Review

A Perspective on the Structural and Functional Constraints for Immune Evasion: Insights from Influenza Virus

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Abstract

Influenza virus evolves rapidly to constantly escape from natural immunity. Most humoral immune responses to influenza virus target the hemagglutinin (HA) glycoprotein, which is the major antigen on the surface of the virus. The HA is composed of a globular head domain for receptor binding and a stem domain for membrane fusion. The major antigenic sites of HA are located in the globular head subdomain, which is highly tolerant of amino acid substitutions and continual addition of glycosylation sites. Nonetheless, the evolution of the receptor-binding site and the stem region on HA is severely constrained by their functional roles in engaging the host receptor and in mediating membrane fusion, respectively. Here, we review how broadly neutralizing antibodies (bnAbs) exploit these evolutionary constraints to protect against diverse influenza strains. We also discuss the emerging role of other epitopes that are conserved only in subsets of viruses. This rapidly increasing knowledge of the evolutionary biology, immunology, structural biology, and virology of influenza virus is invaluable for development and design of more universal influenza vaccines and novel therapeutics.

Introduction

Influenza virus imposes a substantial health and socioeconomic burden globally [1]. There are four known types of influenza virus, named as A–D. Type A and B viruses circulate in human population and are responsible for pandemics (type A), epidemics and seasonal outbreaks (types A and B), while type C and the recently discovered type D [2] viruses do not cause significant disease or epidemics. Influenza A viruses are further classified into subtypes according to the antigenic properties of their two viral surface glycoproteins, namely the hemagglutinin (HA) and neuraminidase (NA). There are 18 known HA subtypes (H1 to H18) and 11 known NA subtypes (N1 to N11) [3]. The 18 HA subtypes can further be classified into group 1 (H1, H2, H5, H6, H7, H8, H9, H11, H12, H13, H16, H17, and H18) or group 2 (H3, H4, H7, H10, H14, and H15) [4]. Out of the 198 (11 × 18) possible combinations, only three (H1N1, H2N2, H3N2) are known to have caused human pandemics. The main natural reservoir for influenza A viruses is wild aquatic birds, but domestic poultry also become infected and hence harbor influenza A viruses [5,6]. Pigs and other mammals, such as horses, dogs, seals, minks, and bats [7], can also be infected by influenza A viruses and contribute to possible sources of viruses that infect humans [5]. Certain subtypes found in natural reservoirs occasionally emerge in the human population, as exemplified by recent H5N1, H5N6, H6N1, H7N7, H7N9, H9N2, and H10N8 viruses. Some of these zoonotic subtypes can be highly pathogenic and have a high mortality rate (>50% of hospitalized individuals) when infecting humans [8,9].

Influenza has been a long-term threat to humans, and the first major pandemic that was documented was that of the 1918 H1N1 Spanish flu that was responsible for more than 50 million deaths worldwide [10,11]. Since then, there have been three pandemics, namely the Asian flu (H2N2) pandemic in 1957, the Hong Kong flu (H3N2) pandemic in 1968, and the most recent swine flu (H1N1)

pandemic in 2009. Over the past 5 decades, annual (seasonal) outbreaks have been caused by influenza A H1N1 and H3N2 subtypes and the two lineages of influenza B virus (B/Victoria/2/87 and B/Yamagata/16/88). As compared to influenza B, influenza A generally results in higher morbidity and mortality [12,13]. In addition, influenza A viruses evolve three times faster than influenza B viruses [14,15]. Therefore, influenza A viruses have often received more attention and concern as a global threat compared to influenza B viruses [16]. Of note, the quadrivalent influenza vaccine that is recently licensed in many countries [17] now offers protection against both lineages of influenza B virus and the two influenza A subtypes [18].

