Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Immunology

Eliciting broadly protective antibody responses against influenza

Greg A Kirchenbaum and Ted M Ross

Antibodies recognizing the hemagglutinin (HA) protein, which are elicited following infection or vaccination, confer protection against influenza virus infection. Although annual seasonal influenza vaccines provide some protection against currently circulating influenza strains, they lack efficacy against viruses expressing divergent globular head domains of HA. Moreover, antigenic drift within the globular head of circulating viruses necessitates frequent reformulation of the seasonal vaccine, a process that is both expensive and time-consuming. In this regard, vaccine strategies that generate antibodies with reactivity against an array of influenza viral strains could reduce the need for yearly influenza vaccination and increase our preparedness for potential pandemics. In this review, recent progress toward the generation of an influenza vaccine capable of eliciting hemagglutinin specific and broadly protective antibody responses is summarized.

Addresses
Vaccine and Viral Immunity Department, Vaccine and Gene Therapy Institute of Florida, Port St. Lucie, FL, USA

Corresponding author: Ross, Ted M (tross@vgtifl.org)

Current Opinion in Immunology 2014, **28**:71–76

This review comes from a themed issue on **Vaccines**

Edited by **Jay Kolls** and **Shabaana Khader**

0952-7915/$ – see front matter,

http://dx.doi.org/10.1016/j.coi.2014.02.005

Introduction

Influenza viruses are members of the *Orthomyxoviridae* family and have a negative-sense single-strained segmented RNA genome. They are further classified as A, B or C viruses [1]. At present, 17 antigenically unique influenza A hemagglutinin (HA) subtypes have been described, which can be further divided into two main phylogenetic groups based on HA amino acid sequence. Group #1 influenza A viruses (IAV) express HA belonging to the H1, H2, H5, H6, H8, H9, H11, H12, H13, H16 and H17 subtypes, whereas group #2 IAV express HA belonging to the H3, H4, H7, H10, H14 and H15 subtypes [1,2]. However, only a limited number of group #1 (H1, H2, H5, H6 and H9) and group #2 (H3 and H7) IAV viruses have been implicated with human infection [3,4]. Moreover, human influenza B viruses have been categorized into 2 lineages (Yamagata-like and Victoria-like) since 1987 [1].

Prophylactic influenza vaccination provides a significant benefit through reducing disease severity and transmission in the population [5]. Although seasonal influenza vaccines generate an antibody response, the antibodies are focused on the globular head domain of HA and therefore are subject to evasion by viral isolates that have modified their targeted epitopes through mutation [1]. Accumulation of mutations in the HA and neuraminidase (NA) genes, the major surface proteins on the influenza virus, is termed antigenic drift and reduces or eliminates the binding of pre-existing antibodies through altering amino acid sequences that comprise immunodominant epitopes [6].

In addition to antigenic drift, IAV also have the potential for antigenic shifts in which a novel HA subtype circulating in the swine or avian reservoirs reassorts with a human influenza strain and is re-introduced into the human population. Antigenic shift events pose a significant threat to influenza pandemic because seasonal vaccination does not generate cross-protective antibodies, and consequently the general population would be immunologically naïve [7].

Hemagglutinin remains a viable target for broadly protective immunity

The HA glycoprotein exists on the surface of the influenza virion as a trimer and mediates cell attachment and subsequent membrane fusion. Each HA monomer consists of two disulfide-linked polypeptides derived from the precursor HA0 protein, HA1 and HA2, which encode the globular head domain and fusion peptide [1]. The membrane distal component of the HA1 subunit mediates virus attachment following an interaction between the receptor-binding site of HA and sialic acids on the host cell surface [8]. The membrane proximal HA2 subunit encodes the fusion peptide and undergoes a pH-dependent conformational change that facilitates release of the influenza genome into the cytosol [1]. Antibodies specific for the globular head of HA have long been appreciated for their ability to neutralize the virus, and can be detected by the hemagglutination inhibition (HI) assay [9]. By contrast, antibodies specific for the stalk domain of HA have only recently been recognized for their protective capacity [10–12,13•].

Antibodies that recognize conserved epitopes present within either the HA1 or HA2 domains significantly reduce morbidity and mortality *in vivo* [14–17,18•,19••]. By contrast, antibodies directed at more conserved influenza proteins such matrix (M2e) or nucleoprotein (NP) were not as protective following a high dose influenza challenge [20]. Although these approaches and many other approaches have merit, several research groups,

including ours, continue efforts toward the generation of an influenza vaccine capable of eliciting a broadly reactive HA-specific antibody response that protects against both drifted seasonal and novel pandemic influenza strains. In the following sections, we review recent findings pertaining to the generation of a broadly protective influenza vaccine targeting the HA protein.

