Significant cross reactive antibodies to influenza virus in adults and children during a period of marked antigenic drift

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Abstract

Background: Little is known about the development of cross-reactive antibodies following natural exposure. This is an important issue in the development of new universal influenza vaccines.

Methods: To study the possibility of the presence of cross-reactive antibodies to influenza viruses which underwent a major antigenic drift between the years 1999 and 2007 sera from samples of 80 children and 400 adults were selected at random from the Israeli national serum bank. The sera was obtained in 2002 and in 2007, two time points that were selected following a major drift in the H3N2 influenza virus infection (A/Panama/2007/99 to A/Wisconsin/67/2005).

Results: In the summer of 2002, 13% of the children had HI antibodies of 40 and more directed against both A/Panama/2007/99 and against A/Wisconsin/67/2005 that was first circulating in Israel only in 2006. In 2007, 29% of the children had had HI antibodies of 40 and more directed against A/Wisconsin/67/2005, and against A/Panama/2007/99, although they had never been exposed to the later virus. In adults, 58% and 68% had antibodies against A/Panama/2007/99 in 2002 and in 2007 respectively, while 8% and 39% had antibodies against A/Wisconsin/67/2005 respectively.

Conclusions: The presence of naturally occurring cross-reactive influenza virus antibodies in a significant percentage of children has important implications for the development of a universal influenza vaccine.
Background

The influenza virus is responsible for annual epidemics which result in increased primary care visits, hospitalizations, loss of work days and death, especially in the elderly and chronically ill population [1, 2]. The respiratory symptoms that result from infection by influenza viruses are usually self-limiting. However, a small percentage of patients may develop primary pneumonia, which can progress to acute respiratory distress syndrome (ARDS) [3]. The combination of pneumonia and ARDS usually occurs in high risk people, such as those with chronic lung diseases, but also has been described in healthy individuals [4]. The majority of deaths during a seasonal outbreak occur from primary pneumonia or secondary bacterial pneumonia and excess cardiovascular disease [5].

For many years, the seasonal vaccine has comprised of three of the most common circulating influenza viruses, A(H1N1), A(H3N2) and B. The objective of vaccination is to induce antibodies effective against the current viruses. As a result of antigenic drift, the virus strains included in the vaccine are "updated" to the most recent circulating viruses. This is based on the assumption of limited cross-reactivity of antibodies against strains that are significantly different from the older strains. When there is an antigenic shift, there could be an influenza pandemic, which will threaten the entire population due the lack of immunity against the new virus [6]. Four major pandemics occurred in the last 100 years, all resulted from influenza A infections and included the Spanish Flu pandemic (1918-1920, H1N1) [7], the Asian Flu pandemic (1957–1958, H2N2) [8], the Hong Kong Flu pandemic (1968–1969, H3N2) [9] and the Swine Flu pandemic (2009-2010, H1N1pdm09) [10].

Influenza viruses contain eight genome segments which encode for 12 proteins [11]. Two of these, glycoproteins named hemagglutinin (HA) and neuraminidase (NA) are expressed on the surface of the influenza virus itself and on infected cells and are involved in eliciting neutralizing antibodies against the homologous virus [12]. Therefore, both proteins are
considered as the main targets for vaccination and are included in all types of influenza vaccines (although antibodies directed against NA are not considered to be neutralization antibodies). Unfortunately, the NA and especially the HA proteins, are subjected to frequent antigenic drifts and occasional antigenic shifts. Thus the development of a universal influenza vaccine that will be effective against various influenza viruses is problematic. Antibody cross-reactivity among various influenza virus strains have been detected in several studies following immunization with influenza vaccines [13-15]. Recently, antibodies were discovered that recognize different influenza viruses [16]. Some of these antibodies bind the HA stem region of H1, H2, H5, H6, H8, H9, H11, H12, H13, H16 influenza A viruses and others bind to the stem region of most of group H3, H4, H7, H10, H14, H15 influenza A viruses. Cross reactive antibodies were also detected against the NA and the M proteins. Antibodies raised against the N1 subtype of human influenza viruses cross-reacted with the N1 from avian influenza and partially protected mice against lethal influenza A/H5N1 virus infection [17]. Broad-reactive antibodies against the M2 protein, raised by vaccination, provided protection against heterologous influenza virus infection in mice [18, 19]. However, to the best of our knowledge, little is known about the existence of anti-influenza antibody cross-reactivity following natural exposure to seasonal influenza viruses. In this study, we examined cross-reactivity of influenza antibodies in children and adults following natural exposure to the viruses during a period of marked antigenic drift in the A(H3N2) virus.
Methods

Sample collection

Since the late 1990's, Serum samples have been collected in an ongoing process from samples of the Israeli population, which are kept frozen at -70 in the Israel Center for Disease Control (ICDC) repository. The samples were collected from children age 1-17 years, but only samples from 1-3 years old children were used in this study. The sera obtained are residual sera from diagnostic laboratories while the adult sera are derived from residual sera samples from routine tests of healthy blood donors.

