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Infectivity and reactogenicity of reassortant cold-adapted influenza A/Korea/1/82 vaccines obtained from the USA and USSR

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The safety and immunogenicity of two live influenza A virus vaccine strains, the CR 59 and 17/25/1 cold-adapted (ca) reassortants, were evaluated in 170 healthy young adult volunteers. The vaccines were produced by recombining A/Korea/1/82 (H3N2) wild-type virus with either A/Ann Arbor/6/60 (H2N2) or A/Leningrad/134/17/57 (H2N2) ca donors of attenuation. Both vaccines were well tolerated in volunteers. The 17/25/1 strain, prepared from A/Leningrad, infected at least 70% of seronegative volunteers after the first dose and 84% after the second; the CR 59 strain infected 62% and 72% of volunteers after first and second doses, respectively. Among the vaccinees who were initially seropositive, 17/25/1 infected 66% after one dose and 85% after two, while CR 59 infected 62% and 71%, respectively. Despite differences in temperature sensitivity, genetic composition, and serological reactivity to monoclonal antibodies, both vaccines behaved almost identically in animal models and man. We conclude that both donors of attenuation may be of great potential value.

Because the existing inactivated influenza vaccines are relatively ineffective, even when given annually (*1*), there is increasing interest in live attenuated vaccines administered by the intranasal route. In the USA and USSR an approach now being pursued for the rapid attenuation of new influenza A viruses involves the use of an empirically derived donor of attenuation which grows well at a temperature that is suboptimal for the replication of wild-type virus (*2*, *3*). Reassortant viruses derived from co-infection with cold-adapted (*ca*) donor strains and virulent influenza A virus can be rapidly and reliably obtained and, when evaluated in adults and children, have been found to be safe, stable, and antigenic in both the USA and USSR (*4*). Although based on the same concepts, the methods used in the two countries show many differences in detail and it was uncertain whether the two *ca* vaccine strains really had similar properties of attenuation and ability to induce protective antibody when administered to man.

We therefore prepared, under identical conditions, two influenza viruses from seed *ca* viruses provided

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by Dr H. Maassab in the USA and Dr Y. Ghendon in the USSR. The two seed viruses were derived from the same "wild" human influenza parent, A/Korea/1/82, but different *ca* viruses were used as the donors of attenuation. The vaccines were administered to healthy young adults whose responses were evaluated clinically and by determining the anti-haemagglutinin antibody (HI) titres. In addition, we sought to compare the strains by laboratory procedures.

METHODS

The seed *ca* reassortant (CR 59) was produced by Dr H. Maassab by recombining A/Ann Arbor/6/60 (H2N2) *ca* virus with A/Korea/1/82 (H3N2) wild-type virus using methods previously described (*5*). The seed *ca* reassortant (17/25/1) was produced by Dr Y. Ghendon by recombining the *ca* donor of attenuation for adults, A/Leningrad/134/17/57 (H2N2), with A/Korea/1/82 (H3N2) wild-type virus that had twice been cloned by terminal dilution in embryonated hens' eggs.

Vaccine preparation in specific pathogen-free eggs and quality control tests were carried out at the Wellcome Research Laboratories, Beckenham, Kent. Bulk CR 59 vaccine, prepared from the American seed, had a titre of $10^{7.5}$ EID_{50} (50% egg infectious doses) per ml; bulk 17/25/1 vaccine, prepared from the Russian seed, had a titre of $10^{7.8}$ EID_{50}/ml. Neither vaccine seed for this study underwent prior selection in preliminary human studies and both can be considered as random clones selected only on the basis of laboratory properties.

The study protocols were approved by Northwick Park Hospital and Leicestershire Area Health Authority Ethical Committees. Informed consent was obtained for all human studies. In a preliminary study at the Common Cold Unit, Salisbury, 23 healthy adults between the ages of 18 and 40 years were randomly assigned to receive $10^{7.5}$ EID_{50} of either CR 59 or 17/25/1 *ca* reassortant virus intranasally by drops. The vaccine was administered in 1-ml volumes to volunteers lying with their necks hyperextended; they remained supine for a minute after immunization and were asked to sniff rather than blow their nose for a further hour. Volunteers were examined daily for systemic and respiratory symptoms and signs and nasal washings were collected for three days after immunization. Paired sera were collected for determining pre- and post-immunization serum haemagglutination-inhibition (HI) antibody titres.

