Travel, infection and immunity

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No cross-reactive serum antibodies to 2009 pandemic influenza A (H1N1) after seasonal influenza vaccination in the virus neutralization assay

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BACKGROUND

The surface hemagglutinin (HA) and neuraminidase proteins in recent seasonal trivalent inactivated influenza vaccines (TIV) are antigenically very distant from those of 2009 pandemic influenza A(H1N1) (pH1N1). Therefore seasonal TIV is generally not expected to confer a significant degree of cross-protection to pH1N1.[1] Only older age by way of exposure to pre-1957 influenza strains has consistently been found to confer a relevant degree of cross-reactive antibodies to pH1N1.[2-6] We were surprised to find that in our study, 2009–2010 seasonal TIV induced cross-reactive antibodies to pH1N1 in a sizeable proportion of subjects.[7] In the hemagglutination-inhibition (HI) assay, the seroprotection rate to pH1N1 increased from 22% to 49% following vaccination with TIV with 31% showing seroconversion. This effect was age dependent. Using virus neutralization assays, others have shown that cross-reactive antibodies that are induced by seasonal TIV are functional against pH1N1.[8] This suggests that these antibodies confer protection against pH1N1. There is epidemiological evidence that supports this claim, although there is also evidence to the contrary.[9-12] We determined whether the cross-reactive antibodies to pH1N1 that were detected in the HI assay, were also present in the virus neutralization (VN) assay.

METHODS

Study design and source population

From the original cohort, we selected 14 HIV-infected individuals who had been vaccinated with 2009–2010 seasonal TIV, a median of 19 days before being vaccinated with pH1N1 vaccine (interquartile range, IQR 15–24 days). Their pH1N1 antibody titer was below the detection limit before vaccination with seasonal TIV. Of these subjects 8 of 14 developed cross-reactive antibodies to pH1N1 after vaccination with TIV, according to the HI assay. Antibody responses before seasonal TIV (day -140), after seasonal TIV (day 0), after the first dose of pH1N1 vaccine (day 21) and after the second dose of pH1N1 vaccine (day 56) were measured with HI assays and VN assays.

Virus neutralization (VN) assay

50 µl volumes of heat-inactivated serum samples were diluted 1:10 and serially diluted two-fold and incubated with an equal volume of virus suspension containing 100 $TCID_{50}$ for two hours at 37 ◦C. The virus A/California/4/2009 (H1N1) was used. Subsequently the mixture was transferred to confluent MDCK cells grown in 96-well plates, incubated for two hours at 37 ◦C and then aspirated. The cells were washed once with infection medium and then cultured for 3–7 days at 37 ◦C. Then the culture supernatants were tested for HA activity as a measure for residual virus replication. The serum titers were expressed as the reciprocal of the dilution that still prevented virus replication. If there was no inhibition of virus replication, the titer was assigned a value of 1:5.

Hemagglutination-inhibition (HI) assay

Antibodies to the vaccine strain A/California/7/2009 (H1N1) were measured using the hemagglutination-inhibition (HI) assay, according to standard methods.[13] Titers below the detection limit (i.e., 1:10) were assigned a value of 1:5. Seroconversion was defined by a post-vaccination HI titer of at least 1:40 combined with at least a four-fold increase in titer in accordance to European and international guidance.

RESULTS

The median age of this group of 14 HIV-infected subjects was 57 (IQR 48–67) years. The median CD4+ T-lymphocyte count was 529 (IQR 324-706) cells/mm^3. The titers obtained in the VN assay correlated reasonably well with those obtained in the HI assay for most serum samples tested (Figure 1). Pearson's correlation coefficient was 0.64 (95% Confidence Interval, CI 0.46–0.77, p-value <0.0001). There were a number of samples with discrepant values. These were mainly seen if titers were at the lower end of the spectrum. Some of the samples with discrepant values had a negative HI titer and a weak VN titer. Others had a negative VN titer and a moderate HI titer. Most of the discrepant titers were seen in the pre-vaccination samples that had been

obtained during routine outpatient visits a median of 140 (IQR 65-205) days before vaccination with TIV and in the samples obtained after vaccination with TIV (day 0).