Ethics Statement

The research was approved by the Sheba Medical Center Helsinki committee (Number 94-11-12-SMC)

Hemagglutination inhibition assay

All sera were treated with receptor destroying enzyme (RDE) (Sigma C8772), 1:4 dilution, for 16 h prior to heat inactivation for 30 min at 56 °C and absorption with erythrocytes to remove non-specific hemagglutination, in accordance with WHO recommended protocol [20].

Sera were serially two fold diluted (1:20–1:2560) in 25µl of PBS in V-shaped well plates, and an equal volume of four hemagglutinin (HA) units of viral antigen were added. The mixture was then incubated at room temperature for 1 h. Fifty microliters of 0.5% chicken erythrocytes suspended in PBS solution was added to the wells, and mixed by shaking the plates on a mechanical vibrator. Agglutination patterns were read after 30 min and the HI titer was defined as the reciprocal of the last dilution of serum that completely inhibited hemagglutination. The cut-off value selected for a positive result was 1:40. The antibodies against the following influenza strains were supplied by the WHO. The following viruses
were tested: A/Panama/2007/99(H3N2) active in 2001-2002 and A/Wisconsin/67/05 (H3N2), active in the 2006-2007 season.

Data analysis

The significance of the differences in the percentages of sera containing antibodies in figures 1 and 2 were calculated using the Chi test. P value of <0.05 was considered statistically significant. Student paired T test was used in figure 3 and P value of <0.05 was considered statistically significant.
Results

Circulating influenza strains in Israel between 1998-9 until 2007-8

Since 1996, the ICDC has operated a network of sentinel clinics in the community in Israel, during the winter months. Swabs from patients with influenza-like illness are transported to the National Influenza Center at the Central Virology Laboratory in the Ministry of Health for virus identification, isolation and typing. A description of the H3N2 viruses isolated between 1999 and 2009 are shown in Table 1 and a phylogenetic tree of all viruses is presented in figure 1.

Until the summer of 2002, the dominant H3N2 viruses were A/Panama/2007/99 or the closely related virus A/Sydney/5/97 (Fig 1 and [21]). In the winter of 2002-2003, only a few cases of A/Panama/2007/99 infections were observed (Table 1). A major drift occurred during the winter of 2003-2004 when a new virus strain, A/Fujian/411/02 appeared which is genetically different from A/Panama/2007/99 (Fig. 1 and [22]). A continuous drift occurred for the following 4 winter seasons (the A/Wyoming and the A/California viruses are similar to A/Fujian) and in the winter season of 2006-2007, the A/Wisconsin/67/05 strain circulated similar to A/Fujian/411/02 (Fig. 1). Therefore we decided to investigate the presence of anti-influenza antibodies against A/Panama/2007/99 at the summer of 2002, following several years in which the A/Panama or viruses that are similar to A/Panama were present in the country (Fig. 1 and Table 1) and in the summer of 2007, following 4 years in which in which A/Fujian or viruses which are similar to A/Fujian were present (Fig. 1 and Table 1).

Anti-influenza antibodies in Children

To test for the present of anti-influenza antibodies in the population random samples of 400 sera from adults age 30-50 and 80 from children age 1-3 were obtained for each of the years 2002 and 2007. Samples were selected for the months between April to November when
influenza virus is not usually active in the country. Importantly, none of the children that evaluated in this manuscript were vaccinated against influenza.

As can be seen in figure 2, in the summer of 2002, 45% of the sera derived from children had detectable antibodies against the influenza strain A/Panama/2007/99 (H3N2), circulating in the population in the preceding winters (Table 1). It is therefore likely that the tested children were indeed exposed to these viruses. However, surprisingly, 13% of the children had antibodies against A/Wisconsin/67/2005 (H3N2), a virus that was not isolated in Israel prior to 2006.

In 2007, 58% of the tested children had detectable antibodies against A/Wisconsin/67/05 (H3N2) (the dominant strain in the winter of 2006-7). Unexpectedly, 29% of the sera obtained from children under 3 in 2007 showed reactivity against A/Panama/2007/99 (H3N2), although this strain was not detected in the population after the year 2003 and they had never been exposed to this virus.

