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Substitution of Coxsackievirus A16 VP1 BC and EF Loop Altered the Protective Immune Responses in Chimera Enterovirus A71

Xiu Hui Tan [1], Wei Lim Chong [2], Vannajan Sanghiran Lee [2], Syahril Abdullah [3,4], Kartini Jasni [4], Saiful Qushairi Suarni [4], David Perera [5], I-Ching Sam [1] and Yoke Fun Chan [1,*]

1 Department of Medical Microbiology, Faculty of Medicine, Universiti Malaya, Kuala Lumpur 50603, Malaysia; mmf190017@siswa.um.edu.my (X.H.T.); jicsam@um.edu.my (I.-C.S.)
2 Department of Chemistry, Center of Theoretical and Computational Physics, Faculty of Science, Universiti Malaya, Kuala Lumpur 50603, Malaysia
3 Department of Biomedical Sciences, Faculty of Medicine & Health Sciences, Universiti Putra Malaysia, Serdang 43400, Malaysia; syahril@upm.edu.my
4 Comparative Medicine and Technology Unit, Institute of Bioscience, Universiti Putra Malaysia, Serdang 43400, Malaysia
5 Institute of Health and Community Medicine, Universiti Malaysia Sarawak, Kota Samarahan 94300, Malaysia; dperera@unimas.my
* Correspondence: chanyf@um.edu.my

Abstract: Hand, foot and mouth disease (HFMD) is a childhood disease caused by enterovirus A71 (EV-A71) and coxsackievirus A16 (CV-A16). Capsid loops are important epitopes for EV-A71 and CV-A16. Seven chimeric EV-A71 (ChiE71) involving VP1 BC (45.5% similarity), DE, EF, GH and HI loops, VP2 EF loop and VP3 GH loop (91.3% similarity) were substituted with corresponding CV-A16 loops. Only ChiE71-1-BC, ChiE71-1-EF, ChiE71-1-GH and ChiE71-3-GH were viable. EV-A71 and CV-A16 antiserum neutralized ChiE71-1-BC and ChiE71-1-EF. Mice immunized with inactivated ChiE71 elicited high IgG, IFN-γ, IL-2, IL-4 and IL-10. Neonatal mice receiving passive transfer of WT EV-A71, ChiE71-1-EF and ChiE71-1-BC immune sera had 100%, 80.0% and no survival, respectively, against lethal challenges with EV-A71, suggesting that the substituted CV-A16 loops disrupted EV-A71 immunogenicity. Passive transfer of CV-A16, ChiE71-1-EF and ChiE71-1-BC immune sera provided 40.0%, 20.0% and 42.9% survival, respectively, against CV-A16. One-day-old neonatal mice immunized with WT EV-A71, ChiE71-1-BC, ChiE71-1-EF and CV-A16 achieved 62.5%, 60.0%, 57.1%, and no survival, respectively, after the EV-A71 challenge. Active immunization using CV-A16 provided full protection while WT EV-A71, ChiE71-1-BC and ChiE71-1-EF immunization showed partial cross-protection in CV-A16 lethal challenge with survival rates of 50.0%, 20.0% and 40%, respectively. Disruption of a capsid loop could affect virus immunogenicity, and future vaccine design should include conservation of the enterovirus capsid loops.

Keywords: enterovirus A71; coxsackievirus A16; enterovirus; hand; foot and mouth disease; capsid loop; vaccine; immunogenicity

Citation: Tan, X.H.; Chong, W.L.; Lee, V.S.; Abdullah, S.; Jasni, K.; Suarni, S.Q.; Perera, D.; Sam, I.-C.; Chan, Y.F. Substitution of Coxsackievirus A16 VP1 BC and EF Loop Altered the Protective Immune Responses in Chimera Enterovirus A71. *Vaccines* **2023**, *11*, 1363. https://doi.org/10.3390/vaccines11081363

Academic Editor: Laurent Andreoletti

Received: 11 July 2023
Revised: 9 August 2023
Accepted: 11 August 2023
Published: 14 August 2023

1. Introduction

Hand, foot and mouth disease (HFMD) is a common disease in children aged below five years old worldwide, especially in the Asia-Pacific region [1]. Enterovirus A71 (EV-A71), coxsackievirus A16 (CV-A16), CV-A2, CV-A4, CV-A6 and CV-A10 are common causative agents of HFMD. Amongst these viruses, EV-A71, CV-A6 and CV-A16 are most frequently associated with HFMD in Malaysia [2–4]. Unlike other enteroviruses causing HFMD, EV-A71 can be associated with severe neurological complications, including aseptic meningitis, brainstem encephalitis and pulmonary edema with high fatality

rates [5]. To date, no vaccine is available for HFMD-associated enteroviruses other than EV-A71. Furthermore, the inactivated EV-A71 vaccine is currently only marketed in China.