A second trial in 147 healthy students was carried out when results from the first trial became available. Healthy volunteers between the ages of 18 and 40 years were randomly assigned to receive $10^{7.5}$ EID_{50} of either CR 59 or 17/25/1 *ca* reassortant virus intranasally, as described above. Volunteers were given a standard diary card to complete daily for three weeks and venous blood was taken for serology. Four weeks later they returned for a second dose of the same vaccine as before and were bled again and given another diary card. Blood samples were obtained 5 weeks thereafter.

Standard methods were used to measure the HI antibody in coded sera from volunteers. In the first trial, A/England/40/83 was used as antigen in the HI test because of its greater sensitivity in detecting serum antibody than that of the CR 59 and 17/25/1 reassortant vaccines and the parent A/Korea/1/82 strain. All four strains were used as antigen in the HI tests in the second trial, and here seronegative individuals were defined as having pre-immunization titres of $<1:10$ against all four test antigens. Laboratory evidence of influenza A virus infection was defined either by isolation of influenza A virus (attempted in the first study only), or by a fourfold or greater rise in serum antibody (HI) against A/Korea/1/82, A/England/40/83, or the reassortant virus administered. This decision was based on the greater sensitivity of the A/England strain in the HI test compared with the parental wild-type virus and the homologous vaccine strain. The use of four test antigens also helped establish the comparability of the two experimental groups before immunization and, with antibody rises generally occurring with two or more antigens, it reduced the likelihood of spurious results.

Dilutions of nasal washings for virus isolation were inoculated into 10-day embryonated eggs by the allantoic route. In certain experiments the canine kidney cell line (MDCK) was used to detect virus in nasal washings. After incubation at 36 °C for 72 hours, the allantoic fluids or tissue culture fluids were harvested and tested for virus haemagglutinin using 0.5% chick cells.

Wild-type A/Korea/1/82 virus and the two vaccine strains, CR 59 and 17/25/1, were tested for virulence in two animal models. First, groups of four ferrets were each inoculated intranasally under ether anaesthesia with 10^5 EID_{50} of test virus in 0.5 ml of Hanks' saline. The temperature response, virus replication, and changes in nasal wash protein were assessed as described previously (*6*). Secondly, groups of newborn rats aged 24–48 hours were inoculated intranasally with 0.01 ml of CR 59, 17/25/1, or wild-type A/Korea/1/82 virus containing 10^8 EID_{50}/ml. Forty-eight hours later, 10^5 cfu of a virulent strain of *Haemophilus influenzae* type b were inoculated into the anterior nares of each rat; the incidence of bacteraemia was determined 48 hours later, as described previously (*7*).

The temperature-sensitivity of replication of the

wild parent strain, A/Korea/1/82, the two reassortants CR 59 and 17/25/1, and one other parental virus (namely, A/Ann Arbor/6/60) were determined by comparing virus replication in MDCK cells incubated at temperatures in the range of 32–39 °C. Viruses were titrated in the presence of 4 μg/ml of trypsin on microwell cultures of MDCK cells.

Haemagglutinins of CR 59, 17/25/1 and the wild parent A/Korea/1/82 virus were compared using a panel of 27 monoclonal antibodies and 4 post-infection ferret sera raised against recent influenza A H3N2 strains including A/England/23/76, A/Texas/1/77, and A/Bangkok/1/79. Similarly, neuraminidase antigens of the two reassortants and the wild parent A/Korea/1/82 virus were compared in the lectin test (*8*) using 3 post-infection ferret sera and 3 monoclonal antibodies directed against the neuraminidase of recent isolates. Finally, the two reassortants CR 59 and 17/25/1, the wild parent strain A/Korea/1/82, and one *ca* donor of attenuation A/Ann Arbor/6/60 were all subjected to polyacrylamide gel analysis of virus polypeptides (*9*).