In the HI assay, none of the 14 subjects had antibodies to pH1N1 before vaccination with seasonal TIV. Eight subjects (57%) developed a cross-reactive anti-pH1N1 titer ≥1:40 after vaccination with TIV. No such response was seen in any of these subjects when measured with the VN assay. This was reflected in the geometric mean titers (GMT), as is depicted in Figure 2. In the HI assay, GMT for cross-reactive antibodies to pH1N1 increased from 5 (95% CI 5–5) to 30 (95% CI 15-61) after vaccination with seasonal TIV. In the VN assay there was no increase in cross-reactive antibody titers: pre-vaccination GMT 13 (95% CI 8-22), post-vaccination GMT 11 (95% CI 7-19).

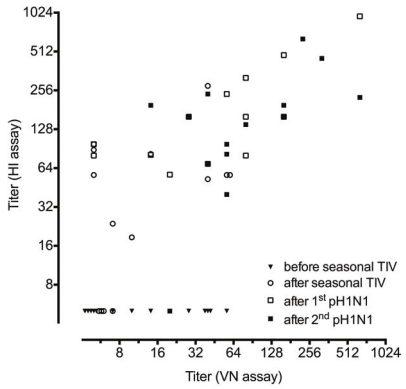

Figure 1. Comparison of serum antibody titers against influenza virus A/California/7/2009 (pH1N1) obtained in virus neutralization (VN) assay with those obtained in the hemagglutination-inhibition (HI) assay.

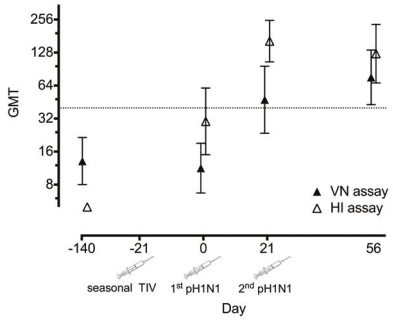

Figure 2. Comparison of geometric mean titers (GMT) for serum antibody titers against influenza virus A/California/7/2009 (pH1N1) obtained in virus neutralization (VN) assay with those obtained in the hemagglutination-inhibition (HI) assay, in response to seasonal TIV and to two doses of monovalent MF59-adjuvanted pandemic influenza vaccine (A/California/7/2009) (pH1N1), in a group of 14 HIV-infected patients.

DISCUSSION

Seasonal TIV did not induce cross-reactive antibodies to pH1N1 according to the VN assay. This was in contrast to the result obtained from the HI assay. The discrepancy between the results of the HI assay and VN assay, is unexpected, since HI titers for influenza virus antibody in human sera closely match VN titers.[14,15] However, VN and HI antibody repertoires do not fully overlap.[16] In our study, discrepant values were mainly seen if titers were at the lower end of the spectrum. Most of these samples had a negative HI titer and a weak VN titer. One can speculate on the cause of the discrepancy. Antibody assays such as the HI assay and VN assay have limited sensitivity to distinguish small difference in antibody titers, such as a two-fold dilution step difference. Since ours was a fairly small study, the play of chance may have magnified the inherent limitations of the assay and introduced a bias that led us to believe that TIV induced a significant degree of cross-protection to pH1N1 based on the HI assay results. Alternatively, aspecific binding of nonimmune or immune factors may prevent hemagglutination.[17]

The VN assay is a functional assay and is considered the gold standard. Therefore, we conclude that seasonal TIV did not confer a significant degree of cross-reactive protective antibodies to pH1N1. However, to complicate matters, there is compelling new evidence that supports our previous observation of a relevant increase in pH1N1 titer after vaccination with seasonal TIV. Li et al. have recently shown that memory B cells, reactive to pH1N1 are present in many people, before pH1N1 emerged.[18] They also show that pH1N1 influenza vaccination induces a recall response of certain memory B cells, that leads to broadly cross-reactive antibodies that bind to conserved regions of hemagglutinin.[18-20] One can speculate that in our study, seasonal TIV activated cross-reactive memory B cells in older individuals which led to the production of antibodies that cross-reacted with the HA protein of pH1N1. Maybe these antibodies were capable of inhibiting hemagglutination in the HI assay, but were not capable of neutralizing pH1N1 virus in the VN assay.

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