Institut Pasteur
Microbes and Infection 17 (2015) 123–134
www.elsevier.com/locate/micinf

Advancements in the development of subunit influenza vaccines

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Received 12 November 2014; accepted 8 December 2014
Available online 18 December 2014

Abstract

The ongoing threat of influenza epidemics and pandemics has emphasized the importance of developing safe and effective vaccines against infections from divergent influenza viruses. In this review, we first introduce the structure and life cycle of influenza A viruses, describing major influenza A virus-caused pandemics. We then compare different types of influenza vaccines and discuss current advancements in the development of subunit influenza vaccines, particularly those based on nucleoprotein (NP), extracellular domain of matrix protein 2 (M2e) and hemagglutinin (HA) proteins. We also illustrate potential strategies for improving the efficacy of subunit influenza vaccines.

Keywords: Influenza A virus; Subunit vaccines; Nucleoprotein; Matrix protein; Hemagglutinin

1. Introduction

Influenza viruses belong to the family *Orthomyxoviridae*, consisting of five genera that include influenza A virus, influenza B virus, influenza C virus, Thogotovirus and Isavirus, as defined by the International Committee on Taxonomy of Viruses [1]. On the basis of their nucleoprotein (NP) and matrix (M) protein antigens, influenza A and B viruses contain hemagglutinin (HA) and neuraminidase (NA) activities, whereas influenza C virus has no NA, but does have a hemagglutinin–esterase fusion (HEF) protein [1]. Only influenza A and B viruses can cause clinical diseases. Influenza B viral infections are often limited to localized outbreaks, while influenza A virus is the primary pathogen for humans and is thus the principal cause of larger epidemics and pandemics.

Influenza A virus is further divided into different subtypes based on the antigenicity of the two surface glycoproteins, HA and NA. Currently, influenza A virus has 18 HA subtypes (H1–H18) and 11 NA subtypes (N1–N11) [2], theoretically leading to 198 potential combinations. In fact, not all of these subtypes cause human diseases. Seroarchaeology data from the late 19th and early 20th centuries indicated that the H1, H2 and H3 influenza virus subtypes were successfully transmitted among humans [3]. Particularly, the H5 subtype has threatened to emerge as a human pandemic pathogen since 1997, when it killed 6 out of 18 infected humans [3]. The H7 subtype is also worthy of concern because of the newly emerged 2013 avian influenza A/H7N9 pandemic in China [4]. The highly pathogenic H5 and H7 subtypes possess a unique ability to accumulate multiple basic amino acids at the HA cleavage site, increasing the ability of the viruses to spread systemically in an infected host and cause significant disease [5].

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http://dx.doi.org/10.1016/j.micinf.2014.12.006
1286-4579/

2. Influenza A virus structure and life cycle

The influenza A virus genome consists of eight negative sense, single-stranded RNA segments encoding eleven viral proteins which are essential for viral replication and packaging of progeny virus. Major viral proteins include structure proteins HA, NA, M1, M2 and NP; three RNA polymerases, such as polymerase basic protein 1 (PB1), PB2 and polymerase acidic protein (PA); as well as three non-structural proteins (NS) named NS1, NS2 (nuclear export protein, NEP) and PB1-F2 (Fig. 1) [6]. These proteins play significant roles in influenza virus replication, including their assistance in cell membrane recognition and endosomal fusion and acidification, inducing ribonucleoprotein (RNP) delivery into the nucleus, catalyzing polymerase holoenzyme of transcription and replication, and promoting protein and RNA binding and sialidase activity. Specific functions of these proteins are listed below.

Influenza virus M1 protein is important in RNP coating during viral assembly [7], while M2 protein is a transmembrane protein that forms an ion channel tetramer, exhibiting pH-inducible proton transport activity. During initial virus infection, M2 regulates the pH of viral core after virus uptake into the host cell's endosomal compartment, and at the late stage of infection, it transports viral transmembrane proteins to the cell surface [8]. HA glycoprotein forms spikes on the surface of virions, mediating attachment of the virus to host cell receptors, thereby enabling the virus to gain entry through membrane fusion. NA glycoprotein forms knob-like structures on the viral surface to catalyze progeny virus from infected cells, thereby allowing the virus to spread with resulting infection of new host cells. NP protein is a core antigen that plays an essential role in viral replication and transcription. NS2 protein may help catalyze the nuclear export of newly synthesized viral RNPs from nucleus to cytoplasm, where assembly of the progeny virions occurs [9].

