Atypical antibody responses to influenza

Dalton Hermans¹, Richard J. Webby², Sook-San Wong²

¹Department of Pediatrics, University of Wisconsin-Madison, Madison, WI 53715, USA; ²Department of Infectious Diseases, St. Jude Children’s Research Hospital, Memphis, TN 38105, USA

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Correspondence to: Sook-San Wong, PhD. Department of Infectious Diseases, St. Jude Children’s Research Hospital, 262 Danny Thomas Pl, Memphis, TN 38105, USA. Email: sook-san.wong@stjude.org.

Abstract: Influenza viruses undergo rapid antigenic evolution and reassortment, resulting in annual epidemics and the occasional pandemics. Exposure to influenza virus hemagglutinin (HA) and neuraminidase (NA) antigen, either through vaccination or infection, induces an antibody response able to recognize only the homologous antigenic subtype. However, atypical antibody responses recognizing non-homologous influenza subtypes have been reported during infection and vaccination. Here, we review the incidence of these phenomena in published literature and discuss the potential mechanisms underlying them.

Keywords: Influenza; antibody; heterosubtypic; neutralizing


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Introduction

Influenza virus is an RNA virus that circulates commonly among humans, causing acute respiratory infections. Each year it is estimated that three to five million people are infected with influenza virus, causing a substantial health burden and economic impact through loss of productivity (1). The young and the elderly are particularly susceptible to severe disease (2). Additionally, completely new antigenic strains can emerge from animal reservoirs through reassortment of the segmented viral genome to cause influenza pandemics (3). For these reasons, influenza viruses pose a constant and significant public health threat.

Antigenic classification of influenza viruses

Influenza viruses belong to the family Orthomyxoviridae and can be classified into four distinct types: influenza A, influenza B, influenza C and the newly identified ( provisionally named) influenza D (4,5). Although Influenza A, B, and C viruses commonly circulate and cause disease in humans, only Influenza A and B are of significant concern [influenza C is usually only associated with mild respiratory infections in children (6)]. Due to their ability to rapidly evolve, influenza A and B viruses undergo antigenic drifts to cause annual seasonal epidemics. This, along with the specificity of the induced antibody response, necessitates annual influenza vaccination against these seasonal influenza viruses.

Influenza A virus (IAV) has been the cause of some of the most devastating infectious outbreaks in history (7). Aquatic birds are the natural reservoir of most, if not all, IAV and it is from this reservoir that viruses sporadically infect other hosts, sometimes establishing stable lineages within the new host species. IAV is classified into distinct subtypes based on its two major surface glycoproteins: hemagglutinin (HA) and neuraminidase (NA). The combination of HA and NA subtypes form the diverse strains of IAV and are the major antigenic targets of the host humoral immunity. HA is the most abundant protein on the virus surface and is responsible for binding the cellular receptor and mediating entry into the host cell (8). It is a tetrameric protein and each monomer contains a globular head and a stalk domain (9) (Figure 1A). Though the viral functionality of
the HA protein is conserved, it can be phylogenetically and antigenically distinguished into multiple different subtypes. Currently, there are 18 subtypes of IAV HA (H1–H18) and 11 subtypes of IAV NA (N1–N11) identified, although the newest identified subtypes, H17, H18, N10 and N11 are of bat-origins (10). The IAV HA proteins are subclassified into two groups based on phylogenetic similarities: group 1 consists of H1, H2, H5, H6, H8, H9, H11, H12, H13, H16, H17 and H18 while group 2 consists of H3, H4, H7, H10, H14 and H15 (Figure 1B).

Aside from genetic and antigenic classification, the HA proteins can also be distinguished based on their receptor binding preference. Human strains of influenza virus recognizes the α(2,6)-linked sialic acid on host cells while avian strains preferentially binds the α(2,3)-linked sialic acid. Some swine-origin influenza viruses can also recognize both moieties (11,12).

The globular head of the HA molecule contains the receptor binding site where the virus attaches to sialic acid on the surface of cells to initiate infection. As the main mechanism of host invasion and the most protrusive molecule on the virus, the head is the most targeted region by the humoral immune response (13). As a result of this immune pressure, the globular head region has the highest mutation rates of all viral proteins, helping evade antibodies targeting it (antigenic drift) (14). Due to antigenic distinction across the various subtypes of HA, the serological response against one subtype typically does not confer reactivity against another (15). In contrast, the HA stalk region is more conserved and antibodies targeting this region are often capable of neutralizing influenza viruses from different IAV subtypes within the same phylogenetic group, and less commonly across groups 1 and 2 (Figure 1B) (16,17). Highlighting the conserved nature of stalk epitopes, one antibody, CR9114, has been identified that binds to the stalk region of influenza A and B (18). These cross-reactive antibodies, termed broadly neutralizing antibodies, are the subject of intense research as they represent a strategy to counter the threat of a diverse and highly mutable virus. For an up-to-date review of broadly neutralizing influenza antibodies and its mechanisms of action, see Corti et al. (17,19).

