JOURNAL OF CLINICAL MICROBIOLOGY, June 1989, p. 1413–1416
0095-1137/89/061413-04$02.00/0

Vol. 27, No. 6

Laboratory Characterization of a Swine Influenza Virus Isolated from a Fatal Case of Human Influenza

PAUL A. ROTA,[1]* ELISABET P. ROCHA,[1] MAURICE W. HARMON,[1] VIRGINIA S. HINSHAW,[2] MARTHA G. SHEERAR,[2] YOSHIHIRO KAWAOKA,[3] NANCY J. COX,[1] AND THOMAS F. SMITH[4]

Influenza Branch, Division of Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333[1]; Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, Wisconsin 53706[2]; Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101[3]; and Section of Clinical Microbiology, Mayo Clinic, Rochester, Minnesota 55901[4]

Received 28 December 1988/Accepted 9 March 1989

A swine influenza virus-like type A (H1N1) virus, designated A/Wisconsin/3523/88, was isolated in September 1988 from a Wisconsin woman who had died with primary viral pneumonia. Antigenic analyses with hemagglutinin-specific monoclonal antibodies and postinfection ferret serum indicated that the hemagglutinin of A/Wisconsin/3523/88 was antigenically closely related to viruses currently circulating in swine. Genetic analysis of the A/Wisconsin/3523/88 virus by RNA fingerprinting and partial RNA sequence analysis of seven of the eight segments indicated that the genome of the human isolate was similar to that of enzootic swine viruses. These laboratory data supported the epidemiologic findings that this human infection occurred by transmission of an enzootic swine influenza virus and that the virus showed no major genetic changes potentially related to increased pathogenesis.

Human epidemic strains of influenza type A (H1N1 and H3N2) viruses can naturally infect animals that are frequently in contact with humans, for example, pigs and some species of birds. Also, a reservoir of enzootic swine influenza virus type A (H1N1) exists, and these classical swine influenza viruses are antigenically distinguishable from type A (H1N1) strains of human and duck origin (14). Direct transmission of these enzootic viruses from swine to humans has occurred occasionally since 1975. However, all of the laboratory-proven cases of swine influenza in humans have been sporadic, and person-to-person spread of the virus has rarely been documented (8, 9, 20). It has been proposed that genetic reassortment between viruses that infect animals and those that infect humans could occur within animal reservoirs. Since such reassortant viruses might have altered pathogenic potential and could be capable of causing widespread influenza epidemics in humans (14), the epidemiologic circumstances of cases of human infection with swine influenza viruses must be investigated rapidly to evaluate the potential for further spread of the virus in humans. In addition, the antigenic and genetic compositions of viruses that are isolated from these cases should be analyzed to differentiate between infection by enzootic swine influenza virus strains and infection by reassortants that might have acquired new pathogenic potential.

In September 1988, a swine influenza virus-like virus was isolated from a 32-year-old woman in Wisconsin who had died of primary viral pneumonia shortly after giving birth. While attending a county fair, the woman visited an exhibit where pigs reported to have an influenzalike illness were housed. Serum specimens from this patient showed a fourfold rise in antibody titer to swine influenza viruses but not in antibody titer to recent influenza virus H1N1 strains of human origin (3). In this report, we describe in greater detail the antigenic properties of this human isolate, A/Wisconsin/3523/88, and confirm its similarity to viruses currently circulating in swine by examining its total genome.

* Corresponding author.

Antigenic analyses. The viruses used in this study included A/Wis/3523/88, which was obtained from the Mayo Clinic; A/Sw/Ind/1726/88, A/Sw/Ky/2269/88, and A/Sw/Wis/1915/88, which were isolated from nasal swabs collected from sick pigs; and A/Ty/NC/1780/88, which was isolated from lung tissue from a sick turkey. All other viruses in Tables 1 and 2 were from the virus repositories at the University of Wisconsin and the Centers for Disease Control. Before being used in hemagglutination inhibition tests (19), all viruses were cultivated in 10- to 11-day-old embryonated chicken eggs.

