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Effect of Simultaneous Administration of Cold-Adapted and Wild-Type Influenza A Viruses on Experimental Wild-Type Influenza Infection in Humans

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On the basis of the ability of the attenuated cold-adapted strain of influenza A virus to suppress disease production in ferrets simultaneously infected with epidemic influenza virus (P. Whitaker-Dowling, H. F. Maassab, and J. S. Youngner, J. Infect. Dis. 164:1200–1202, 1991), an evaluation of the ability of the cold-adapted virus to modify clinical disease in humans was made. Adult volunteers with prechallenge serum hemagglutination-inhibition titers to the influenza A/Kawasaki/86 (H1N1) virus of ≤1:8 received either 10^7 50% tissue culture infective doses of the wild-type A/Kawasaki virus or a mixture of 10^7 50% tissue culture infective doses of each of the wild-type virus and a cold-adapted A/Kawasaki reassortant virus by intranasal drops in a randomized, double-blind fashion. Symptoms and wild-type virus shedding were assessed daily for 6 days following challenge. Results were compared with those derived from another group of volunteers who received only cold-adapted virus. Volunteers who received the mixed inoculum of cold-adapted and wild-type viruses had lower symptom scores than those who received wild-type virus alone, suggesting that coinfection with the cold-adapted virus may modify wild-type virus infection, but the differences were not statistically significant in this small study. The data demonstrate that administration of cold-adapted influenza A virus to humans at the time of wild-type virus infection is a safe procedure.

The cold-adapted (ca) mutant of the A/Ann Arbor/6/60 (H2N2) strain of influenza A virus is adapted to grow efficiently at 25°C and has a temperature-sensitive phenotype that restricts its growth at 37°C (2). Reassortment of this virus with wild-type (wt) influenza A viruses allows the selection of reassortant viruses that contain the hemagglutinin (HA) and neuraminidase (NA) gene segments of the wt parent virus and the other six genome segments of the ca parent virus. Convincing documentation suggests that such reassortant viruses are attenuated in animals and humans (3, 5, 6). Clinical studies have demonstrated that the ca reassortant viruses are safe and effectively induce immunity against influenza virus when they are administered by the intranasal route (4).

In addition to their immunizing effects, the ca reassortant viruses act as dominant-negative mutants; i.e., in mixed infections of cell cultures or embryonated eggs, they directly interfere with the growth of pathogenic influenza A viruses (8, 10; unpublished data). Experiments in cell culture have demonstrated that this interference occurs at the level of gene expression and that in mixed infections the viral proteins synthesized are predominantly those of the ca virus. Cold-adapted reassortant viruses are dominant over wt viruses, which bear completely unrelated transmembrane glycoproteins, indicating that the dominance is independent of the HA and NA genes (8). Several lines of evidence, including the use of single-gene reassortants, show that gene segment 7, encoding the M1 and M2 proteins, plays a primary role in the dominance phenotype of the ca virus (11).

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Most recently, it has been found that the ca reassortant virus suppresses clinical disease in ferrets when given simultaneously with a virulent influenza A virus (9). The ca virus effectively prevents disease even when the virulent virus is of a different subtype than the ca virus, confirming the irrelevance of the transmembrane glycoproteins to the dominance phenotype. Interestingly, ferrets produce antibodies to both subtypes under these circumstances in the absence of clinical disease. These findings suggest the possibility of the development of a novel class of antiviral agents for influenza, namely, a live, dominant-negative, attenuated mutant virus that interferes with the replication of epidemic strains of virus. The current report describes a randomized, double-blind study which assessed the ability of ca influenza virus to modify disease caused by a homologous wt influenza A virus when both viruses were administered simultaneously to seronegative human volunteers.

MATERIALS AND METHODS

Subjects. A total of 27 healthy adult volunteers with prechallenge serum hemagglutination-inhibition (HI) titers of ≤1:8 to A/Kawasaki/86 (H1N1) virus participated in the inpatient challenge portion of the study. After informed consent was obtained, volunteers underwent a complete physical examination and screening laboratory tests, including complete blood count, chemistry profile, and urine pregnancy tests. Volunteers with normal results were then admitted to the challenge facility and observed overnight prior to challenge.

