JOURNAL OF CLINICAL MICROBIOLOGY, June 2005, p. 3027–3029 Vol. 43, No. 6
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.6.3027–3029.2005

Fast-Track Communication

New A/H3N2 Influenza Variant: a Small Genetic Evolution but a Heavy Burden on the Italian Population during the 2004-2005 Season

Received 29 March 2005/Accepted 31 March 2005

Recurrent epidemics of influenza are due to the continuous emergence of new antigenic variants. Antigenic drift, which occurs when the accumulation of point mutations results in amino acid changes in surface glycoproteins, is the mechanism used by influenza viruses to escape from immunological pressure induced by previous natural exposures and vaccination. Since the appearance of A/H3N2 influenza viruses in 1968, surveillance has shown that new drift variants of epidemiological importance present a mean of 13.2 ± 2.9 amino acid substitutions on the main antigenic determinant, i.e., hemagglutin, and an average of 8.1 ± 4 amino acid substitutions over antigenic sites. Only A/Beijing/353/89 and A/Wuhan/359/95 drift variants showed a single amino acid change in position 145 over antigenic site A (5).

As far as concerns the recent drift strains, a new virus, A/Fuijian/411/02, appeared during the 2002-2003 season, and its variant, A/Christchurch/28/03, predominated during the following winter (Fig. 1A). The Fuijian-like virus A/Wyoming/3/03 first appeared in 2004 and formed the 2004-2005 vaccine composition for the Southern and Northern Hemispheres (1, 2). Most of the viruses isolated during the 2004-2005 season, identified as A/California/7/04 cluster (Fig. 1A), appeared phylogenetically close to A/Fuijian/411/02, showing 6 to 7 amino acid changes in comparison with the reference strain. Interestingly, several strains isolated during the last weeks of the 2003-2004 epidemic, i.e., A/Genoa/11/04 and A/Parma/75/04 (shown in italics in Fig. 1a), belonged to the A/California/7/04 cluster, representing the "herald wave" (3). The number of changes observed in the 2004-2005 isolates, relative to A/Fuijian/411/02, is the expected value, considering that the rate of amino acid substitutions in isolates belonging to the same cluster ranged between 2 and 4 per year (data from reference 5) and that A/Fuijian/411 appeared 2 years ago; this finding is completely different from the above-mentioned number of amino acid substitutions usually observed in new drift variants.

As far as the major epitopes of A/H3N2 virus are concerned, strains isolated in 2004-2005 had amino acid changes, relative to A/Fuijian/411/02, in positions distributed over antigenic sites A, B, and D. All isolates had an asparagine residue in position 145 (K145N), which created an additional glycosylation site in antigenic site A; a phenylalanine and an asparagine residue in positions 159 (Y159F) and 189 (S189N), respectively, in antigenic site B; and an isoleucine and a proline residue in positions 226 (V226I) and 227 (S227P), respectively, in antigenic site D. The A/H3N2 strain A/California/7/04, selected for the 2005-2006 vaccine composition, showed identical residues at the above-mentioned positions. Interestingly, the single amino acid substitution in position 145 is the amino acid change that was responsible for antigenic drifts between A/Sichuan/2/87 and A/Beijing/353/89 (N145K) and between A/Beijing/32/92 and A/Wuhan/359/95(K145N). With regard to serological characterization, the results of the hemagglutination inhibition test showed that viruses isolated in Italy gave >4-fold reduced titers with antisera to the 2004-2005 vaccine strain A/Wyoming/3/03. Regional surveillances recorded laboratory-confirmed cases in immunized subjects as well as outbreaks in nursing homes where vaccine coverage is close to 100%, suggesting the possibility of a suboptimal protection, as far as the A/H3N2 component is concerned, for immunized subjects.