The antigenic properties of A/Wis/3523/88 were analyzed in hemagglutination inhibition tests by using a series of hemagglutinin (HA)-specific monoclonal antibodies that had been generated against a contemporary swine virus. The monoclonal antibodies were prepared by immunizing BALB/c mice intramuscularly with 10,000 HA units of sucrose gradient-purified A/Sw/Ind/1726/88 virions. Mice were antigenically boosted 3 days before removal of their spleens. Hybridomas were prepared by fusing the immune spleen cells to NS-1 myeloma cells by the method of Kohler and Milstein (15). The HA specificity of the hybridomas was established by radioimmunoprecipitation as previously described (13). Hybridomas producing monoclonal antibodies were cloned twice by limiting dilution and grown as ascites in BALB/c mice. These monoclonal antibodies could distinguish between recent swine influenza virus isolates and earlier influenza virus H1N1 strains isolated from swine, birds, and humans (Table 1). The results indicated that the recent human isolate A/Wis/3523/88 was antigenically indistinguishable from viruses currently circulating in swine and turkeys but could be distinguished from A/New Jersey/8/76, a swine influenza virus that was isolated from a human in 1976 (8).

Postinfection ferret serum against A/Wis/3523/88 reacted in hemagglutination inhibition tests with swine influenza viruses, including viruses isolated from swine and turkeys in 1988, but not with a recent H1N1 virus of human origin. Also, in tests using ferret serum against a number of swine

TABLE 1. Antigenic analysis of influenza type A (H1N1) viruses isolated from humans, swine, and birds with monoclonal antibodies derived against a recently isolated swine influenza virus

Virus	Hemagglutination inhibition titer with the following monoclonal antibody:			
	3F2c	7B1b	2G1n	4A12a
Influenza A (H1N1) viruses[a]				
A/Sw/Wis/1/61	1,000	1,000	1,000	1,000
A/Sw/Wis/11/76	64,000	64,000	32,000	800
A/Sw/Wis/27/86	16,000	32,000	16,000	800
A/Sw/Wis/1915/88	51,200	25,600	51,200	800
A/Sw/Ind/1726/88	32,000	32,000	32,000	6000
A/Ty/NC/1780/88	16,000	16,000	8,000	1600
A/Dk/Alb/35/76	<100	12,800	12,800	<100
A/Ty/Ks/4880/81	32,000	100	<100	<100
Swine influenza A (H1N1) viruses[b]				
A/NJ/8/76	6,400	800	<100	<100
A/Wis/263/76[c]	25,600	51,200	51,200	1600
A/Wis/3523/88	25,600	12,800	25,600	800
Human influenza A (H1N1) virus A/Chile/1/83	<100	<100	<100	<100

[a] Isolated from swine and birds.
[b] Isolated from humans.
[c] Reference 18.

influenza viruses, A/Wis/3523/88 showed a pattern of reactivity that was very similar to those of recent swine influenza viruses isolated from pigs, turkeys, and humans (Table 2). Neuraminidase inhibition tests (12; data not shown) indicated that A/Wis/3523/88 contained an N1 neuraminidase antigen related to that of recently isolated swine influenza virus. The results (Tables 1 and 2) indicate that virus strain A/Wis/3523/88 has antigenic properties similar to those of recently isolated enzootic virus from swine but also establish that some antigenic drift from previous swine influenza viruses has occurred.

TABLE 2. Antigenic reactivities of influenza A (H1N1) viruses isolated from swine and humans with postinfection ferret serum against swine influenza viruses

Virus	Hemagglutination inhibition titer with postinfection ferret[a] antiserum to:					
	Sw/IA/30	Sw/Cam/39	Sw/Wis/67	NJ/76	Nev/82	Wis/3523/88
Influenza A (H1N1) viruses[b]						
A/Sw/IA/15/30	320	<10	40	20	20	40
A/Sw/Cam/39	160	640	160	80	160	160
A/Sw/Wis/1/67	160	<10	160	40	160	320
A/Sw/Wis/1915/88	640	20	160	320	1,280	1,280
A/Sw/Ind/1726/88	320	40	160	320	1,280	1,280
A/Ty/NC/1780/88	320	40	160	320	1,280	2,560
Swine influenza A (H1N1) viruses[c]						
A/NJ/8/76	640	80	160	320	2,560	5,120
A/Nevada/101/82[d]	160	80	160	640	2,560	5,120
A/Wis/3523/88	320	80	160	320	2,560	5,120
Current human influenza viruses						
A/Chile/1/83 (H1N1)	80	<10	40	<10	<10	10
A/Taiwan/1/86 (H1N1)	<10	<10	<10	<10	<10	<10
A/Sichuan/2/87 (H3N2)	<10	<10	<10	<10	<10	<10
B/Victoria/2/87	<10	<10	<10	<10	<10	<10

[a] Ferrets were inoculated intranasally with a 1:10 dilution of influenza virus in phosphate-buffered saline, and serum samples were obtained from the animals at 14 days postinfection. The underscored values represent homologous titers.
[b] Isolated from swine and birds.
[c] Isolated from humans.
[d] Reference 20.