RESULTS

Properties of the vaccines

Gene analysis. The CR 59 virus contained six genes that code for internal and non-structural proteins from the donor cold-adapted strain and the genes that code for the haemagglutinin and neuraminidase surface glycoproteins from the influenza A/Korea virus (Dr H. Maassab, personal communication). The 17/25/1 virus inherited genes 1, 2, 3 and 7 from the *ca* parent and genes 4, 6 and 8 from the A/Korea strain. Gene 5 could not be differentiated by means of cRNA/DNA hybridization, although since gene 5 of 17/25/1 has the same temperature-sensitive (*ts*) mutation as gene 5 of the *ca* parent, it is thought to be inherited from the *ca* parent. The 17/25/1 virus contains *ts* mutations in genes 1, 5 and 7 (Dr Y. Ghendon, personal communication). Preliminary biochemical analysis of the viruses confirmed that both the CR 59 and 17/25/1 viruses were reassortants since, for example, neither virus possessed the nucleoprotein (NP) or matrix (M) protein of A/Korea/1/82.

Virulence studies. Four separate experiments were carried out to determine the virulence of CR 59, 17/25/1, A/Korea/1/82 wild-type virus, and a control virulent virus, A/Finland/74 (H3N2), in an infant rat model of infection (*7*). *H. influenzae* bacteraemia occurred in 32 out of 35 (91%) rats following inoculation with A/Finland/74. In contrast, bacteraemia developed in 6% of rats (3 of 51) inoculated with A/Korea/1/82, 3% (1 of 37) inoculated with CR 59, and 19% (7 of 37) with 17/25/1; there were no significant differences between the rates of bacteraemia for A/Korea/1/82, CR 59, and 17/25/1 viruses.

Inoculation of the viruses into ferrets showed similar results: both reassortant viruses and A/Korea/1/82 wild-type virus induced little or no febrile reaction and little change in nasal protein. All three viruses grew to equally high titre in ferret nasal washings and induced comparable titres of post-infection serum HI antibody.

Temperature sensitivity. A total of four experiments showed the CR 59 reassortant to have marked *ts* properties, with a reduction in titre of at least 4.0 $\log_{10}$ $TCID_{50}$ (50% tissue culture infectious doses) per ml when titrated in MDCK cells at 39 °C compared to 32 °C. In contrast, the 17/25/1 vaccine virus showed a 2.25 $\log_{10}$ reduction in titre under comparable conditions; the parental wild virus, A/Korea/1/82, showed no *ts* properties.

Serological analysis of the CR 59 and 17/25/1 haemagglutinin. Serological analysis of the viruses using monoclonal antibodies to haemagglutinin (HA) and the HI test demonstrated that the HA of the CR 59 vaccine virus was antigenically similar to that of the A/Korea/1/82 virus. In contrast, the HA of the 17/25/1 vaccine virus showed a number of antigenic differences. Post-infection ferret sera also distinguished the two vaccine strains but to a much lesser extent than the monoclonal antibodies. One hundred human sera failed to detect any fourfold differences between 17/25/1 and CR 59; however, there was a statistically significant difference between the geometric mean reciprocal HI titres (GMT) against CR 59 and 17/25/1 (23.8 versus 28.8 respectively, $P<0.005$, paired t test). Serological analysis of the neuraminidase showed the CR 59 and 17/25/1 vaccine strains to be closely related to the neuraminidase of the parental A/Korea/1/82 virus.

Concomitant with serological differences in the HA of the 17/25/1 vaccine strain compared to CR 59 and A/Korea/1/82, electrophoretic migration differences in HA were also detected.