Virus challenge. On the following morning, the volunteers were randomly assigned to receive either the A/Kawasaki/9/86 wt virus (CI 2-1, lot E-262; PRI/DynCorp, Rockville, Md.) at a dose of 10^7 50% tissue culture infective doses ($TCID_{50}$s) in a

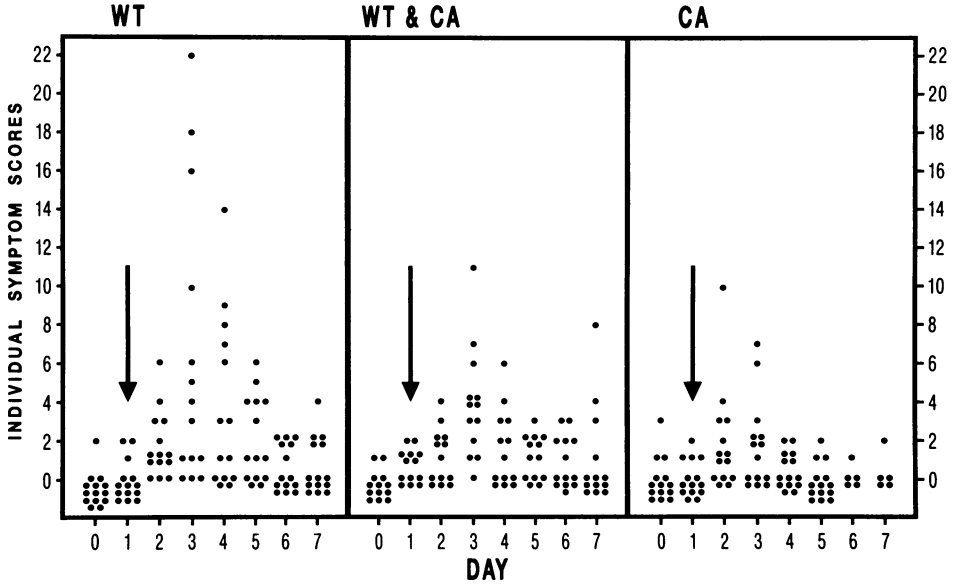

FIG. 1. Individual symptom scores for volunteers who were given intranasally wt virus alone (WT), a mixed inoculum containing wt virus plus ca virus (WT & CA), or ca virus alone (CA). Arrows indicate the day of infection.

volume of 0.5 ml administered intranasally or a mixture of the wt A/Kawasaki/86 virus and the ca A/Kawasaki/86 virus (CR 125, lot E-271; PRI/DynCorp), each at a dose of 10^7 $TCID_{50}$s (total dose, $10^{7.3}$ $TCID_{50}$s of virus) in a total volume of 0.5 ml. The titer of each virus preparation was adjusted by dilution in sterile veal infusion broth (VIB) immediately prior to administration. Two separate challenges were performed. In the first challenge, which was conducted in May 1991, seven volunteers received wt virus alone and seven volunteers received wt and ca viruses. In the second challenge, which was conducted in October 1991, seven volunteers received wt virus alone and six volunteers received wt and ca viruses. Each study was conducted in a double-blind fashion, and the data were combined for presentation here.

Physical assessment. The volunteers were observed for 7 days after challenge for signs and symptoms of influenza. Each morning, the subjects completed a symptom report card in which symptoms were graded on a scale of 1 to 3, with 1 being mild (definitely present but mild), 2 being moderate (interfering with normal activities), and 3 being severe (worst ever; would consider missing work or school). Symptom report cards were reviewed with the subject on each morning by a physician who was blinded as to the subject's treatment assignment and who also performed a brief physical assessment. Symptoms were classified as upper respiratory, including photophobia or eye pain, earache, stuffy nose, runny nose, sneezing, postnasal drip, and sore throat; lower respiratory symptoms, including hoarseness, cough, and dyspnea; and systemic symptoms, including fever, chills, muscle ache, malaise, and anorexia. Each symptom was scored separately. Individuals with a score of 4 or greater for upper respiratory symptoms or with a total score of 3 or greater for lower respiratory symptoms were considered to have a respiratory illness. Individuals with a total upper respiratory score of 3 or greater on 2 consecutive days were also considered to have a respiratory illness. Individuals with a respiratory illness and a total score of 3 or greater for systemic symptoms and/or fever were considered to have flu-like illness. Fever was defined as an oral temperature of 38.0°C or greater, which was confirmed by repeat oral measurement within 1 h.