Genetic analysis. The entire genomes of A/Wis/3523/88 and three recently isolated swine influenza viruses were compared by T1 nuclease digestion of purified viral RNA followed by two-dimensional gel electrophoresis as previously described (5, 6). Analysis of the genomic fingerprints of A/Wis/3523/88, A/Sw/Wis/1915/88, A/Sw/Ky/2269/88, and A/Sw/Ind/1726/88 indicated that of 60 specific oligonucleotide spots, A/Wis/3523/88 had only 4 to 11 spot differences from the three recent enzootic swine viruses. The fingerprints of A/Wis/3523/88, A/Sw/Ind/1726/88, and A/Sw/Wis/1915/88 are shown in Fig. 1. The three swine viruses also had 4 to 10 spot differences among themselves. In contrast, A/Wis/3523/88 had 20 spot differences from A/New Jersey/8/76, and the three swine viruses also differed from A/New Jersey/8/76 by 18 to 21 spots. Therefore, A/Wis/3523/88 showed very strong overall genetic similarity to the recent swine viruses. The degree of difference between A/Wis/3523/88 and the other 1988 swine viruses is similar to that observed when human isolates within one epidemic were compared (5a, 6, 26).

T1 mapping alone might not have detected viruses having reassorted *M* or *NS* genes, because these segments are quite conserved and account for relatively few of the oligonucleotide spots. Therefore, partial RNA sequence analysis (1, 22) was used to compare the *M* and *NS* gene sequences of A/Wis/3523/88 with those of A/Sw/Ind/1726/88 and A/Sw/Wis/1915/88. Segment-specific primers for sequencing *M* and *NS* genes were based on the published sequences of H3N2 viruses and have been previously described (7). Table 3 summarizes the results of partial sequence analysis, which indicated that the *M* and *NS* genes of A/Wis/3523/88 had a high degree of nucleotide homology with the *M* and *NS*

TABLE 3. Partial sequence analysis of A/Wis/3523/88

Gene and strain	% of A/Wis/3523/88 sequenced	% Homology to indicated gene
PA; A/Sw/TN/26/77[a]	82	96
PB1; A/Sw/Ont/2/81[a]	74	97
HA1		
A/Sw/Ind/1726/88	100	99
A/Sw/Wi/1915/88	100	99
A/NJ/8/76	100	94
NP; A/Sw/TN/26/77[a]	22	98
NA; A/Sw/Hok/2/81[a]	93	97
M		
A/Sw/Ind/1726/88	16	100
A/Sw/Wi/1915/88	16	100
A/Sw/IA/15/30[a]	34	92
NS		
A/SW/Ind/1726/88	21	98
A/Sw/Wi/1915/88	21	97
A/Sw/Ont/2/81[a]	98	98

[a] This comparison was based on sequences obtained from cDNA clones of A/Wis/3523/88; all others were based on sequences obtained from viral RNA.