Infectivity of volunteers

For evaluation of the results of both clinical trials, the volunteers were divided into two groups according to the vaccine received and further subdivided into categories according to their initial antibody status. Among the 13 persons given CR 59 in the preliminary study, 10 had pre-immunization titres to A/England/40/83 of $<1:40$ and 5 (50%) of these had fourfold or greater rises in HI antibody after infection. Among the 10 volunteers given

Table 1. Antibody status of vaccinees before immunization

Vaccine	No. receiving vaccine	No. with reciprocal antibody titre to: A/Korea/1/82 <10	A/Korea/1/82 <40	A/Eng/40/83 <10	A/Eng/40/83 <40	A/Korea/1/82, A/Eng/40/83 and vaccine strain <10
CR 59	63	45	56	36	44	29
17/25/1	58	32	53	28	35	20

17/25/1 vaccine, 7 had initial titres of <1:40 and 4 (57%) of these had serological evidence of infection. None of six volunteers with pre-immunization titres of >1:40 was infected.

Serological data are available from 121 out of 147 vaccinees in the second clinical trial. There were no significant differences in the initial distribution of antibody to either A/Korea/1/82 or A/England/40/83 between the two vaccine groups (Table 1) ($P<0.05$, χ^2 test).

Among the 20 recipients of 17/25/1 vaccine who were initially seronegative to A/Korea/1/82, A/England/40/83, and 17/25/1 viruses, 14 (70%) were infected by the first dose and 2 out of 5 (40%) were infected by the second; 16 out of 19 (84%) recipients of two doses were infected overall: one person developed fourfold rises in antibody after both the first and second doses. Of 13 seropositive individuals with pre-immunization titres of <1:40 against A/Korea/1/82, A/England/40/83, and 17/25/1 viruses, 11 (85%) were infected by the first dose and 1 out of 2 was infected by the second; 12 out of 13 (92%) individuals given both doses were infected overall. Among 25 volunteers with pre-immunization titres of >1:40 against A/Korea/1/82, A/England/40/83, or 17/25/1 viruses, 14

Table 2. HI antibody status before and after intranasal inoculation with 17/25/1 and CR 59 attenuated influenza vaccines

Vaccine and initial HI-antibody status	HI test antigen	Percentage infected after: 1st dose	2nd dose	Geometric mean reciprocal antibody titre: Before 1st dose	After 1st dose	After 2nd dose
17/25/1 vaccine:						
Seronegative	A/England/40/83	70	84	<10	69	135[a]
	A/Korea/1/82			<10	17	19
Seropositive (<1:40)	A/England/40/83	85	92	9	70	154[b]
	A/Korea/1/82			14	29	54
Seropositive (>1:40)	A/England/40/83	56	80	71	224	278
	A/Korea/1/82			17	36	44[c]
CR 59 vaccine:						
Seronegative	A/England/40/83	62	72	<10	31	31[a]
	A/Korea/1/82			<10	13	16
Seropositive (<1:40)	A/England/40/83	71	83	14	76	76[b]
	A/Korea/1/82			9	32	39
Seropositive (>1:40)	A/England/40/83	55	63	106	276	413
	A/Korea/1/82			16	61	82[c]

[a] $P<0.005$.
[b] $P<0.025$.
[c] $P<0.05$.

(56%) were infected by the first dose and 2 out of 6 were infected by the second; 16 out of 20 (80%) recipients of both doses were infected overall. The infection rate for all 33 persons, initially seronegative or having titres of <1:40, was 76% (25 of 33) after the first dose and 87.5% (28 of 32) after the second; for all 58 recipients of one and two doses of 17/25/1, the infection rates were 67% (39 of 58) and 85% (44 of 52) respectively. The geometric mean antibody titres are presented in Table 2.

Among the 29 recipients of CR 59 vaccine who were initially seronegative to A/Korea/1/82, A/England/40/83, and CR 59 viruses, 18 (62%) were infected by the first dose and 3 out of 11 (27%) were infected by the second; 21 out of 29 (72%) recipients of two doses were infected overall. Of 14 seropositive individuals with pre-immunization titres of <1:40 against A/Korea/1/82, A/England/40/83, and CR 59 viruses, 10 (71%) were infected by the first dose and none of 2 was infected by the second; 10 out of 12 (83%) individuals given both doses were infected overall. Among 20 volunteers with pre-immunization titres of >1:40 against A/Korea/1/82, A/England/40/83, or CR 59 viruses, 11 (55%) were infected by the first dose and 1 out of 8 was infected by the second; 12 out of 19 (63%) recipients of both doses were infected overall. The infection rate for all 43 persons initially seronegative or having titres of <1:40 was 65% (28 of 43) after the first dose and 76% (31 of 41) after the second; for all 63 recipients of one and two doses of CR 59, the infection rates were 62% (39 of 63) and 72% (43 of 60) respectively.

