Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Virology

Influenza virus antigenicity and broadly neutralizing epitopes

Gillian M Air

A vaccine formulation that would be effective against all strains of influenza virus has long been a goal of vaccine developers, but antibodies after infection or vaccination were seen to be strain specific and there was little evidence of cross-reactive antibodies that neutralized across subtypes. Recently a number of broadly neutralizing monoclonal antibodies have been characterized. This review describes the different classes of broadly neutralizing antibodies and discusses the potential of their therapeutic use or for design of immunogens that induce a high proportion of broadly neutralizing antibodies.

Address
Department of Biochemistry & Molecular Biology, University of Oklahoma Health Sciences Center, PO Box 26901, Oklahoma City, OK 73126-0901, United States

Corresponding author: Air, Gillian M (Gillian-air@ouhsc.edu)

Current Opinion in Virology 2015, **11**:113–121

This review comes from a themed issue on **Preventive and therapeutic vaccines (B Cell Epitope Vaccine)**

Edited by **Mansun Law**

http://dx.doi.org/10.1016/j.coviro.2015.03.006

Introduction

Influenza vaccines have been used since the 1940s. They are safe but need to be multivalent to protect against the multiple circulating viruses, and the components need to be updated nearly every year in response to mutations of the virus. The holy grail for influenza vaccine would be a single formulation that cross-protects against all current and future strains. Recent discoveries of cross-reactive monoclonal antibodies have given hope that a universal influenza vaccine may be possible.

This review covers recent work (approximately 2009–2014) to characterize neutralizing antibodies against influenza with emphasis on those that show some level of cross-reactivity between different subtypes.

Early observations

Human influenza virus was first isolated in 1933. Memories of the devastating death toll of the 1918–1919 epidemic fuelled efforts to develop a vaccine, spurred even more by the advent of the Second World War. By 1936 it had been recognized that influenza viruses are antigenically diverse. Methods to inactivate the virus with formalin overcame the inherent safety concerns of live virus vaccines and the vaccine given to troops in World War II was trivalent, containing A/PR/8/34, A/Weiss/43, and B/Lee/40. This vaccine was shown to provide protection against type A and B viruses until 1947, when it dramatically failed. The 1947 viruses were originally classified as 'A prime' but eventually were grouped into the H1N1 subtype, despite the marked change in antigenic properties. By 1954 there were two fundamental questions on antigenic variation [1]. One was whether the virus mutates in response to environment (such as infection of a new host, or presence of antibodies), versus the ideas of G.K. Hirst and J.Y. Sugg that a pre-existing variant is selected out by environmental pressure. The second question was whether there are a limited number of variants of influenza virus that wax and wane in the human population (J. Salk, T. Francis), or whether the virus is continually changing (F.L. Horsfall, F.M. Burnet). A finite number of variants would imply that a vaccine containing all of them would be effective. Unfortunately this is not the case, and we now know that influenza evolves linearly by selection of escape mutants, usually by antibodies, from a small population of variants generated by random mutation from the preceding virus. This means that development of a universal influenza vaccine requires a strategy other than including all known strains.

Antigenic drift and shift, neutralizing antigens, current vaccine strategies

Influenza viruses are classified by serological cross-reactivity, or lack thereof. Types A, B and C do not cross-react by any serological test. Type A viruses all share cross-reactivity of internal proteins, nucleoprotein (NP) and matrix (M1), but the surface glycoproteins hemagglutinin (HA, or H) and neuraminidase (NA or N) are divided into serological subtypes H1–H16 and N1–N9 that do not cross-react with serum antibodies. Only H1, H2 and H3 with N1 or N2 circulate in the human population. Recent influenza sequences from bats proposed as H17, H18, N10 and N11 have functionally different glycoproteins and the viruses have not yet been isolated [2]. A new subtype entering the human population is described as antigenic shift, such as when H2N2 viruses replaced H1N1 in 1957 and H3N2 replaced H2N2 in 1968. Antigenic shift is facilitated by the large variety of influenza viruses in bird populations and by the segmented nature

of the genome that allows reassortment of genes in a mixed infection. Following antigenic shift, the new virus undergoes progressive changes due to antibody selection, known as antigenic drift.