Virus isolation. Nasal washes for virus isolation were obtained prior to inoculation and daily for 7 days after virus isolation. The nares were washed with 10 ml of Ringer's lactate, and the washing returns were mixed with 5× VIB to a final concentration of 1× VIB and were transported to the laboratory on wet ice. Samples were immediately inoculated on Madin-Darby canine kidney (MDCK) cells and were incubated at 33°C, while the remainder was frozen in aliquots at −70°C. The level of wt virus replication was then determined by plaque assay of a fresh aliquot of each sample exhibiting a viral cytopathic effect as well as of the sample from the day prior to and the day after such samples were obtained. This assay was carried out on MDCK cells at 38.5°C by using an overlay of 0.6% agarose–L-15 nutrient medium. In preliminary experiments it was determined that incubation at this temperature produces a greater than 10,000-fold reduction in the level of replication of the ca virus, with no significant effect on the replication of the wt virus (data not shown). Each titration was performed twice on fresh aliquots, and the higher of the two titrations was taken as the PFU per milliliter value. In a subset of samples, plaque assays of nasal washes were also carried out at 34°C to evaluate the presence of reassortant viruses (see below). Sera obtained prechallenge and 3 weeks postchallenge were tested for the level of antibody to the challenge virus by HI by using the ca A/Kawasaki/86 virus as antigen.

Studies of the ca A/Kawasaki/86 virus. The results for subjects who received wt virus alone or a mixture of wt and ca viruses in the inpatient challenge studies described above were compared with the results for subjects who received the ca A/Kawasaki/86 virus alone (lot E-271; PRI/DynCorp) as outpatients during the period of time that the inpatient challenge studies were performed. A total of 14 healthy adults with HI titers in serum of ≤1:8 received the ca A/Kawasaki/86 virus at a dose of 10^7 $TCID_{50}$s intranasally. Subjects in this group completed the same symptom diary card used in the challenge studies and measured their own temperatures daily for 7 days, but they were not examined daily. Nasal washings for virus isolation were obtained prior to inoculation and daily on days

TABLE 1. Clinical responses of volunteers challenged with wt or ca A/Kawasaki/86 virus or simultaneously challenged with both viruses

Virus shedding	No. of subjects	No. of subjects with:			Peak temp (°C)[a]	Peak symptom score[b]	No. shedding wt virus[c]	No. ill shedding wt virus
		Illness[d]	Flu-like illness[e]	Fever[f]				
wt alone	14	6	4	3	38.9, 38.5, 38.4	6.5 ± 1.9	10	5
wt + ca	13	5	2	2	38.0, 38.0	4.2 ± 0.8	8	4
ca alone	14	2	0	0	NA[g]	2.6 ± 0.7	NA[h]	NA[h]

[a] Of those with fever.
[b] Values are means ± standard errors.
[c] wt virus, virus able to form plaques at 38.5°C.
[d] Subjects with a score of 4 or greater on any day or 3 or greater on 2 consecutive days were considered ill.
[e] Flu-like illness was defined as a score of 4 or greater for respiratory symptoms plus a score of 3 or greater for systemic symptoms and/or fever.
[f] Fever was defined as a confirmed oral temperature of 38.0°C or greater.
[g] NA, not applicable.
[h] Both individuals with symptoms shed ca virus only.

3 to 7 after virus inoculation and were handled as described above for the challenge studies.

Analysis of viral proteins. Monolayer cultures of MDCK cells in 96-well trays were inoculated with agar plugs from well-separated virus plaques and were incubated at 34°C. The monolayers were examined by light microscopy and were radiolabeled with [^{35}S]methionine for 2 h at the first sign of virus-induced cytopathology, usually between 24 and 48 h after the addition of the agar plug to the well. A cell lysate was prepared and the viral proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described previously (8).