The life cycle of influenza A virus involves the participation of major viral proteins in several important steps (Fig. 2). First, influenza virus infects host cells via HA-mediated binding to cell surface sialic acids, internalizing via receptor-mediated endocytosis. Then, the virus with proteolytically activated HA fuses with endosomal membrane through an acidic pH-promoted conformational change, followed by acidification of the viral core via M2 proton channel, leading to dissociation of the M1 coat protein and release of viral RNPs into the cytoplasm. Nuclear-localized signal on NP facilitates the transport of RNPs to the nucleus, where viral mRNA transcription and genomic replication occur. Once translated, NS1 protein binds to double-stranded RNA and host mRNA processing factors to inhibit cellular interferon-induced antiviral responses [10]. Subsequently, the polymerase performs host mRNA cap-recognition, providing capped mRNA primers for initiation of viral transcription [11]. Replicated RNPs, assisted by NEP, are then exported from the nucleus and transported to plasma membrane for assembly with envelope proteins (HA, NA, M1 and M2). Finally, NA protein plays a crucial role in removing sialic acid from sialyl oligosaccharides, thereby releasing newly assembled virions from the cell surface and preventing self-aggregation of the viral particles [6].

3. Influenza A virus-caused pandemics and the importance of developing effective influenza vaccines

Antigenic shift causes emergence of new influenza viruses, resulting in efficient human-to-human transmission and influenza pandemics. Influenza pandemics occur frequently, with three major outbreaks in the 20th century and two outbreaks in the 21st century, including the 2009 pandemic H1N1 and the newly emerged 2013 avian influenza A/H7N9 pandemic. In addition, the highly pathogenic avian influenza (HPAI) A/H5N1 with pandemic potential has caused serious diseases. This section introduces major pandemics caused by

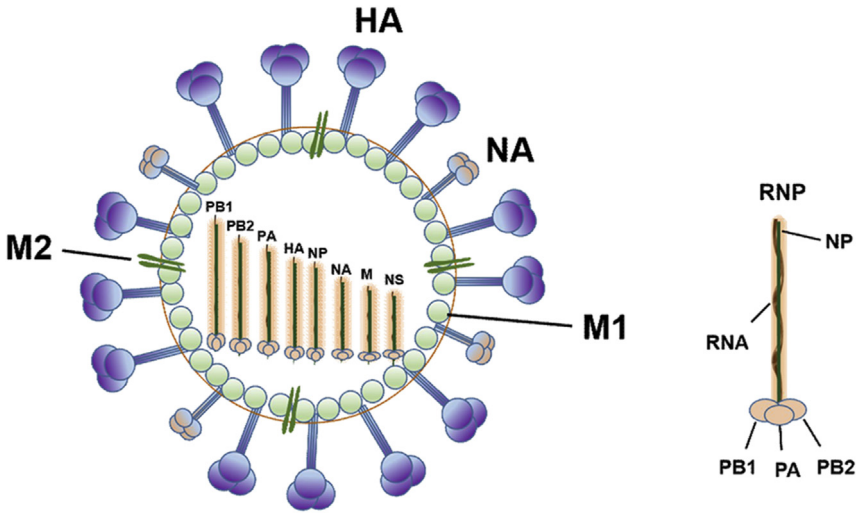

Fig. 1. Schematic diagram of the influenza A virus structure. The virus contains three trans-membrane proteins, including HA, NA, and M2 ion channel. The matrix protein M1 forms the protein layer beneath the lipid bilayer. Within the viral envelope is ribonucleoprotein (RNP) consisting of RNA segments associated with NP and the PA, PB1, and PB2 polymerase proteins.