Among all influenza virus proteins, HA evolves at the highest rate [19,20] due to it being the major target of the immune response. Phylogenetic analysis suggests that different HA subtypes of influenza A virus diverged around 2000 years ago [21]. Although the protein sequences of their HAs share as low as 40% sequence identity, they adopt the same protein fold [22]. As a class I viral fusion protein, HA plays an important role for viral entry by binding to the host receptor, sialylated glycans on endothelial cells in the respiratory tract, and by facilitating membrane fusion in the low pH environment of the endosomal compartments after cell entry via endocytosis. During virus replication, the uncleaved precursor of the HA, namely HA0, is synthesized and then cleaved by cellular proteases into two subunits, HA1 and HA2, to produce the fully functional form of the protein [23]. Although this cleavage is usually catalyzed by trypsin-like serine endoproteases [24,25], HAs from highly pathogenic H5 and H7 subtypes that contain a polybasic cleavage site can also be cleaved by the ubiquitous protease furin [26–28]. This maturation process is a prerequisite to attain the fusion-competent, metastable form of the HA that undergoes the large conformational rearrangements required for the membrane fusion process.

While a large portion of the HA1 amino acid sequence is highly variable [22] and intrinsically tolerable to mutations, the receptor-binding site (RBS) is an exception [29,30]. The HA RBS is composed of the 130-loop, 150-loop, 190-helix, and 220-loop, named after their relative positions on the HA amino acid sequence. The stem region, which is composed primarily of HA2 with some residues from the N and C termini of HA1, is even more conserved [22]. During the membrane fusion process that is triggered by the acidic pH in the endosomal compartments [31–33], the α-helices in stem region [34] undergo large conformational rearrangements to form a 100-Å triple-helical coiled coil before collapsing to its 6-helix bundle fusion conformation [35]. This molecular machine consists of many moving parts that impose strong evolutionary constraints on many residues in the stem region.

Influenza H1N1 and H3N2 viruses entered the human population in 1918 and 1968, respectively [36]. While the seasonal H3N2 virus continues to circulate until the present day, the history of human H1N1 virus is more complex [36,37]. In the last century, the human H1N1 virus first appeared in 1918 but discontinued circulating in human population in 1957 for around 20 years. It remerged in 1977 as a relatively benign epidemic and continued to circulate as a seasonal virus until 2009, when the pandemic swine flu [A(H1N1)pdm09] emerged and displaced the seasonal H1N1 virus. Of note, HAs from both seasonal H1N1 virus and A(H1N1)pdm09 are derived from the HA of 1918 Spanish flu, while the other genes have different origins [38]. Nonetheless, the seasonal H1N1 virus had mutated so much that it was antigenically very distant from both the 1918 Spanish flu and the A(H1N1)pdm09. Thus, when the A(H1N1)pdm09 emerged, immunity was lacking in the younger to middle-aged population [39]. Despite the difference in circulation history between H1N1 and H3N2 subtypes in human population, both subtypes are subjected to continual pressure to escape from the human immune system.

In this review, we will discuss the interplay among immune evasion associated with influenza virus, the countermeasures offered by the humoral immune system to combat ongoing variation in influenza viruses, and the requirement for the virus to maintain function throughout this complex evolutionary process. We will mostly focus on the HA protein due to its role as the main antigen of influenza virus and the major seasonal vaccine target, but we will also touch on the emerging role of anti-NA antibodies. Throughout this review, all residues on HA are named according to H3 numbering.

Antigenic Drift: Point Mutations and Glycosylation

Most antibodies elicited by influenza virus by natural infection and vaccination target the globular head domain of HA1, which is distal from the virus surface and readily accessible for immune recognition. Influenza virus HA, in turn, mutates to escape from preexisting immunity. This mutation-based immune evasion process is known as antigenic drift. Early studies proposed five major antigenic sites in the HA1 globular head domain for both H1 and H3 HAs [40–44], namely Sa, Sb, Ca1, Ca2, and Cb for H1 HA [40,41] (Fig. 1a), and sites A–E for H3 HA [42–44] (Fig. 1b). The locations of Sa, Sb, and Ca2 of H1 HA, and antigenic sites A and B of H3 HA partially overlap with the RBS, whereas Ca1 and Cb of H1 HA, and antigenic sites C–E of H3 HA are more distant from the RBS. These antigenic sites provide a structural framework to understand the evolutionary dynamics and constraints of influenza virus in response to humoral immunity.