The B-cell responses to influenza virus exposure

Infection with any pathogen elicits an innate followed by an adaptive immune response. The adaptive immune response is mediated primarily by lymphocytes recognizing antigens, or more specifically, epitopes specific to the infecting pathogens. During a primary infection, where an antigen is
head of the HA. These antibodies are capable of neutralizing only the immunizing antigen and other antigenically similar viruses, likely of the same HA subtype (homosubtypic seroconversion). However, in recent years, atypical antibody responses have been documented where more cross reactive profiles have been observed. In this review, we focus on the phenomena of atypical antibody responses after influenza exposures that result in seroconversion to a wider range of HAs. This includes induction of antibody responses to virus subtype that are different from the immunizing antigen (heterosubtypic seroconversion). The focus of this review is strictly on the serological response, and no other forms of heterotypic immunity such as that afforded by T-cells or other less antigenically-specific immune mechanisms. More specifically, we will consider heterosubtypic antibody responses as measured by a four-fold increase in antibody titer (seroconversion) to a non-infecting/exposed strain when measured by hemagglutination-inhibition assay (HAI) or neutralization assay.

**Heterosubtypic antibody responses after infection**

We became interested in this phenomenon after conducting a serological study on patients enrolled through a longitudinal-observational cohort study that was based at the Le Bonheur Hospital in Memphis, Tennessee (25). This cohort was initiated after the 2009 pandemic, and enrolled more than 300 participants over 5 influenza seasons. In this study, patients were tested for influenza positivity and subsequently subtyped by polymerase-chain-reaction (PCR). Serological testing was performed against H1N1, H3N2 and influenza B strains that were likely to be circulating in the Northern Hemisphere for that particular season. Our data revealed a small subset of individuals that showed an atypical antibody response (Figure 2) by seroconverting to more than one influenza subtype after infection. Although this heterosubtypic seroconversion first caught our attention after the 2009 pandemic, we noted that it occurred at varying degrees over the next 4 influenza seasons (Figure 2). A review of published literature revealed that this phenomenon had also been observed by others, albeit in very limited studies (26–28). The first of these studies were reported by Baz et al. (26) in a Canadian cohort during the 2009 H1N1 pandemic. They reported that 8 out of 67 (12%) individuals infected with the 2009 pandemic strain A(H1N1)pdm also seroconverted to the seasonal A/Brisbane/59/2007 (H1N1) strain, which was antigenically distinct from A(H1N1)pdm. More intriguingly however,
5 individuals that were positive by PCR and serology to A(H1N1)pdm also seroconverted to the seasonal H3N2 strain, A/Panama/2007/99. Similarly, a seroepidemiological study of antibody responses to A(H1N1)pdm in a Singaporean cohort identified 20%, 18% and 16% of the A(H1N1)pdm—confirmed cases (N=45) that seroconverted to antigenically-distinct A/Brisbane/59/2007 (H1N1), A/Brisbane/10/2007 (H3N2), and A/Wisconsin/15/2009 (H3N2), respectively (27). The authors also drew attention to a similar observation made in Singapore during the 1968 H3N2 pandemic, whereby at least 4 adults showed concomitant HAI-seroconversion to the newly emerged pandemic strain A/Hong Kong/1/1968 (H3N2) and the circulating A/Singapore/1/1957 (H2N2) strain (28).

**Heterosubtypic antibody response after vaccination**

Atypical antibody responses have also been reported in vaccine trials. For example, in the early vaccine trials of an A/Viet Nam/1203/2005 (H5N1)-based vaccine, 15 participants (3% of entire cohort) had positive H5 HAI-antibody titers at baseline (29). As the study was conducted in United States, it was highly unlikely that these individuals have been exposed to the H5N1 strain that was circulating only in Asia at that time.

How then do these individuals have H5N1 antibodies, particularly those that target the globular head, without prior exposure? Cross-reactive neutralizing antibodies have been shown to exist at low levels in pre-immune human serum (30) and can be boosted after vaccination with influenza strains possessing a divergent globular head (17,31-33). For example, during vaccine trials with an A(H1N1)pdm monovalent inactivated vaccine, some recipients were shown to seroconvert to the antigenically distinct seasonal A(H1N1) strains. However, those that were vaccinated with seasonal A(H1N1) did not have any
Co-infections or an immunological phenomenon?