**Anti-influenza antibodies in Adults**

The results for the adults are shown in figure 2b. In 2002, the antibody distribution of the adults was similar to that obtained in children and 58% of the adults had antibodies directed against A/Panama/2007/99. In 2007, however, the results in adults differed from the results in children. High percentages (68%), of healthy adults had antibodies directed against A/Panama/2007/99, a virus which was not detected in the Israeli population after the year 2003. Thirty-nine percent of the adults had antibodies directed against the dominant strain in the preceding winter, A/Wisconsin/67/05.

**Cross reactivity among various anti-influenza antibodies**

Since antibodies were detected against viruses to which the children were never exposed, both in 2002 and in 2007 (the children were not immunized against influenza), we speculated that these are cross-reactive antibodies. Indeed, strikingly, the antibodies to
A/Wisconsin/67/05 (13% of the children) that were found in 2002 in children were detected only in children who also had antibodies to A/Panama/2007/99 (Fig. 3), which must represent cross-reactivity since they had not been exposed to this virus. In 2007, all of the 29% children that had antibodies against A/Panama/2007/99 (Fig. 2), also had antibodies to A/Wisconsin/67/05 (Fig. 3). The oldest children that were evaluated in 2007 were born in 2004, a year after A/Panama/2007/99 strain was last documented in Israel, suggesting that the antibody recognition of A/Panama/2007/99 observed in children in 2007 was also due to cross-reactivity.

In contrast, in adults, in 2007, 50% of the adults’ antibodies against A/Panama/2007/99 were found to cross-react with A/Wisconsin/67/05, while in 2002 all of the adults (8%, Fig. 2) that had antibodies against A/Panama also had antibodies against A/Wisconsin (Fig. 3). We thus concluded that while in adults the antibodies observed might result from various influenza virus strains to which they were exposed to during their life, in children they must represent “true” cross-reactive antibodies.

Titers of anti-influenza antibodies

To try and understand why antibodies against a particular strain were detected in the population when the virus is absent we examined the titers of the anti-influenza antibodies in children and adults, as an estimation of the effectiveness and the strength of the immune response (Fig. 4). In 2002, the highest antibody titers observed both in adults and in children were directed against A/Panama/2007/99. In contrast, in 2007, the highest antibody titers observed in children were directed against A/Wisconsin/67/05, the dominant strain in the preceding winter. In adults, in 2007, the highest antibody titers observed were against A/Panama/2007/99, while A/Wisconsin/67/05 was recognized to a slightly lesser extent, probably due to repeated exposure to A/Panama/2007/99 like viruses in previous years (Fig. 4b and d).
Also of interest is the difference in the distribution of the titers between children and adults. In children, the antibody titers observed against A/Panama/2007/99 and against A/Wisconsin/67/05, in 2002 and in 2007 respectively resembles a bell-like shape (Fig. 4a and b), supporting the assumption that the children were indeed infected by the viruses. In contrast, in adults, bell-like graphs were not observed, probably because the adult sera contains antibodies directed against several influenza virus strains they were exposed to during the years (Fig. 4).
Discussion

In this manuscript we evaluated the anti-influenza antibodies present in the Israeli toddlers and adult population at the summer seasons of 2002 and 2007, following a marked antigenic drift in the influenza virus. We tested the anti-influenza antibodies in 2002 after a period that was dominated by one H3N2 strain A/Panama/2007/99, and following 4 years of infections at the end of 2007 that were dominated by a markedly different H3N2 virus, A/Wisconsin/67/2005 (as seen in our phylogenetic tree and as acknowledged previously, [22-24]). We demonstrated in children age 1-3, that a significant percentage of those with naturally occurring antibodies against the currently circulating strains, also had antibodies against markedly different strains to which they had never been exposed. Because these children were not vaccinated against influenza, this provided evidence of substantial antibody cross-reactivity against seasonal influenza virus strains.

To the best of our knowledge, the presence of naturally occurring cross-reactive antibodies against seasonal infections following significant antigenic drift has not been reported. It was suggested that some elderly people that were probably exposed to the 1918 pandemic influenza infection had cross reactive antibodies against the pandemic 2009 swine origin influenza virus [25]. However, cross-reactive antibodies against seasonal influenza infections were not observed [25].

The results of the current study can contribute to the understanding of the epidemiology of influenza, the immune responses and the transmission rates. For example, in a recent survey around 12,000 individuals were tested and antibodies against the avian H5N1 virus were present in the population in a much higher percentages than previously thought [26]. The authors of the paper concluded that this virus, considered being highly dangerous (60% mortality), can also cause mild or subclinical infection. However, it is possible that some or all of the antibodies detected were cross-reactions with other circulating influenza viruses.
It is difficult to assay for antibody cross-reactivity in adults, since they may have been exposed to the virus during their life. One of the strengths of the current study was the use of sera from young children that had not previously been exposed to the virus tested. Thus, the sera contained antibodies directed against influenza strains that were not circulating during their short life, it should indicate antibody cross-reactivity.

