizing antibodies in monkeys		National Taiwan University	[37]
VLP	G082 (C4)	Sf9	Protect mice against lethal challenge		IPS-CAS (China)	[38]
VLP	AH08/08 (C4)	Saccharomyces cerevisiae	Protect mice against lethal challenge		IPS-CAS (China)	[40]
Attenuated live virus	EV71 BrCr (A)	Vero	Protect monkeys against lethal challenge		NIID (Japan)	[42]

NHRI: National Health Research Institute, Taiwan.
CAMS: Chinese Academy of Medical Sciences, China.
IPS-CAS: Institut Pasteur of Shanghai, Chinese Academy of Sciences, China.
NIID: National Institute of Infectious Diseases, Japan.