The fact that all infection-associated heterosubtypic seroconversion events were reported during a pandemic raises an interesting question: are heterosubtypic seroconversions a consequence of dramatic antigenic shift or merely undetected incidences of co-infections? In the published studies, circulation of seasonal influenza strains was negligible, or absent altogether during the peak of the pandemic activity (Figure 3), suggesting that co-infection was unlikely. However, co-infection of a single individual with A(H1N1)pdm and seasonal influenza strains did occur during the 2009 pandemic (37,38). Historically, the most striking epidemiological support for co-infections was the emergence of the H1N2 virus that had limited global spread between 2000 and 2003 [reviewed in (39)]. This reassortant virus was generated from an A/Moscow/10/1999 (H3N2)-like virus that had acquired the HA from an A/New Caledonia/20/1999 (H1N1)-like virus (40).

Analysis of influenza subtype circulation in the United States between 2009 and 2016, suggests that a single subtype typically dominates at greater than 80% prevalence during peak activity in a typical influenza (non-pandemic) season. The exception to this was in the year after the pandemic (the 2010–2011 influenza season) where both the A(H1N1)pdm and H3N2 subtypes were detected at almost similar proportions (Figure 3). Thus, epidemiological data at the population level suggests that co-circulation of multiple subtypes certainly can occur during some influenza seasons.

How often then, are co-infections within an individual detected? Review of the few published manuscripts suggests that co-infections by different influenza subtypes are only rarely detected; studies by Perez-Garcia and Falchi reported a 1.6% and 3.2% co-infection rates respectively (41,42). The highest co-infection rate (7.3%) was reported by Goka et al., from over 25,000 respiratory samples analyzed over a period of 4 years (43). While these data suggest that co-infection can be a plausible explanation for heterosubtypic seroconversion, the lack of a reliable estimate of its occurrence and any systematic study to link this precludes any conclusive association. Furthermore, co-infection will not explain the observation in the study by Yin-Murphy (28), whereby seroconversion was detected against a strain that was no longer in circulation.

Original antigenic sin

The possibility that these heterosubtypic seroconversions may be a result of an immunological phenomenon should be considered. It is now evident that the antibody response to influenza is complex due to the influence of the host memory response and the antigenic variability in the HA. The ability to produce HA stalk-reactive antibodies in the absence of a highly similar globular head has been attributed to the effects of original antigenic sin (OAS) (17). OAS, first coined by Thomas Francis (44), refers to the phenomenon in which, following exposure via vaccination or infection to a virus that is antigenically similar to a previously encountered strain, the body will preferentially recall the originally encountered memory B-cell clones, resulting in an increase in antibody response to the original antigen.

Two recent studies have provided intriguing new insights into the effects of OAS after influenza vaccination. In the first, Huang et al. showed that although the induced antibody response upon influenza vaccination is polyclonal, a majority of these antibody clones recognized epitopes that were common to the strain that the host was most likely to have been first exposed to (45). The authors proposed that OAS was the reason why the donor made antibodies that failed to neutralize recently emerged strains that possess a single major mutation that had evaded neutralization. In a similar vein, the second study by Schmidt et al. showed the germline B-cell precursor, the “unmutated common ancestors” (UCA) of six clonal lineage of broadly neutralizing antibodies produced antibodies that recognized strains that the host would most likely have been exposed to. Upon vaccination however, the increased breadth of reactivity was due to clonal proliferation and diversification of the original clone (46). These two studies illustrate how the memory B-cell response can impact the diversity and breadth of antibody response upon re-exposure to influenza virus antigen.

However, while OAS effects can result in a misdirected
Figure 3 Annual prevalence of influenza viruses, by subtypes, in the United States between 2009 and 2016. With the exception of 2009/2010 and 2010/2011 influenza season, a single subtype typically dominates during peak influenza activity period. During the 2009/2010 season, only A(H1N1)pdm09 circulated while in 2010/2011, both A(H1N1)pdm and H3N2 subtypes were detected at similar proportions. This suggests that co-infections with multiple influenza virus subtypes can occur at low frequencies. Data was retrieved from World Health Organization's (WHO)'s Global Influenza Surveillance and Response System (GISRS) online reporting portal, FluNet (http://www.who.int/influenza/gisrs_laboratory/flunet/en/).
antibody response during infection or vaccination, it cannot yet account for the heterosubtypic seroconversion against multiple antigenically distinct subtypes observed after infection.

