Neutralizing and Hemagglutination-Inhibiting Activities of Antibodies Elicited by the 2004-2005 Influenza Vaccine against Drifted Viruses

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Evaluation of the antibody responses induced by the 2004-2005 influenza vaccine strain against the homologous variant, the 2004-2005 field isolates, and a previous circulating strain showed that a correlation between neutralizing and hemagglutination-inhibiting activities exists only when the antigen is very close to the vaccine strain.

The prevention of influenza is based on annual vaccination with an inactivated virus vaccine, the effectiveness of which largely depends on the match between the vaccine strains and the circulating viruses (9, 11). During the last 5 years (2000 to 2005), two antigenic drifts occurred, and two A/H3N2 drifted variants, i.e., Fujian/411/02 and California/7/04, appeared on the epidemiological scene during the 2002-2003 and 2004-2005 seasons, respectively, taking the place of previously circulating Panama/2007/99-like strains (6, 10, 14). Fujian/411/02-like viruses, such as Wyoming/3/03 or Kumamoto/102/02, were included in the composition of the 2003-2004 influenza vaccine for the Southern Hemisphere, and as early as February 2005, the WHO recommended the inclusion of a California/7/04 virus component in the vaccine for the 2005-2006 winter (2, 3).

The appearance of these two drift variants was reflected in the epidemiological patterns observed in Italy in the 2002-2003 and 2004-2005 seasons; about 5,000,000 cumulative cases of influenza-like illness were reported in each of these two seasons, while during the mild epidemic season, the estimated number of cases ranged between 2.5 million and 4 million. In particular, during the early months of 2005, the Italian surveillance network observed the highest incidence in the 65-year-old group, since the surveillance network was established in 1999, and regional surveillances recorded lab-confirmed cases in immunized subjects as well as outbreaks in nursing homes where vaccine coverage was close to 100%, suggesting the possibility of suboptimal protection as concerns the A/H3N2 viruses, such as Wyoming/3/03 or Kumamoto/102/02, were included in the composition of the 2003-2004 influenza vaccine for the Southern Hemisphere, and as early as February 2005, the WHO recommended the inclusion of a California/7/04 virus component in the vaccine for the 2005-2006 winter (2, 3).

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In the present study, the antibody responses induced by the Wyoming/3/03 2004-2005 vaccine strain against the homologous variant, the 2004-2005 field isolate, and a previously circulating strain were investigated by comparing the antibody responses using hemagglutination inhibition (HI) and neutralization tests.

The serum samples from vaccinees used in the present study had been obtained in the course of a previous vaccination study including 20 free-dwelling elderly individuals aged >60 years. All subjects had been vaccinated with one dose of a commercial trivalent influenza subunit vaccine containing 15 μg hemagglutinin from each component. The vaccine contained a Wyoming/3/03-like reassortant as the A/H3N2 component. A blood sample was collected just before vaccination and 28 ± 1 days later. HI and neutralization tests were performed, as previously described (5, 18), using three isolates collected during the 2001-2002, 2002-2003, and 2004-2005 seasons. Strains A/Genoa/47/02 (Pan-field), A/Genoa/1961/03 (Wyo-field), and A/Genoa/12/05 (Cal-field) were chosen as representative of the Panama/2007/99, Wyoming/3/03, and California/7/04 strains, respectively.

The molecular characterization of the globular head region of hemagglutinin was carried out by the sequence analysis of the HA1 subunit, and the antigenic characterization of isolates was carried out by HI testing, as described elsewhere (4, 15). The phylogenetic tree that includes the isolates, vaccine, and reference strains is shown in Fig. 1, and the antigenic and molecular distances between the Wyoming/3/03 vaccine strain and Pan-field, Wyo-field, and Cal-field are shown in Table 1. The amino acid changes relative to Wyoming/3/03 observed in Pan-field and Cal-field that had a major impact on virus antigenicity are those in positions 155 and 156 (Pan-field) and in position 145 (Cal-field), which created an additional glycosylation site (6, 10, 14).

HI and neutralization titers were transformed into binary logarithms and corrected for prevaccination state, as described by Beyer et al. (8). The observed distributions were confirmed to be normally distributed by the one-sample Kolmogorov-Smirnov goodness of fit test procedure. Correlations between HI and neutralization titers for Pan-field, Wyo-field, and Cal-field were tested by the Pearson test, and comparisons between viruses were analyzed by analysis of variance with a post hoc Bonferroni test (titors) and a chi-square test (seroprotection-subject proportions).

As shown in Table 2, the HI titer of the homologous strain was the highest and the proportion of patients showing seroprotection against Wyo-field was higher than that against Pan-
field. The antibody response to the Wyoming/3/03 vaccine strain versus that to the Cal-field strain was higher than that to Pan-field as concerns both proportions of patients showing seroprotection and titers, although the difference did not reach statistical significance ($P = 0.1$). The neutralizing activities against Wyo-field and Pan-field were higher than that against Cal-field. Although the protective neutralization titer remains to be established, the proportions of titers that were $>3, >4$, and $>5$ reflected a better response against Wyo-field and Pan-field than against Cal-field.