RESULTS

Clinical responses to virus challenge. Individual symptom scores by day for the subjects in the three challenge groups are plotted in Fig. 1. The patterns of symptoms in the group given the mixed inoculum of wt plus ca viruses suggest that clinical disease was milder in those individuals than in the group given the wt virus alone. The individual symptom scores for volunteers who received the wt virus alone reached severity levels not seen for the other groups of subjects. In addition, the pattern of symptom scores for the group given the mixed inoculum was quite similar to that for the group given only the ca virus, suggesting that the level of symptoms in the mixed inoculum group was only slightly above that expected for the group given the ca virus alone. It is of interest that the difference in the pattern of symptom scores could be detected in both the challenge experiment performed in May 1991 and the second challenge performed in October 1991. The difference in mean peak symptom score between those receiving wt virus alone (6.5 ± 1.9) and those receiving wt and ca viruses simultaneously (4.2 ± 0.8) did not achieve statistical significance (P = 0.27; two-tailed t test) (Table 1). However, the difference in the mean peak symptom scores between those receiving wt virus alone and those receiving ca virus alone (2.6 ± 0.2) also was not statistically significant in this small study (P = 0.07). The incidence of fever, defined by a temperature of greater than 38.0°C, was similar in those who received the wt virus alone (three subjects) and those who received the mixed inoculum of wt and ca viruses (two subjects). However, the fevers that were observed were higher in those who received the wt virus alone (Table 1). None of the subjects in the group given the ca virus alone showed a febrile response.

Virologic and serologic responses. The geometric mean prechallenge HI titers in the sera of the subjects in the three challenge groups were similar. All of the volunteers given wt virus alone, 12 of 13 volunteers in the group given wt and ca viruses, and 9 of 14 volunteers given ca virus alone had a fourfold rise in antibody titer following challenge (Table 2). The replication of wt virus in the respiratory tract was assessed by determining the titer of virus in nasal secretions at 38.5°C, a temperature at which the replication of the ca virus is restricted by more than 10,000-fold. In the group receiving wt virus alone, plaque-forming viruses were recovered at 38.5°C from at least one sample from 10 of 14 subjects (71.4%), and in the group given the mixed inoculum of wt and ca viruses, plaque-forming viruses were recovered from 8 of 13 subjects (61.5%). There was no significant difference in the mean peak titer of virus shed by the two groups (Table 2). However, there was some difference in the pattern of virus shedding, as shown in Fig. 2. In subjects given the wt virus alone, peak virus titers were recorded 2 days after infection, while in the group given the mixed inoculum, the peak of virus shedding was delayed until day 4 after infection. It must be pointed out that a delayed pattern of virus shedding in the mixed inoculum group was

TABLE 2. Antibody response and wt virus shedding of volunteers challenged with wt or ca A/Kawasaki/86 virus or simultaneously challenged with both viruses

Virus challenge	No. of volunteers	Mean $\log_2$ HI titer in serum[a]		wt virus replication	
		Preinoculation	Postinoculation	No. of subjects shedding wt virus	Mean peak titer[a,b]
wt alone	14	2.2 ± 0.2	5.8 ± 0.3[c]	10	2.25 ± 0.38[c]
wt + ca	13	1.8 ± 0.2	6.0 ± 0.4[c]	8	2.02 ± 0.46[c]
ca alone	14	1.7 ± 0.2	4.1 ± 0.4	NA[d]	NA

[a] Values are means ± standard errors.
[b] $\log_{10}$ PFU per milliliter assayed at 38.5°C.
[c] P = not significant when comparing the wt and ca group with the wt alone group.
[d] NA, not applicable.