The Italian sentinel-based Network for Surveillance of Influenza, run under the auspices of the Italian Centre for Disease Control (part of the Health Ministry), covers about 2% of the national population. A total of about 900 caring physician and pediatricians reports on a weekly basis the number of new cases of influenza-like-illness according to a standard case definition: abrupt onset of fever (>38.0°C), one or more respiratory symptoms (nonproductive cough, sore throat, rhinitis), and one or more systemic symptoms (myalgia, headache, severe malaise) (4). In the current influenza season the network reported low activity until week 51 in 2004. Subsequently, an increasing incidence was observed, reaching the peak (14.5 cases/1,000 inhabitants) at week 6 in 2005. The epidemic curve was different in the different age groups: the incidence rate was higher in children (29.2 cases/1,000 inhabitants and 28.6 cases/1,000 inhabitants in the age groups 0 to 4 years old and 5 to 14 years old, respectively, at week 6 in 2005) than in adults (12.4 cases/1,000 inhabitants) or in the age group >65 years old (8.3 cases/1,000 inhabitants). The overall weekly incidence was the highest incidence observed since the surveillance network was established in 1999, a period including the appearance of two drift variants, i.e., Sydney/5/97 and A/Fuijian/411/02. The appearance of these two drift variants was reflected in the epidemiological pattern with peaks in the 1999-2000 and 2002-2003 seasons (Fig. 1B). So far, each influenza season has caused in Italy an estimated number of cases ranging between 2.5 and 5 million, while in the current season, not yet completed, the cumulative number of cases has already reached 5 million.

Taking into consideration these molecular and antigenic data, together with the epidemiological findings, the new variant isolated in Italy, although showing only a slight genetic change, can be defined as an epidemic strain. The A/H3N2 vaccine strain