The correlations between HI and neutralization titers with respect to the three strains are shown in Fig. 2. As concerns Pan-field and Cal-field, no correlation emerged either with the scatter plot or with the extrapolated regression lines ($R$ values of $<0.25$ for both strains) or using the Pearson correlation test ($P$ values of 0.29 and 0.42, respectively). In contrast, the Pearson correlation test (correlation coefficient, 0.7; $P < 0.01$) showed a clear correlation between the hemagglutination-inhibiting and neutralizing activities of antibodies elicited by Wyoming/3/03 against Wyo-field. The regression line extrapolated by the HI and neutralization data indicated a positive linear relationship ($R = 0.7$), although only about 50% ($R^2 = 0.49$) of the observed HI titers can be explained by the linear regression.

Although the HI assay is routinely used for the detection of antibodies to human influenza, several studies have demonstrated that neutralization assays may be more sensitive than the HI test both in detecting a higher rate of antibody increases and in detecting antibody in individuals seronegative according to the HI assay (7, 12, 17). Data emerging from the present study clearly showed that a correlation between hemagglutination-inhibiting and neutralizing activities exists only if the antigen is very close to the vaccine strain. The finding of a seroprotective HI titer against Cal-field in 60% of the subjects vaccinated with Wyoming/3/03, comparable to that against the homologous strain (70%), was unexpected, considering the mismatch between the vaccine strain and California/7/04-like viruses and the epidemiological data showing the suboptimal efficacy of the vaccine during the 2004-2005 season (6, 10). On the other hand, the neutralizing activity by the antibody against Wyoming/3/03 appeared to be similar to those against Wyo-field and Pan-field and clearly lower than that against Cal-field. These discordant HI and neutralization patterns could be explained, in part, by the different neutralizing and hemagglutination-inhibiting activities by antibodies: the electron microscopy findings indicated that HI and non-HI monoclonal antibodies bind to the top of the globular head and the external surface of the HA molecule, respectively. Non-HI monoclonal antibodies play a fundamental role in the neutralizing activity, inhibiting the fusion with the intracellular vacuolar membrane (13). In particular, the amino acid changes relative to Wyoming/3/03 in antigenic site A, observed in Cal-field, such as the change in position 145 (K145N), seem not to play an important role in hemagglutination-inhibiting activity impairment, whereas changes in the B or E site, such as that observed in Pan-field, affected the result of the HI test (13, 16). Interestingly, vaccination with Wyoming/3/03 elicited antibodies with a high neutralization activity against Pan-field. The well-known “antigenic sin” phenomenon, which, with exposure to Wyoming/3/03, could have boosted immunity against the previous Pan-field strain, did not justify this pattern since the HI assay did not show high titers against Pan-field (1). The cross-protection determined by antibodies elicited by Wyoming/3/03 against Pan-field, in consideration of the high antigenic and molecular distances between these strains, remains a subject for discussion and worthy of further studies.

In conclusion, the HI test did not appear to be an optimal gold standard to quantify the immune response of an influenza vaccine against antigens different from the homologous strains, such as drifted variants. The neutralization assay, offering the

### TABLE 1. Antigenic and molecular distances between Wyoming/3/03 and Wyo-field, Pan-field, and Cal-field

| Strain    | HI titer of ferret serum after infection with Wyoming/3/03 | Nucleotides (no. ± SE) | Amino acids (no. ± SE) | Change relative to Wyoming/3/03 |
|-----------|-----------------------------------------------------------|-------------------------|------------------------|--------------------------------|
|           |                                                            |                         |                        | A                   | B     | C     | D     | E     |
| Wyoming/3/03 | 5,120                                                      |                         |                        | T131A               | T155H | G50R  | Q75H  | K75E  |
| Wyo-field  | 2,560                                                      | 6 ± 2.4                 | 4 ± 2                  | N144D               | H156Q | G50E  | S227P |
| Pan-field  | 160                                                       | 36 ± 5.9                | 18 ± 4.1               | K145N               | Y159F | G50E  | S227P |
| Cal-field  | 640                                                       | 13 ± 3.7                | 9 ± 2.9                | Excluding the substitution related to egg adaptation in positions 189 and 226.
advantage of better sensitivity and detection of the functional antibody, contributed to a better understanding of some of the epidemiological evidence and could be a valid alternative to the HI assay.

FIG. 2. Correlations between HI and neutralization titers with respect to Wyo-field, Pan-field, and Cal-field.

### TABLE 2. HI and neutralization test postvaccination titers corrected according to Beyer et al. (8)

| Test and parameter | Value for indicated strain used in titrations |
|--------------------|-----------------------------------------------|
|                    | Wyo-field | Pan-field | Cal-field |
| HI Mean ± SD       | 4.14 ± 1.58 | 2.18 ± 1.46 | 3.22 ± 1.57 |
| 95% CI             | 3.4–4.88   | 1.5–2.87   | 2.49–3.96   |
| Neutralization     | 14 (70)    | 5 (25)b    | 12 (60)c    |
| Mean ± SD          | 5.95 ± 1.79 | 5.1 ± 1.45 | 5.75 ± 2.51d |
| 95% CI             | 4.4–6.79   | 3.8–5.78   | 4.0–7.51    |
| Titer >3 (%)       | 20 (100)   | 17 (85)    | 10 (50)     |
| Titer >4 (%)       | 18 (90)    | 17 (85)    | 8 (40)      |
| Titer >5 (%)       | 12 (60)    | 11 (55)    | 4 (20)      |

a CI, confidence interval.
b Significantly different from value for Wyo-field strain at a P of <0.01.
c Significantly different from value for Pan-field strain at a P of <0.01.
d Significantly different from value for Pan-field strain at a P of <0.05.

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