All the genes of influenza virus undergo some degree of variation, all occurring by the same basic mechanism. Influenza has an RNA genome that codes for its own RNA polymerase. RNA polymerases in general lack the editing feature of DNA polymerases, an exonuclease domain that removes a mismatched 3′ nucleotide before elongation can continue. Without this exonuclease activity, the intrinsic error rate of RNA polymerases is relatively high. Most of the resulting variants are lost in the population, but a few may be fixed by chance ('random drift'). Some mutations are positively selected, for example, to escape from antibody neutralization or for more efficient replication or better interaction with a specific host protein, and these variants rapidly take over the population because they confer an advantage. Changes in proteins selected for improved function may change their antigenic properties. There is a marked distinction between antigenic selection (resistance to the immune system) and antigenic change that is a consequence of some other selective pressure.

Definitions of 'neutralizing' and 'epitope'

Protection against influenza is mediated by innate systems and by T cells and antibodies. The relative roles of these vary with the patient's genetic profile and history of influenza, but overall the most important contribution to protection is from neutralizing antibodies, and current vaccines are measured by their power to induce neutralizing antibodies. The classical definition of 'neutralizing' is to block the ability of a virus to attach to a cell; that is, to block the first step in viral infection. Such antibodies sterically interfere with the receptor-binding site on the hemagglutinin (HA) so it cannot bind to its sialic acid receptor on the cell surface. However, there are antibodies that interfere in infection at later stages. Although these do not meet the classical definition, they effectively neutralize the infection and in laboratory studies are equally protective.

What are the targets of neutralizing antibodies that do not block attachment to sialic acid? Although antibodies can be raised that block various viral activities (RNA-dependent RNA polymerase, assembly of nucleoprotein complexes or of the viral matrix protein), these antibodies are not neutralizing or protective because they cannot access their targets during the normal course of infection. Neutralization targets are those outside the viral membrane and so exposed on the virus; the surface proteins HA, neuraminidase (NA) and M2 ion channel. The HA's first function is to bind sialic acid receptors, but after internalization, in the low pH environment of the endosome, the HA undergoes a conformational change to enable a fusion activity that allows release of the viral genome. So antibodies that efficiently block either binding to receptor, conformational change or the fusion function will also be neutralizing.

Antibodies can only neutralize if they block a function. It follows that they bind to native proteins. Typically they bind to multiple segments of the polypeptide chain that may be dispersed in the primary sequence but come together in the three-dimensional structure. Therefore most neutralizing antibodies bind to a so-called 'conformational' epitope, that is lost if the protein is denatured or even partially unfolded.

Mapping antibody epitopes

For this review, an epitope is defined as the amino acids on the antigen that make contact with antibody. Some of the interactions of antibody with antigen are more important than others. The crucial contacts, with highest interaction energy, can be identified by selection of escape mutants, or by exhaustive mutagenesis, or by hydrogen exchange methods. These studies only give a partial view of the epitope, albeit the most energetically important view. Full descriptions of epitopes can be made from X-ray crystal structures of the antigen–antibody complex; this is currently the only available method that shows all the atomic interactions between antigen and antibody. An excellent discussion of broadly neutralizing epitopes characterized by X-ray crystallography is given by Lee and Wilson [3•].

How polyclonal is human serum?

Much of our knowledge of neutralizing epitopes comes from studies with monoclonal antibodies (mAbs), that allow selection of escape mutants that can be attributed to a single selecting antibody. A monoclonal antibody also allows complexes of antigen and antibody to be crystallized for structural analysis. The human response is, of course, polyclonal, raising questions about the applicability of monoclonal studies (usually mouse) to human protection. Mouse monoclonal antibodies were used to map broad antigenic regions on the HA of H3N2 and H1N1 viruses by using competition assays and cross-reactivities of the mAbs with escape mutants selected by other mAbs. Five sites were found on the H3 HA (sites A through E, [4,5••]) and four on H1 HA (Sa, Sb, Ca, Cb, [6]). An antibody that recognizes a change in Site A, for example, did not recognize changes in sites B–E. The deduction is that changes in all antigenic sites would be needed for a virus to escape the human immune system and begin a new epidemic. For some antigenic drift strains of H3N2 viruses this was the case, but more commonly, especially more recently, only one or two changes are found between epidemic viruses. It has been noted for some time that human H3N2 viruses show a changing pattern of immunodominance and it appears that for any given virus, the human response does not