EV-A71 and CV-A16 are positive-stranded RNA viruses under the family of *Picornaviridae* and the genus of *enterovirus*. The genomic RNA comprises a single open reading frame flanked by 5′ and 3′ untranslated regions. Translation of genomic RNA produces a single polypeptide which is further cleaved into structural capsid (VP1-VP4) and non-structural (2A–2C and 3A–3D) proteins [6]. Only VP1-3 capsid proteins are exposed, whereas VP4 is completely buried. Capsids are important for host receptor binding and can trigger host immune responses.

For EV-A71, both IgM and IgG from EV-A71-infected patients recognize all the structural proteins. Of these, the VP1 protein of EV-A71 is the most immunodominant viral protein recognized by HFMD patients [7]. Other than immunodominant binding epitopes, neutralization epitopes are usually located in the capsid surface-exposed loops, where neutralizing antibodies bind, leading to the inactivation of the virus. In other enteroviruses such as the poliovirus, all the neutralizing sites are located at the capsid loops, and recent studies showed that both the D antigen (infectious virus) and C antigen (non-infectious empty particles) are immunogenic [8–10]. In total, there are seven well-characterized loops, namely the VP1 BC, DE, EF, GH and HI loops, VP2 EF loop and VP3 GH loop (Figure 1).

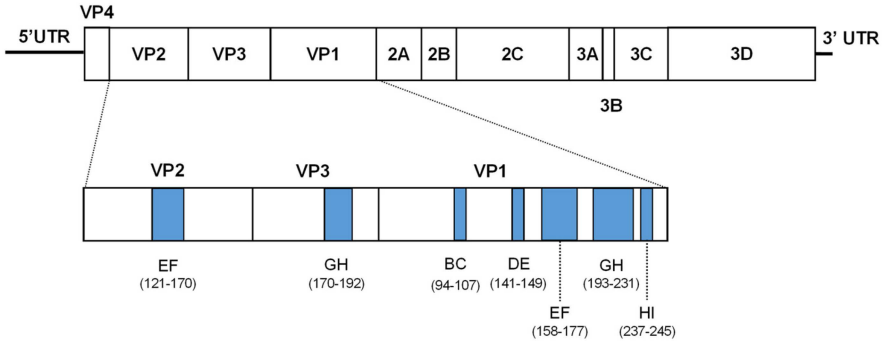

Figure 1. Schematic representation of EV-A71 genome and architecture of capsid loops. Structural protein consists of VP1–VP4. Blue-colored regions represent the location of each capsid loop constructed in this study, and the numbers indicate the amino acid position within each viral protein.

Most enterovirus-neutralizing epitopes are located within the capsid loops in the form of conformational or linear epitopes. Both EV-A71 and CV-A16 have some similar neutralizing epitopes at VP1 EF loop (SP55 of EV-A71 and PEP 55 of CV-A16) [7,11–13] and GH loop (SP70 of EV-A71 and PEP71 of CV-A16) [12,14]. The PEP27 spanning part of the VP1 DE loop (aa 142–156) was also reported as an IgM-neutralizing epitope of EV-A71 [7]. These previous studies also reported several other CV-A16 neutralizing epitopes within the capsid loops, such as aa 94–108 in the VP1 BC loop and aa 176–190 in the VP3 GH loop. However, these are linear epitopes, and the study on EV-A71 and CV-A16 conformational epitopes is limited and would benefit the future development of broad-spectrum HFMD vaccine design.

Reverse genetics have been used to engineer chimeric EV-A71 (ChiE71) to identify neutralization epitopes, antigenicity and virulence in other picornaviruses [14–16]. Previous studies have shown that poliovirus capsid loop engineered with HIV-1 gp41 epitopes could elicit neutralizing antibodies against HIV-1 [17]. Human poliovirus 3 which is one of the three serotypes of poliovirus could also tolerate insertion at the BC loop without affecting virus production [18]. We hypothesized that the capsid loops between enteroviruses can be exchanged and substitution of capsid loops of CV-A16 into EV-A71 will not alter the immunogenicity of EV-A71. In this study, chimeric EV-A71 (ChiE71) carrying capsid loops of CV-A16 were constructed and characterized. We further evaluated the homologous and heterologous immune responses and protection efficacy against ChiE71 in a mouse model.