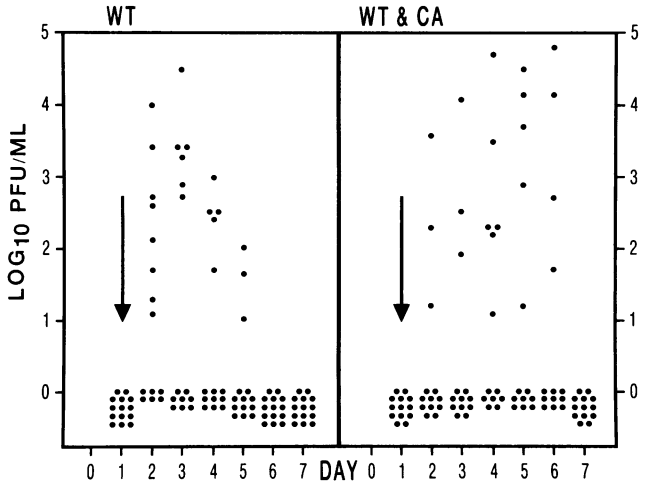

FIG. 2. Titers (in PFU per milliliter) of virus present in nasal washings obtained from volunteers who were given intranasally wt virus alone (WT) or a mixed inoculum containing wt virus plus ca virus (WT & CA). Arrows indicate the day of infection.

seen only in the second challenge performed in October 1991 and not in the challenge experiment performed in May 1991. There is no ready explanation for this variation. In addition, there was no correlation between the virus titers that were recorded and the severity of symptoms in the individuals from whom the specimens were obtained.

A random sampling of single clones of virus isolated at 34°C from different nasal washings confirmed that the majority of the viruses recovered from subjects given the mixed inoculum were wt virus. The absence of ca virus in the nasal washings is not surprising. It has been shown that volunteers given ca virus shed virus for only a short time (mean, 0.2 days) and at a low peak titer (mean, $10^{0.6}$ $TCID_{50}$/ml) in comparison with the times and titers for volunteers given wt virus (mean, 4.0 days and $10^{3.6}$ $TCID_{50}$s/ml, respectively) (7). In the present study, the mean titer of virus in the volunteers given ca virus alone was $10^{1.1}$ $TCID_{50}$s/ml. Under these conditions it would be unlikely that the ca virus could be detected by the endpoint dilution methods used in the present study unless its growth had been significantly rescued by coinfection with the wt virus.

Further clonal analysis was carried out on nasal washings obtained from five volunteers given the mixed inoculum of wt and ca viruses. The virus-positive specimens tested were taken on different days (days 2 to 6) after infection. Single clones obtained at the permissive temperature (34°C) were randomly selected and further amplified at 34°C in MDCK cells in 96-well trays. The cells were labeled after infection with [^{35}S]methionine, and the proteins were analyzed by SDS-PAGE. Under these conditions, the PA, NP, M1, and NS1 proteins of the ca reassortant A/Kawasaki/86 virus (these proteins were derived from the A/Ann Arbor/6/60 virus) can be distinguished from those of the wt A/Kawasaki/86 virus (8). These migration differences were used to detect the presence of reassortants in the viruses recovered from the nasal washings of the volunteers given the mixed inoculum of wt and ca viruses.

A total of 340 virus clones were isolated from the nasal washings, and 8 of these (2.4%) were identified as reassortants by analysis of the migration of their proteins. Of the eight reassortants, seven were apparent single-gene reassortants, five of which derived the M gene segment from the ca virus and two of which derived the NS gene segment from the ca virus; the other reassortant derived two gene segments from the ca virus (PA and M genes). One of the single-gene reassortants containing the ca virus M gene was tested in a mixed wt and ca infection of MDCK cells and was found to be dominant over the wt A/Kawasaki/86 virus (data not shown). This finding is in agreement with the previous finding that segment 7 (M gene) of the ca virus is associated with the dominance phenotype (11).

DISCUSSION

Studies demonstrating the ability of the attenuated ca virus to interfere with epidemic strains of influenza A virus in animals have suggested a potentially expanded prophylactic use of ca virus vaccine (8, 9, 10). This potential is particularly pertinent in the case of influenza since the incubation period of this disease is short (1 to 2 days) in comparison with the time required for the vaccine to induce immunity (1 to 2 weeks). For this reason, we evaluated the effect of simultaneous administration of ca virus and wt virus on the course of experimental influenza of humans.