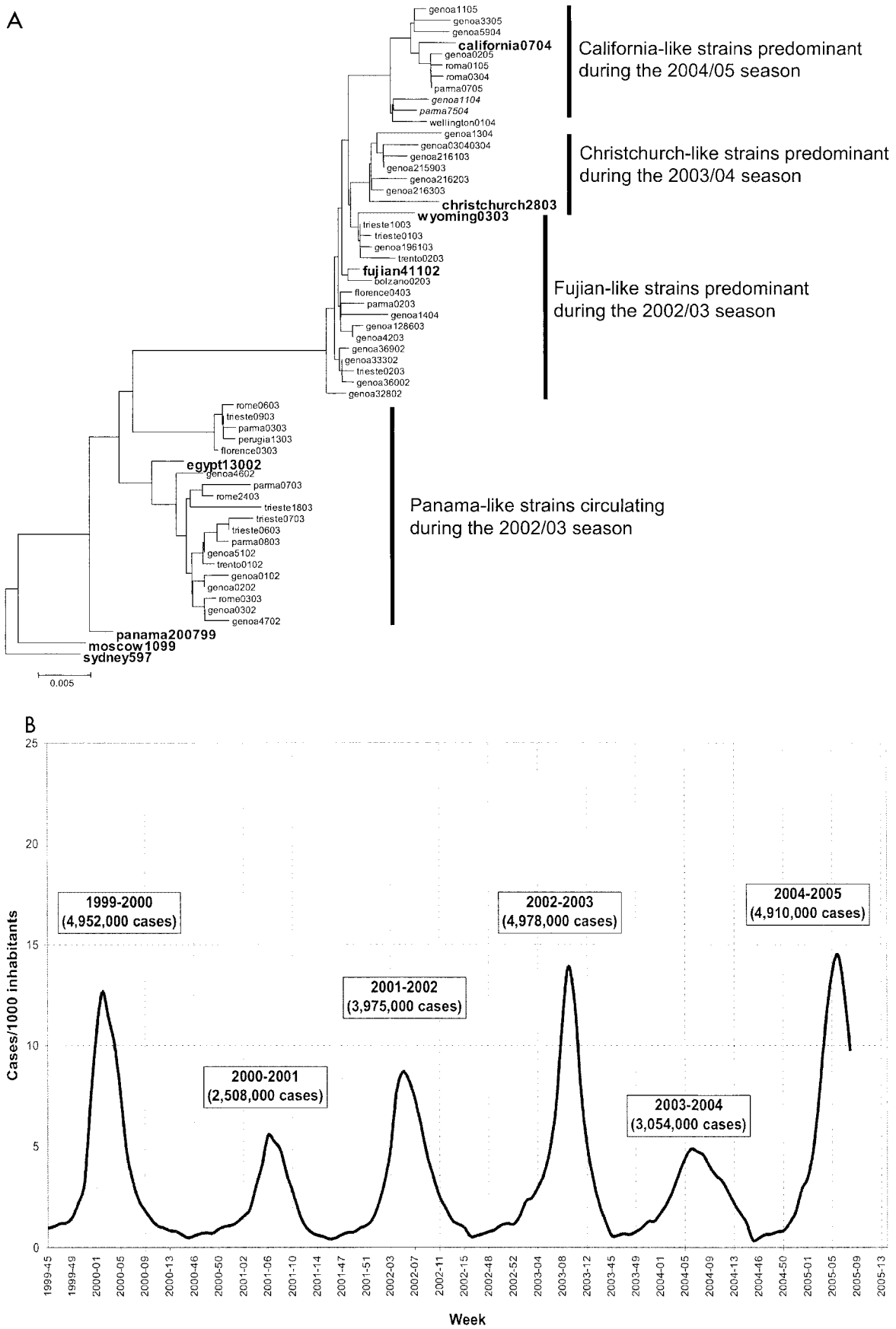

FIG. 1. (A) Phylogenetic tree including representative viruses isolated in Italy during the 2002-2003, 2003-2004, and 2004-2005 seasons, along with reference strains; (B) epidemic curves related to the last six seasons.

A/California/7/04, selected for the next season, appears to be the optimal choice to protect against these new circulating strains.

This work was supported in part by grants from CCM, Italian Centre for Disease Control.

REFERENCES

1. **Anonymous.** 2003. Recommended composition of influenza virus vaccines for use in the 2004 influenza season. Weekly Epidemiol. Rec. **78:**373–380.
2. **Anonymous.** 2003. Recommended composition of influenza virus vaccines for use in the 2004-05 influenza season. Weekly Epidemiol. Rec. **78:**89–92.
3. **Glezen, W. P., R. B. Couch, and H. R. Six.** 1982. The influenza herald wave. Am. J. Epidemiol. **116:**589–598.
4. **Manuguerra, J. C., and A. Mosnier.** 2000. Surveillance of influenza in Europe from October 1999 to February 2000. Eurosurveillance **5:**63–68.
5. **Smith, D. J., A. S. Lapeds, J. C. de Jong, T. M. Bestebroer, G. F. Rimmelzwaan, A. D. M. Osterhaus, and R. A. M. Fouchier.** 2004. Mapping the antigenic and genetic evolution of influenza virus. Science **305:**371–376.

Filippo Ansaldi*
Giancarlo Icardi
Roberto Gasparini
CIRI-IV, Department of Health Sciences
University of Genoa
Genoa, Italy

Cesare Campello
CIRI-IV, Department of Public Medicine Sciences
University of Trieste
Trieste, Italy

Simona Puzelli
National Influenza Center
Dipartimento di Malattie Infettive, Parassitarie e Immunomediate
Istituto Superiore di Sanità
Rome, Italy

Antonino Bella
National Centre for Epidemiology, Surveillance and Health Promotion
Istituto Superiore di Sanità
Rome, Italy

Isabella Donatelli
National Influenza Center
Dipartimento di Malattie Infettive, Parassitarie e Immunomediate
Istituto Superiore di Sanità
Rome, Italy

Stefania Salmaso
National Centre for Epidemiology, Surveillance and Health Promotion
Istituto Superiore di Sanità
Rome, Italy

Pietro Crovari
CIRI-IV, Department of Health Sciences
University of Genoa
Genoa, Italy

*Phone: 390 103535803
Fax: 390 0103538407
E-mail: filippo.ansaldi@unige.it