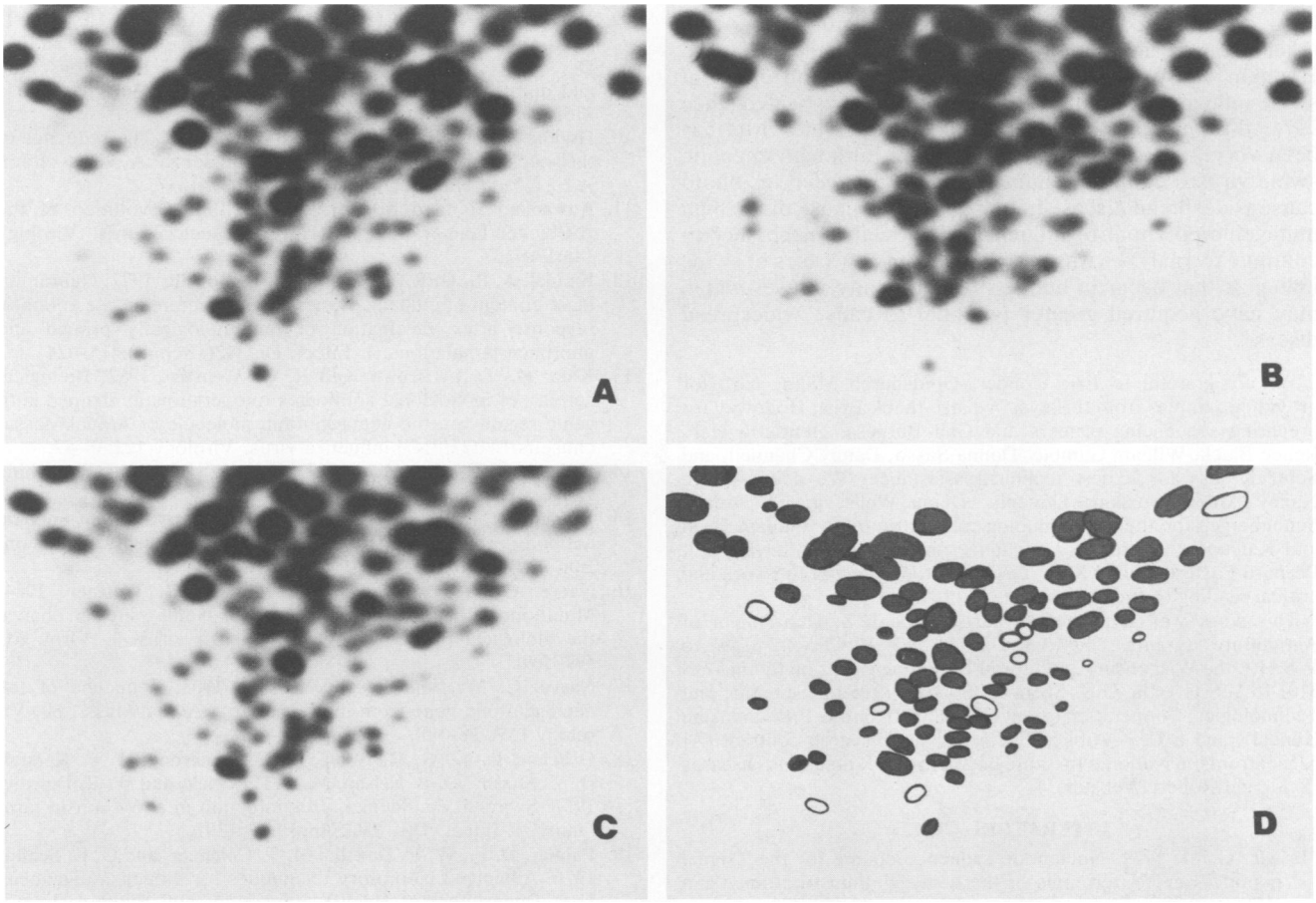

FIG. 1. Genomic comparison of A/Wis/3523/88 with recently isolated enzootic swine viruses. RNA fingerprints were prepared as described by Cox et al. (5). Panels A to C show the fingerprints of A/Sw/Ind/1726/88, A/Sw/Wis/1915/88, and A/Wis/3523/88, respectively. Panel D shows a spot comparison between A/Wis/3523/88 and A/Sw/Wis/1915/88; the dark areas represent spots common to the two viruses, and the open circles represent spots unique to A/Wis/3523/88.

genes of the other two 1988 swine viruses. In addition, full-length cDNA clones of the *PA*, *PB*1, *NA*, *NP*, *M*, and *NS* genes of A/Wis/3523/88 were constructed by reverse transcription of virion RNA by previously described methods (10). Partial sequences obtained from each of the clones (4) had strong homology to the sequence of the same gene of other swine influenza viruses (Table 3). These results confirm the swine origin of these genes and, in combination with the antigenic analyses and oligonucleotide map patterns, rule out the possibility that A/Wis/3523/88 is a reassortant virus containing genes from contemporary human influenza virus strains.

Previous investigations have shown that changes at certain locations in the *HA*1 domain of the gene for HA can affect the host range and pathogenicity of influenza viruses which infect animals (11, 17, 24). Therefore, we sequenced the *HA*1 domain of the HA-encoding genes of A/Wis/3523/88 and the two other swine viruses, A/Sw/Ind/1726/88 and A/Sw/Wis/1915/88 (Table 3). A/Wis/3523/88 had 99% nucleotide homology to the HA-encoding genes of the recent swine viruses and 94% nucleotide homology to A/New Jersey/8/76, which was isolated 12 years previously (2). The predicted amino acid sequences (data not shown) of the *HA*1 domains of A/Wis/3523/88 and the enzootic swine viruses revealed no differences which could affect the potential glycosylation or the receptor-binding ability of the molecule (2, 16, 21, 25). However, since the RNA was purified from virus grown in eggs, it is possible that subpopulations of virus that may grow preferentially in mammalian cell culture were not represented.