**Broad B-cell repertoire or polyreactive antibodies?**

That certain individuals can produce significant titers against cross-reactive epitopes suggests that these individuals, for some unknown reason, may have a broader B-cell repertoire than normal. Intriguingly, researches in the broadly neutralizing antibodies field have shown that the ability to mount these broadly neutralizing antibodies was also linked to a host-genetic component. For example, generation of the Group 1-broadly neutralizing antibodies often involve somatic hypermutation and usage of the Ig heavy-chain variable region VH1-69 gene (32,47). Indeed, a germline encoded polymorphism in this gene was an early requirement for the generation of these antibodies (48). Thus it may be possible that a host-genetic component could predispose certain individuals to generate a highly diverse B-cell repertoire after infection.

Another possible immunological phenomenon that could underlie the atypical antibody response is the generation of polyreactive antibodies. Polyreactive antibodies or “natural antibodies” as its name implies, exist as part of the normal immune repertoire. They can bind to multiple ligands without needing prior antigen exposure. Due to their lack of ligand-specificity, they are considered to have “innate” immune-like function. Although polyreactive antibodies are typically of low affinity and of the IgM-isotype, high affinity polyreactive IgG and IgA isotypes have also been described [reviewed in (49)]. It is important to recognize that these antibodies do not typically exist in high titers as a safeguard against self-reactivity. Polyreactive B-cell clones are generally selected against during the B-cell maturation process, except in malignancies in which this process becomes impaired (i.e., such as in systemic lupus erythematosus, SLE).

Polyreactive antibodies have been described after bacterial and viral infections; and have particularly well-studied in the context of human immunodeficiency virus (HIV). It was found that some broadly neutralizing HIV-antibodies are also polyreactive against self-antigens (50,51). One salient feature of these antibodies is the high-plasticity of their antigen-binding pocket that results in more permissive binding of different ligand structures (such as was described for the influenza A and B cross-reactive antibody CR9114). Despite the wealth of literature for HIV, no similar observations have been made for influenza. The only exception was a study by Kaur et al., which found no statistically significant differences in the levels of broadly neutralizing or polyreactive influenza antibodies in a cohort of influenza vaccinated SLE patients compared to controls, although there were certainly intriguing trends detected (52).

**Current knowledge gaps and future research directions**

While there have been insightful studies into the mechanisms and requirements that drive broadly influenza virus-neutralizing antibody responses—particularly after influenza vaccinations—it is unknown if the same processes underlie heterosubtypic antibody responses seen after naturally acquired influenza virus infections. Furthermore, it is currently unclear whether individuals with heterotypic responses are better protected against infection by diverse influenza virus subtypes.

The studies cited in this review all reported heterosubtypic seroconversion events during a pandemic. Whether this was a chance observation due to increased surveillance and serological testing or a pandemic-associated phenomenon is unknown. Hence, a critical knowledge gap currently is in determining the prevalence of heterosubtypic seroconversion during a typical influenza season. This type of study presents obvious logistical and economical challenges. At the reported prevalence rate of co-infections, any studies attempting to examine this phenomenon will need to enroll a large cohort during an influenza season. Extensive molecular detection and serological analysis against diverse strains will need to be performed to detect these heterosubtypic seroconversion events. When a baseline prevalence rate is established, other factors such as age, subtype, and underlying conditions can be examined to determine if this is an immunological or virological phenomenon.

**Summary**

Atypical antibody responses have been reported in the context of influenza virus infection and vaccination. Many of these reports have focused on the effects that the 2009 A(H1N1)pdm had on eliciting heterotypic antibody response. These studies suggest that the 2009 pandemic virus or exposure to antigenically shifted virus with no prior
immunity may be apposite for inducing heterosubtypic seroconversions. However, there have also been vaccination studies in which the influenza vaccine was able to generate a heterosubtypic response. While some of these observations have an immunological basis, others such as heterosubtypic seroconversion events observed after infection still lack a satisfying explanation. While it is possible that OAS, genetic predisposition to form broad B-cell repertoires, polyreactive antibodies, and/or other currently undescribed immune mechanisms may play a role in these responses, we are still lacking direct evidence to suggest a mechanism. Heterosubtypic seroconversion hitherto represents an unknown and largely unstudied phenomenon of the immune response that should be explored further. If indeed such responses are able to create protection from unencountered strains, the mechanism by which these individuals are able to significantly broaden their antibody repertoire may prove useful both for general vaccine design and pandemic prevention.

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Footnote

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