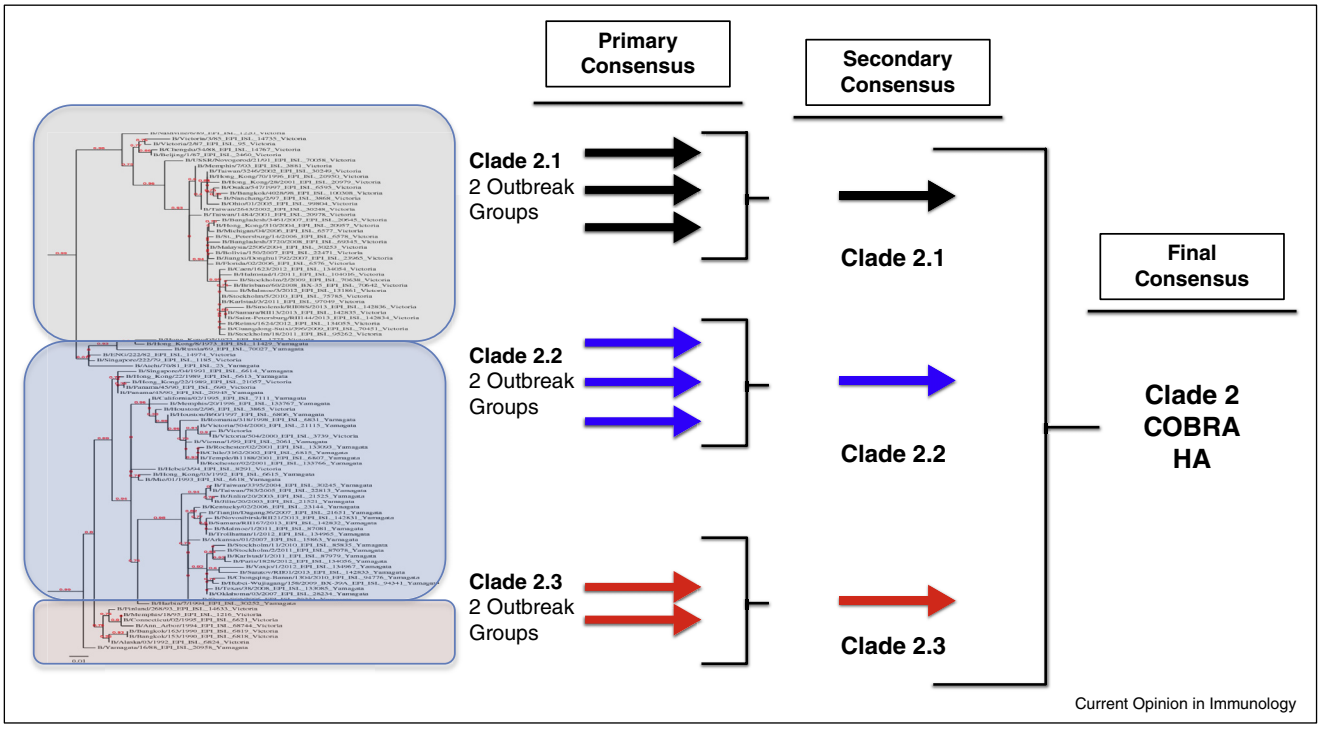
Consensus sequence designed hemagglutinin

Consensus sequences encode the most common nucleotide or amino acid at each position of a gene or protein. Whereas ancestral or center-of-the tree (COT) sequences approximate a sequence that existed in the past, consensus sequence generation captures a sequence that is more relevant to a current epidemic [21]. Moreover, consensus sequence-based vaccines have been investigated as a strategy for eliciting broadly reactive immune responses against a variety of viruses including HIV [21], chikungunya [22] and influenza viruses [23–26,27•].

We and others have previously demonstrated protection against lethal influenza A challenge following vaccination with consensus sequence designed HA [24–26,27•, 28,29•,30]. Specifically, we sought to determine whether a consensus sequence designed H5 would be capable of eliciting antibodies that recognized viral isolates belonging to divergent clades or sub-clades. The H5N1 virus was chosen as a proof-of-principle model system for the establishment of the computationally optimized broadly reactive antigen (COBRA) approach because the HA encoded by these viruses display large antigenic differences [31]. Despite high levels of protein identity (>90%) between the various H5 proteins, receptor-blocking antibodies elicited following vaccination or infection do not exhibit cross-reactivity. As result, H5N1 influenza viruses are further categorized into clades, subclades and even sub-subclades.

In order to generate an H5 HA protein that elicited protective antibodies against H5N1 isolates belonging to divergent subclades/clades, and to avoid bias occurring as result of sampling or outbreak dominance, we utilized a novel centralized sequence generation approach involving sequential rounds of consensus sequence generation (Figure 1) [27•]. H5 sequences representing clade 2 viruses isolated from human infection (n = 129 unique HA sequences) from 2004 to 2006 were first grouped according to their phylogenetic subclade and then further segregated into individual outbreak groups. The amino acid sequences of each individual outbreak group were aligned and the most common amino acid at each position determined. These consensus outbreak sequences for each subclade were then used to perform a second round

Figure 1



Schematic to describe how the COBRA H5 molecule was designed. The phylogenetic tree was inferred from hemagglutinin amino acid sequences using the maximum likelihood method and clade/sub-clade groupings were identified. Primary consensus sequences were generated for each outbreak group. Secondary consensus sequences were then generated for each subclade using the primary sequences as input. The secondary consensus sequences were then aligned and the resulting consensus, designated COBRA, was generated.