The results of this laboratory investigation demonstrate that A/Wis/3523/88 is representative of the enzootic type A (H1N1) viruses that currently circulate in U.S. swine, and they support the epidemiological data suggesting that this case in Wisconsin was caused by transmission of an enzootic swine virus to a human. These laboratory results correspond to those of the investigation (20) of a fatal case of swine influenza in 1982. In that case, a swine influenza virus isolated from the patient was shown by antigenic and RNA fingerprinting analyses to be very similar to a virus isolated from pigs in 1980, although direct contact with swine could not be established. The conclusions of our current report support those of previous investigations which indicated that enzootic swine influenza viruses can infect humans and cause disease that is sometimes fatal.

Laboratory studies have failed to reveal any unusual genetic properties in swine influenza viruses isolated from humans, including some from fatal infections, that suggest that changes are occurring in the virus that affect its potential to cause disease in humans. Rather, it it more likely that the unusual epidemiological or clinical circumstances of the patient are responsible for severe disease. For example, in

the 1982 study (20), the swine influenza virus was isolated from an immunosuppressed child. A case of fatal swine influenza was also reported in 1974 in a child who had Hodgkin's disease (23). Furthermore, in all the cases of swine influenza in humans that have been investigated since 1974, the person-to-person spread of the swine virus has been very limited (9, 20, 23). Therefore, although enzootic swine viruses can infect humans, they have been unable to cause widespread disease in humans (8). Because of the high mutagenic potential of influenza viruses, it is necessary to continue to analyze viruses isolated from new cases of swine influenza that occur in humans to detect any viruses which may have acquired greater potential to cause widespread disease.

We are grateful to Eric Gonder, Goldsburgh Milling Co., for providing samples from turkeys. We also thank Brian Holloway for preparing sequencing primers and Gail Burgess, Henrietta Hall, Renee Black, William Gamble, Donna Sasso, Danny Channell, and Scott Krauss for expert technical assistance. We acknowledge Nancy Arden, Suzanne Gaventa, Diana Wells, and Lawrence Schonberger for their epidemiological contribution; William Bean and Katsunori Okazaki for sequence analysis; and Alan Kendal, Bernard Easterday, and Robert Webster for review of the work and critical reading of the manuscript.

This work was supported in part by the U.S. Department of Agriculture (grants 86-CRCR-1-2194 and 87-CRSR-2-3159 to V.S.H.), the Wisconsin Agricultural Experiment Station (grant WIS 3101 to V.S.H.), the U.S.-Spain Joint Committee for Scientific and Technological Cooperation (grant CCA-8510/116 to E.P.R. and Alan Kendal), and a U.S. Public Health Service research contract (AI 52586) from the National Institute of Allergy and Infectious Diseases (Y.K. and Robert Webster).

LITERATURE CITED

1. **Air, G. M.** 1979. Nucleotide sequence coding for the "signal peptide" and N terminus of the hemagglutinin from an Asian (H2N2) strain of influenza virus. Virology **97**:468–472.
2. **Both, G. W., C. H. Shi, and E. D. Kilbourne.** 1983. Hemagglutinin of swine influenza virus: a single amino acid change pleiotropically affects viral antigenicity and replication. Proc. Natl. Acad. Sci. USA **80**:6996–7000.
3. **Centers for Disease Control.** 1988. Human infection with swine influenza virus—Wisconsin. Morbid. Mortal. Weekly Rep. **37**: 661–663.
4. **Chen, E. Y., and P. H. Seeburg.** 1985. Supercoil sequencing: a fast and simple method for sequencing plasmid DNA. DNA **4**:165–170.
5. **Cox, N. J., Z. S. Bai, and A. P. Kendal.** 1983. Laboratory-based surveillance of influenza A (H1N1) and (H3N2) viruses in 1980–1981: antigenic and genomic analyses. Bull. W.H.O. **61**: 143–152.

5a. **Cox, N. J., R. A. Black, and A. P. Kendal.** 1989. Pathways of evolution of influenza A (H1N1) viruses from 1977 to 1986 as determined by oligonucleotide mapping and sequencing studies. J. Gen. Virol. **70**:299–313.