Although there were no significant differences between the infection rates for CR 59 and 17/25/1 viruses in any of the groups analysed, comparison of the antibody responses (Table 2) revealed significantly higher titres against A/England/40/83 after the second dose of 17/25/1, as compared with CR 59, both among seronegatives and seropositives with initial titres of <1:40.

Virus was isolated from only one of the 23 volunteers inoculated with either reassortant virus in the preliminary studies and this had the *ca* properties of the parental virus. No virus isolation procedures were carried out in the second study.

Adverse effects on volunteers

The frequency and severity of reactions to CR 59 and 17/25/1 vaccines were unaffected by the antibody status before inoculation. The majority of infected and non-infected persons (81–90%) were either asymptomatic or complained of no more than one local reaction (rhinorrhoea, cough, or sore throat) during the 5 days after immunization. Systemic complaints (headache, malaise, myalgia, or feeling feverish) together with two or more upper respiratory symptoms developed after 2% (3/135) of non-infecting immunizations, as compared with 12% (5/43) for the CR 59 infections ($P<0.05$) and 7% (3/43) for the 17/25/1 infections. Two volunteers took time off work during the 5-day period after immunization but in neither case was this attributable to the inoculation: one volunteer, infected with CR 59, had a diarrhoeal illness with no rhinorrhoea or sore throat; the other, infected with 17/25/1, was off work with otitis which developed immediately after the immunization.

DISCUSSION

The most impressive observation of this study was the remarkable similarity of the Russian and American *ca* reassortants in terms of infectivity, immunogenicity and reactogenicity. We found that a single inoculation with CR 59 or 17/25/1 virus was sufficient to infect at least 62% and 70%, respectively, of seronegative volunteers, 71% and 85% of subjects with pre-immunization titres of <1:40, and 55% and 56% of persons with initial titres >1:40. Although infection of persons possessing HI antibody is not without precedent, we cannot rule out the possibility that the antigenic mass of HA in the inoculating material caused a host immune response in persons with pre-existing antibody. Nevertheless, in practical terms a large percentage of the volunteers had, at the end of the study, increased levels of HI antibody which, assuming a correlation between antibody and protection, would be advantageous.

The second inoculation with CR 59 virus infected an additional 10% of volunteers overall and the 17/25/1 reassortant a further 18%, suggesting that second doses may be highly cost-effective. It is also of note that a second dose reinfected only 1 out of 87 vaccinees: although not a formal protection study, the results are comparable to the high levels of protection against illness and infection found in recent studies using similar *ca* reassortants and wild-type virus, with virus challenge occurring up to 3 months after immunization (*10, 11*).

Both the *ca* reassortant virus vaccines used in the present study were well tolerated; 81% of infections with CR 59 and 88% of infections with 17/25/1 were either asymptomatic or were associated with only one upper respiratory symptom. A total of 12% of CR 59 infections, 7% of 17/25/1 infections, and 2% of non-infective inoculations were followed by various combinations of systemic and upper respiratory symptoms; these were generally mild and of short duration and resulted in no time off work; their frequency and severity were uninfluenced by the levels of antibody prior to immunization and, in the

context of protection against influenza, were considered acceptable.

The studies in the rat and ferret animal models confirmed that the two reassortants CR 59 and 17/25/1 were attenuated but failed to establish that the wild-type A/Korea virus was virulent. Although in this study we did not compare the reassortants with wild-type virus in man, colleagues in the United States inoculated 14 seronegative volunteers with A/Korea/1/82 wild-type virus and observed systemic reactions in 29% and rhinitis in 50% (Dr M. H. Snyder, personal communication). Since intranasal infection with 17/25/1 and CR 59 caused significantly less rhinitis (14% and 16%, respectively) and fewer systemic reactions than the wild-type virus, we conclude that the two reassortants were attenuated but acknowledge that the A/Korea/1/82 parent is only of moderate virulence.