We have shown directly that the anti-influenza antibodies directed against A/Panama/2007/99 and against A/Wisconsin/67/05 cross-react with each other. The assays that were performed to test the presence of the anti-influenza antibodies were hemagglutination inhibition tests. In these type of assays 40 HI units is considered to be protective [13], thus these cross-reactive antibodies might provide protective function.

Interestingly, not all antibodies cross-reacted with each other. One possible way to explain this is that in all cases cross-reactive antibodies are generated but we did not detect these as our detection. Regardless of whether this is true or not, it is clear that protective cross-reactive antibodies were probably not generated in every individual. It is possible that the genetic background of the different individuals determines whether or not they will develop cross reactive antibodies. With that regard, it will be interesting to determine in the future the MHC class I and class II haplotypes of the various individuals (those who generate the cross reactive protective antibodies and those that did not).

Cross reactivity has previously been reported following vaccination against influenza [27]. In addition, the presence of cross reactive and vaccine induced antibodies to the new emerging virus swine influenza A(H3N2) was also recently demonstrated [28]. Furthermore, several cross reactive monoclonal antibodies were developed following immunization that cross-react with various influenza A and influenza B viruses. These antibodies recognize distinct conserved epitopes in the head region of the hemagglutinin.
derived from influenza A and B viruses [29]. Finally, it was also reported that more cross-reactive antibodies are generated following infection as compared with vaccination [30]. Thus, the understanding of the mechanisms leading to the antibody cross-reactivity could provide essential information for the design of broadly, cross-reactive vaccine. In vivo studies were also conducted to evaluate the efficiency of cross reactive antibodies in vivo. It was shown that classical swine H1N1 influenza viruses confer cross protection from swine-origin 2009 pandemic H1N1 influenza virus infection. This was demonstrated both in mice and in ferrets [31]. Very promising results were obtained with the broadly cross-reactive antibodies mentioned above as it was demonstrated that these cross reactive antibodies generated following immunization were able to protect against lethal challenge with influenza A and B viruses [29]. Thus, the presence of naturally occurring cross-reactive influenza virus antibodies against markedly different influenza virus strains, in a significant percentage of children suggests that the in vivo cross reactive experiments performed in have human relevancy. Furthermore, our results suggests that it might be physiable to develop a broad reactive anti-influenza vaccine.
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Competing interests

The authors declare that they have no competing interest.

Authors’ contributions

MM, MB, HS, IZ, KY, RB, reviewed and collected the data. MM and MG conceived the study and wrote the paper. RD, was responsible for statistical analysis. DC, TS, EM supervised the work and reviewed the manuscript drafted the manuscript. All authors reviewed the work and approved the final manuscript.
References


Figure legends

Figure 1 Phylogenetic tree of the viruses evaluated in this study

The two antigenically different viruses are highlighted in bold. 1701 nucleotides of the HA protein of each virus were compared.

Figure 2 Anti-influenza antibodies in young children and in young adults, 2002 and 2007

Children (1-3 years, a) and adults (35-50 years, b) sera obtained in 2002 and in 2007 were tested by HI test against A/Wisconsin/67/2005 (black columns), and A/Panama/2007/99 (gray columns). * less than 0.0013, ** less than 0.01, *** less than 0.0001 using Chi test.

Figure 3 Percentages of antibody cross-reactivity

Percentages of antibody cross reactivity in 2002 (a) and in 2007 (b). In 2002 antibodies against A/Wisconsin/67/05 were set to be 100% and the anti-A/Panama/2007/99 were compared to them, while in 2007 the A/Panama/2007/99 antibodies were set to be 100% and were compared with the A/Wisconsin/67/05 antibodies. * less than 0.0001 using Chi test.

Figure 4 Distribution of antibody titers in young children and in young adults, 2002 and 2007

Antibody titers were determined by HI test in children (a and b) and in adults (c and d) in 2002 (a and c) and 2007 (b and d).

Table 1 Influenza viruses circulating in the Israeli population between 1998-2008
Figure 2

a

Percent positive - children

- A/H1N1/68/2005 (H3N2)
- A/Panama/2007/99 (H3N2)

Year

Percent

2002: 13, 45
2007: 58, 29

b

Percent positive - adults

- A/H1N1/68/2005 (H3N2)
- A/Panama/2007/99 (H3N2)

Year

Percent

2002: 8, 58
2007: 39, 68

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Additional files provided with this submission:

Additional file 1: Table 1.TIF, 47K
http://www.biomedcentral.com/imedia/1590254330128477/supp1.tiff