6. **Cox, N. J., and A. P. Kendal.** 1984. Genetic stability of A/Ann Arbor/6/60 cold-mutant (temperature sensitive) live influenza virus genes: analysis by oligonucleotide mapping of recombinant vaccine strains before and after replication in volunteers. J. Infect. Dis. **149**:194–200.
7. **Cox, N. J., F. Kitame, A. P. Kendal, H. F. Maassab, and C. Naeve.** 1988. Identification of sequence changes in the cold-adapted, live attenuated influenza vaccine strain A/Ann Arbor/6/60. Virology **167**:554–567.
8. **Dowdle, W. R., and M. A. W. Hattwick.** 1977. Swine influenza virus infections in humans. J. Infect. Dis. **136**(Suppl.):386–389.
9. **Hodder, R. A., G. C. Graydos, R. G. Allen, F. H. Top, T. Nowosiwsky, and P. K. Russell.** 1977. Swine influenza at Fort Dix, New Jersey (January–February 1976). III. Extent of spread and duration of the outbreak. J. Infect. Dis. **136**(Suppl.):369–375.
10. **Huddleston, J. A., and G. G. Brownlee.** 1982. The sequence of the nucleoprotein gene of human influenza A virus strain, A/NT/60/68. Nucleic Acids Res. **10**:1029–1037.
11. **Kawaoka, Y., and R. G. Webster.** 1985. Evolution of the A/Chicken/Pennsylvania/83 (H5N2) influenza virus. Virology **146**:130–137.
12. **Kendal, A. P., G. R. Noble, and W. R. Dowdle.** 1977. Neuraminidase content of influenza vaccines and neuraminidase antibody response after vaccination of immunologically primed and unprimed populations. J. Infect. Dis. **136**(Suppl.):415–424.
13. **Kida, H., L. E. Brown, and R. G. Webster.** 1982. Biological activity of monoclonal antibodies to operationally defined antigenic regions on the hemagglutinin molecule of A/Seal/Massachusetts/1/80 (H7N7) influenza virus. Virology **122**:38–47.
14. **Kilbourne, E. D. (ed.).** 1987. Influenza. Plenum Publishing Corp., New York.
15. **Kohler, G. R., and C. Milstein.** 1976. Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion. Eur. J. Immunol. **6**:511–519.
16. **Naeve, C. W., V. S. Hinshaw, and R. G. Webster.** 1984. Mutations in the hemagglutinin receptor-binding site can change the biological properties of an influenza virus. J. Virol. **51**: 567–569.
17. **Naeve, C. W., and R. G. Webster.** 1983. Sequence of the hemagglutinin gene from influenza virus A/Seal/Mass/1/80. Virology **129**:298–308.
18. **O'Brian, R. J., G. R. Noble, B. C. Easterday, A. P. Kendal, D. M. Shasby, D. B. Nelson, M. A. Hattwick, and W. R. Dowdle.** 1977. Swine-like influenza virus infection in a Wisconsin farm family. J. Infect. Dis. **136**(Suppl.):390–397.
19. **Palmer, D. F., W. R. Dowdle, M. T. Coleman, and G. C. Schild.** 1976. Advanced laboratory techniques for influenza diagnosis. U.S. Department of Health, Education, and Welfare (Immunology ser., no. 6). Centers for Disease Control, Atlanta.
20. **Patriarca, P. A., A. P. Kendal, P. C. Zakowski, N. J. Cox, M. S. Trautman, J. D. Cherry, D. M. Auerbach, J. McCusker, R. R. Belliveau, and K. D. Kappus.** 1984. Lack of significant person-to-person spread of swine influenza-like virus following fatal infection in an immunocompromised child. Am. J. Epidemiol. **119**:152–158.
21. **Rogers, G. N., and J. C. Paulson.** 1983. Receptor determinants of human and animal influenza virus isolates: differences in receptor specificity of the H3 hemagglutinin based on species of origin. Virology **127**:361–373.
22. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
23. **Smith, T. F., E. O. Burgert, W. R. Dowdle, G. R. Noble, R. J. Campbell, and R. E. Van Scoy.** 1976. Isolation of swine influenza virus from autopsy lung tissue of man. N. Engl. J. Med. **294**:708–710.
24. **Webster, R. G., V. S. Hinshaw, W. J. Bean, K. L. Van Wyke, J. R. Geraci, D. J. St. Aubin, and G. Petursson.** 1981. Characterization of an influenza A virus from seals. Virology **113**: 712–724.
25. **Wiley, D. C., and J. J. Skehel.** 1987. The structure and function of the membrane glycoprotein of influenza virus. Annu. Rev. Biochem. **56**:365–394.
26. **Young, J. F., U. Desselberger, and P. Palese.** 1979. Evolution of human influenza viruses in nature: sequential mutations in the genomes of new H1N1 isolates. Cell **18**:73–83.