Serological analysis of the viruses using monoclonal antibodies to HA and the HI test demonstrated that the HA of the CR 59 vaccine virus was antigenically similar to that of the A/Korea/1/82 virus, whereas the HA of the 17/25/1 virus showed a number of differences. Concomitant with serological differences in the HA of the 17/25/1 vaccine strain compared to CR 59 and A/Korea/1/82, electrophoretic migration differences in HA were also detected. It must be assumed therefore that the A/Korea wild-type virus was a heterogenous mixture and that the laboratory manipulations in the USSR and USA resulted in the emergence of antigenically distinguishable viruses. The haemagglutinins of the Korea wild-type parent and two reassortants are currently being sequenced and the results will be published with other biochemical data.

Previous studies show that the transfer of the six internal genes from the A/Ann Arbor/6/60 *ca* virus reproducibly confers properties of attenuation for man, genetic stability, lack of transmissibility, and the ability to induce resistance to challenge by wild-type virus. The Russian A/Leningrad/134/17/57 *ca* reassortants are said to behave identically to the A/Ann Arbor reassortants and have been given to many thousands of people in the USSR. Despite differences in temperature sensitivity and genetic composition, our findings indicate CR 59 and 17/25/1 to be of equal efficacy in man. We conclude that intranasal immunization with reassortants from both donors of attenuation may be of great value and that comparative field trials should be conducted to determine their clinical efficacy.

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RÉSUMÉ

INFECTIOSITÉ ET RÉACTOGÉNICITÉ DE VACCINS GRIPPAUX A/KOREA/1/82 RÉASSORTIS ET CRYO-ADAPTÉS OBTENUS AUX ÉTATS-UNIS D'AMÉRIQUE ET EN UNION SOVIÉTIQUE

On a évalué chez 170 volontaires adultes, jeunes et en bonne santé, l'innocuité et l'immunogénicité de deux souches de vaccins grippaux vivants, les vaccins cryo-adaptés (*ca*) et réassortis CR 59 et 17/25/1. Ces vaccins ont été obtenus en recombinant le virus de type sauvage A/Korea/1/82 (H3N2), avec des atténuateurs A/Ann Arbor/6/60 (H2N2), ou A/Leningrad/134/17/57 (H2N2) cryo-adaptés. Le virus CR 59 contenait six gènes codant pour les protéines internes non structurales de la souche atténuatrice, cryo-adaptée, A/Ann Arbor/6/60 ainsi que les gènes codant pour les glycoprotéines de surface (hémagglutinine et neuraminidase) provenant du virus A/Korea. Le virus 17/25/1 a hérité les gènes 1, 2, 3 et 7 de la souche mère A/Leningrad/134/17/57 et les gènes 4, 6 et 8 de la souche A/Korea, le gène 5 n'ayant pu être différencié par hybridation ARN/ADN, encore que, puisque le gène 5 du virus 17/25/1 présente la même mutation thermosensible que le gène 5 de la souche cryo-adaptée, on puisse légitimement penser qu'il provient de cette souche.

On a déterminé la virulence du virus A/Korea/1/82 de

type sauvage et des deux souches variantes CR 59 et 17/25/1 chez le furet et le rat. Ces études ont confirmé l'atténuation des deux virus réassortis, mais elles n'ont pu mettre en évidence la virulence du virus A/Korea de type sauvage. La souche réassortie CR 59 présentait des propriétés thermosensibles marquées, avec une réduction de titre d'au moins 4,0 $\log_{10}$ $DICT_{50}$ (dose infectante 50% en culture tissulaire), quand le titrage avait lieu sur cellules MDCK à 39 °C plutôt qu'à 32 °C. Par opposition, le virus vaccinal 17/25/1 présentait une réduction de titre de 2,25 $\log_{10}$ dans des conditions comparables; le virus A/Korea/1/82 de type sauvage, provenant de la même lignée, n'a montré aucune thermosensibilité.