Two important observations can be made on the basis of the data presented here. First, the current study presents no contraindication to the use of the ca virus as a vaccine in the face of an influenza epidemic. There was no evidence of an enhancement of clinical disease observed in the volunteers who were infected with both viruses. The presence of wt virus did not rescue or enhance the growth of the ca vaccine virus to any detectable extent. Although reassortant viruses were generated during the mixed infection, there was no evidence of enhanced pathogenicity caused by these reassortants. These findings indicate that a synergistic virulence effect does not occur during the mixed infection.

Second, when volunteers were challenged intranasally with an inoculum containing a mixture of equal amounts of ca and wt viruses, there appeared to be a slight reduction in the frequency and severity of illness produced in comparison with that seen in volunteers given wt virus alone. In addition, there was some suggestion that the pattern of wt virus shedding was altered by the ca virus, although not consistently. However, within the limitations of the challenge model, the study suggests that the transdominant phenotype of the ca virus may be expressed in humans, as it is in cell culture, embryonated eggs, and ferrets (8–10).

The recovery of reassortant viruses generated in the volunteers provides additional evidence relevant to the expression of the dominance phenotype of the ca virus in humans and records another instance of reassortment of influenza viruses in humans (1). The fact that reassortants were generated at a significant rate (2.4%) implies that double infections of individual cells occur, and such double infections are a prerequisite for the expression of dominance. The finding that most (six of eight) of the reassortants contained segment 7 (M gene) of the ca virus suggests that the dominance phenotype was expressed in doubly infected volunteers. Previous studies in cell culture demonstrated that the dominant gene of the ca virus (M gene) tends to be disproportionately present in reassortants from mixed infections with ca and wt viruses (8; unpublished data). It is noteworthy that segment 7 (M gene) of the ca virus is one of the genes capable of conferring an attenuated phenotype (7).

It should be recognized that demonstration of the transdominant phenotype in humans is not straightforward. First, certain aspects of the human challenge model are not ideal for the evaluation of attenuated transdominant viruses as modifi-

ers of influenza infection. For example, the H1N1 subtype of influenza A virus used in the present study has been in continuous circulation in the human population since 1977; it is unlikely that any of the subjects in the study were immunologically naive, even though their sera contained low levels of HI antibodies to A/Kawasaki/86 (H1N1). This made it necessary to use relatively high doses of wt virus (10^7 $TCID_{50}$s) to induce clinical illness. Even with this inoculum, clinical influenza was produced in very few of the volunteers. The incidence and extent of illness produced by the wt virus in the present study were so low that even when disease produced by wt virus alone was compared with the effect of the ca virus alone, it was not possible to show statistically significant differences.

Second, the large inoculum of wt virus may have limited the demonstration of dominance of the ca virus virologically, although the delayed virus shedding in the doubly infected group is suggestive. Under natural conditions of infection, the amount of wt influenza virus that would make up the infectious dose would likely be at least several orders of magnitude less than the amount used in the challenge experiments described here. For this reason, it is possible that the ca virus would have a more dramatic effect on the ability of the wt virus to produce clinical disease under natural conditions of infection with epidemic strains of virus.

In addition to these factors, human challenge studies with influenza viruses are restricted to the use of the intranasal route of inoculation of virus; under normal circumstances, the major route of infection by influenza virus is via small-particle aerosol. The intranasal route of infection may limit the opportunities for expression of dominance during a double infection.

Several important questions are still unresolved. Although the data presented here suggest that simultaneous infection of the respiratory tract with the ca and wt viruses may reduce the severity of disease, it is not known what the outcome would be if the two viruses were given sequentially. For example, how long after intranasal administration of ca virus can the trans-dominance effect be demonstrated? Conversely, how effective could the ca virus be at preventing disease if the wt virus was given a head start? Would the ca virus be effective in reducing disease in humans, as it is in ferrets, if the ca and wt viruses contain glycoproteins of completely different antigenic subtypes (9)? Since the antigenic subtypes of influenza A virus are irrelevant to the dominance phenotype (8, 9), the attenuated virus might offer short-term protection in the event of an antigenic "shift" and the appearance of a new influenza strain for which there is no available vaccine.

It seems evident that the dominance phenotype is a desirable characteristic for any live virus vaccine. In addition to offering protection against any wt revertants that might arise during the vaccination process, the dominance phenotype may provide short-term protection against any natural exposure to an epidemic virus that occurs before an effective antibody response is mounted. More complete evaluation of the clinical usefulness of this effect will require further studies under field conditions.

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REFERENCES

1. **Cox, N., Z. S. Bai, and A. P. Kendal.** 1983. Laboratory-based surveillance of influenza A (H1N1) and A (H3N2) viruses in 1980–81: antigenic and genomic analysis. Bull. W.H.O. **61:**143–152.
2. **Maassab, H. F.** 1967. Adaptation and growth characteristics of influenza virus at 25°C. Nature (London) **213:**612–614.
3. **Maassab, H. F., A. P. Kendal, G. D. Abrams, and A. Monto.** 1982. Evaluation of a cold-adapted recombinant influenza virus vaccine in ferrets. J. Infect. Dis. **146:**780–790.
4. **Murphy, B. R., R. M. Chanock, M. L. Clements, W. C. Anthony, A. J. Sear, L. A. Cisneros, M. B. Rennels, E. H. Miller, R. E. Black, M. M. Levine, R. F. Betts, R. G. Douglas, Jr., H. F. Maassab, N. J. Cox, and A. P. Kendal.** 1981. Evaluation of A/Alaska/6/77 (H3N2) cold-adapted recombinant viruses derived from A/Ann Arbor/6/60 cold-adapted virus in adult seronegative volunteers. J. Virol. **62:**488–495.
5. **Murphy, B. R., M. L. Clements, H. P. Madore, J. Steinberg, S. O'Donnell, R. Betts, D. Demico, R. C. Reichman, R. Dolin, and H. F. Maassab.** 1984. Dose response of cold-adapted, reassortant influenza A/California/10/78 virus (H1N1) in adult volunteers. J. Infect. Dis. **149:**816.
6. **Murphy, B. R., M. B. Rennes, R. G. Douglas, Jr., R. F. Betts, R. B. Couch, T. R. Cate Jr., R. M. Chanock, A. P. Kendal, H. F. Maassab, S. B. Suwanagool, S. B. Sotman, I. A. Cisneros, W. C. Anthony, D. R. Nalin, and M. M. Levine.** 1980. Evaluation of influenza A/Hong Kong/123/77 (H1N1) ts-1A2 and cold-adapted recombinant viruses in seronegative adult volunteers. Infect. Immun. **29:**348–355.
7. **Snyder, M. H., M. L. Clements, D. De Borde, H. F. Maassab, and B. R. Murphy.** 1985. Attenuation of wild-type human influenza A virus by acquisition of the PA polymerase and matrix protein genes of influenza A/Ann Arbor/6/60 cold-adapted donor virus. J. Clin. Microbiol. **22:**719–725.
8. **Whitaker-Dowling, P., W. Lucas, and J. S. Youngner.** 1990. Cold-adapted vaccine strains of influenza A virus act as dominant negative mutants in mixed infections with wild-type influenza A virus. Virology **175:**358–364.
9. **Whitaker-Dowling, P., H. F. Maassab, and J. S. Youngner.** 1991. Dominant-negative mutants as antiviral agents: simultaneous infection with the cold-adapted live-virus vaccine for influenza A protects ferrets from disease produced by wild-type influenza A. J. Infect. Dis. **164:**1200–1202.
10. **Whitaker-Dowling, P., and J. S. Youngner.** 1989. Dominance of cold-adapted vaccine strains of influenza A viruses in mixed infections with wild-type virus, p. 402–407. *In* D. Kolakofsky and B. Mahy (ed.), Genetics and pathogenicity of negative strand viruses. Elsevier, Amsterdam.
11. **Whitaker-Dowling, P., R. Zvolenski, and J. S. Youngner.** 1991. The genes associated with trans-dominance of the influenza A cold-adapted live virus vaccine. Virology **180:**81–87.