L'analyse sérologique des virus à l'aide d'anticorps monoclonaux hémagglutinants et la réaction d'inhibition de l'hémagglutination ont révélé que l'hémagglutinine du virus de la souche vaccinale CR 59 était antigéniquement identique à celle du virus A/Korea/1/82, tandis que celle du virus vaccinal 17/25/1 présentait un certain nombre de différences antigéniques. En outre, parallèlement à ces différences sérologiques au niveau de l'hémagglutinine de la souche vaccinale 17/25/1 par rapport à CR 59 et A/Korea/1/82, on a observé des différences en ce qui concerne la migration électrophorétique. Les volontaires ont bien supporté les deux vaccins. Le virus 17/25/1 obtenu à partir de A/Leningrad a infecté au moins 70% des volontaires séronégatifs après la première dose et 84% après la seconde; une et deux doses du virus CR 59 ont infecté respectivement 62% et 72% des volontaires. Le virus 17/25/1 a infecté 66% des sujets vaccinés, initialement séropositifs, après une dose et 85% après deux doses; de même, le virus CR 59 a infecté respectivement 62% et 71% de ces sujets. Malgré des différences au niveau de la thermosensibilité, des composants génétiques et de la réactivité sérologique aux anticorps monoclonaux, les deux vaccins se sont comportés pratiquement de la même façon chez les modèles animaux et chez l'homme. Nous en concluons au grand intérêt potentiel de ces deux atténuateurs.

REFERENCES

1. HOSKINS, T. W. ET AL. Assessment of inactivated influenza A vaccine after three outbreaks of influenza A at Christ's Hospital. *Lancet*, **1**: 33-35 (1979).
2. ALEXANDROVA, G. I. & SMORODINTSEV, A. A. Obtaining an additionally attenuated vaccinating cryophilic influenza strain. *Revue roumaine d'inframicrobiologie*, **2**: 179-186 (1965).
3. MAASSAB, H. F. Adaptation and growth characteristics of influenza virus at 25 °C. *Nature*, **213**: 612-614 (1967).
4. KENDAL, A. S. ET AL. Development of cold-adapted recombinant live attenuated influenza vaccines in the USA and USSR. *Antiviral research*, **1**: 339-365 (1981).
5. COX, N. J. ET AL. Comparative studies of wild-type and cold-mutant (temperature sensitive) influenza viruses: nonrandom reassortment of genes during preparation of live virus vaccine candidates by recombination at 25 °C between recent H3N2 and H1N1 epidemic strains and cold-adapted Ann Arbor/6/60. *Virology*, **97**: 190-194 (1979).
6. POTTER, C. W. ET AL. Immunity to influenza in ferrets; response to live and killed virus. *British journal of experimental pathology*, **53**: 153-167 (1972).
7. JENNINGS, R. ET AL. The replication of type A influenza viruses in the infant rat: a marker for virus attenuation. *Journal of general virology*, **49**: 343-354 (1980).
8. LUTHER, P. ET AL. An investigation of antigenic drift of neuraminidases of influenza A(H1N1) viruses. *Journal of hygiene*, **92**: 223-229 (1984).
9. OXFORD, J. S. ET AL. Biochemical and antigenic analysis using monoclonal antibodies of a series of infuenza A(H3N2) and (H1N1) virus reassortants. *Vaccine*, **4**: 9-14 (1986).
10. CLEMENTS, M. L. ET AL. Advantage of live attenuated cold-adapted influenza A virus over inactivated vaccine for A/Washington/80 (H3N2) wild-type virus infection. *Lancet*, **1**: 705-708 (1984).
11. BETTS, R. F. ET AL. Resistance to challenge with influenza A/Hong Kong/123/77 (H1N1) wild-type virus induced by live attenuated A/Hong Kong/123/77 (H1N1) cold-adapted reassortant virus. *Journal of infectious diseases*, **151**: 744-745 (1985).