

Influenza vaccination with live-attenuated and inactivated virus-vaccines during an outbreak of disease*

By G. ROCCHI, G. RAGONA, C. PIGA, A. PELOSIO, A. VOLPI,
S. VELLA

Third Medical Clinic, University of Rome, 00161 Rome, Italy

N. LEGNITI

Servizio di Sanità dell'Esercito, Italy

AND A. DE FELICI

Central Laboratory, 'Lazzaro Spallanzani' Hospital, Rome

(Received 13 November 1978)

SUMMARY

Immunization procedures with live attenuated and inactivated vaccines were carried out on a group of young recruits at the beginning of an outbreak of infection due to an A/Victoria/3/75-related virus strain, which occurred in February 1977 in a military camp. A retrospective investigation on protection from clinical influenza was then performed in order to investigate whether immunization with live virus vaccines, administered at the beginning of an epidemic, could provide early protection from the disease. In the course of the two weeks following vaccination, laboratory-confirmed clinical influenza cases occurred in 4 subjects among the 110 volunteers of the control group which received placebo, and in 8, 7 and 4 subjects respectively of the 3 groups of about 125 individuals, each of which received one of the following vaccine preparations: (a), live attenuated A/Victoria/3/75 influenza virus oral vaccine, grown on chick embryo kidney culture; (b), live attenuated nasal vaccine, a recombinant of A/Puerto Rico/8/34 with A/Victoria/3/75 virus; and (c), inactivated A/Victoria/3/75 virus intramuscular vaccine. These data do not support the hypothesis that, during an epidemic of infection, early protection from clinical influenza can be achieved through immunization with live attenuated or inactivated influenza virus vaccines, in spite of the high immunizing capability of the vaccine preparations.

INTRODUCTION

Several investigations indicated the safety of live attenuated influenza vaccines which were recently developed (McDonald *et al.* 1962; Alexandrova *et al.* 1970). A high degree of stability of attenuation and a virtually absent transmissibility to susceptible contacts has been reported for these vaccines (Murphy *et al.* 1972; Beare *et al.* 1973; Ikić *et al.* 1977). Immunization produces a high incidence of

* This work was supported by contract No. 78.00367.84 within 'Progetto Finalizzato Virus' of the C.N.R. Italy.

specific antibody rise in blood serum and nasal secretion (Prévost *et al.* 1973; Minor *et al.* 1975; Rubin *et al.* 1976; Crifò *et al.* 1978), which is associated with a high degree of protection against artificial challenge and natural infection (Rytel *et al.* 1975; Donikian, McKee & Greene, 1977; MacKenzie *et al.* 1975; Noble *et al.* 1975).

The present study was originally undertaken in the winter of 1977 to investigate the immunogenicity of two live-attenuated strains of the current serotype A/Victoria/3/75 (H₃N₂) virus in a partially immune population. The population was not selected for immunity status to influenza A/Victoria virus, yet it was expected that the majority of candidates would have antibody for influenza A/Victoria virus, since antigenically related strains had been repeatedly isolated in Italy from respiratory illness during the winter of 1976. The trial was carried out on recruits who volunteered to receive either placebo or attenuated or inactivated vaccines. The vaccination procedure was performed when the volunteers entered military camp, at the beginning of February 1977. This was a rather late period for vaccination to be started, since, during interpandemic years, influenza virus is usually prevalent in Italy in January through March. In fact, at nearly the same time when immunization procedures were started, an outbreak of influenza began amongst new recruits, producing a limited number of illnesses. This gave the opportunity to investigate retrospectively whether vaccination against influenza could induce protection from natural infection when the immunization procedure is carried on during an outbreak of disease.

In fact, it has been reported that live attenuated vaccines could protect those susceptible in a community even though the immunization programme is started when the natural infectious agent has already reached the community (Shvetshova *et al.* 1971; Alexieva, Petrova & Jancheva 1971; Shvetshova *et al.* 1973; Alexieva *et al.* 1978).

MATERIALS AND METHODS

Volunteers

Subjects were healthy new recruits between the ages of 18 and 20 years, of the 'Scuola Allievi Sottoufficiali', Viterbo, Italy. This is a basic training post which received 600 new recruits during the last few days of January 1977, and which has a total population of approximately 2000 men. New recruits were grouped in four companies each housed in different lodgings. Each company carried on training activity, which was independent of the others. All recruits, however, had several opportunities to meet with other residents in the camp. Candidates for the trial were selected among new recruits on the basis of willingness to participate in the study. A complete explanation of the nature and benefits of the study was given to the participants in order to obtain consent.

Vaccines and placebo

Live attenuated type A influenza virus vaccine, Influvoral, an A/Victoria/3/75 virus grown on chick kidney embryo, prepared according to the technique of the Moscow Institute for Viral Preparations, was supplied by Istituto Sieroterapico

Milanese 'S. Belfanti', Milan, Italy. This vaccine was prepared for experimental trials. It was given in a 2 ml amount oral dose, i.e. $2 \times 10^{4.5}$ 50% egg infectious doses (EID₅₀).

Live attenuated type A influenza virus vaccine, RIT 4050, a recombinant of A/Puerto Rico/8/34 with A/Victoria/3/75, was supplied by Recherche et Industrie Therapeutiques, Belgium. Attenuated vaccine was given to vaccinees by dropping 0.25 ml in each nostril, i.e. an individual dose of 10^7 EID₅₀.

Inactivated influenza vaccine contained A/Victoria/3/75 virus (600 i.u.) and B/Hong Kong/5/72 (300 i.u.) and aluminium phosphate (5 mg/ml). This vaccine was given by intramuscular injection. It was supplied by the same producer of oral vaccine.

Placebo consisted of the diluent in which nasal vaccine was reconstituted and was indistinguishable in appearance from the nasal vaccine. Placebo was given intranasally. Volunteers received placebo or nasal vaccine in a blind fashion.

Experimental design

A total of 496 volunteers belonging to four different companies participated in the trial. In early February 1977 126 individuals of the first company received inactivated vaccine, 133 and 127 of the second and third company, respectively, were given either live attenuated nasal or oral vaccine, and 110 of the fourth company received placebo. A second dose of vaccines and placebo was given two weeks after the first one.

Clinical evaluation

Before vaccination, and 5 weeks and 5 months later, a complete clinical history was obtained and physical examination was performed for all volunteers. Temperatures were obtained during ten days after administration of vaccine or placebo. Volunteers reporting any complaint were examined by a physician. Volunteers presenting evidence of clinical illness were carefully observed for the presence and severity of signs and symptoms to be recorded.

Antibody studies

Specimens of serum were collected from all participants before vaccination, and 5 and 21 weeks thereafter. The five-week sample of serum was used to determine immunogenicity of vaccines and/or the effect of natural infection. The 21-week sample was used to evaluate the persistence of immunity and to determine the occurrence of further prevalence of influenza virus infections after the February outbreak. Studies of hemagglutination-inhibiting (HI) antibody were done by a standard micromethod using four units of A/Victoria/3/75 virus antigen (Sever, 1962).

RESULTS

During the week before vaccination scattered cases of febrile illness resembling influenza were observed throughout the population of the military camp. On the day when vaccination was performed, 6 volunteers in the four companies were

Table 1. *Symptoms reported by 35 volunteers during the fortnight between first and second administrations of vaccine or placebo*

Symptom	Number of volunteers with symptoms			
	Vaccine			Placebo
	Attenuated		Inactivated	
	Oral	Nasal	Intra-muscular	
Malaise	8	9	5	3
Myalgia	4	4	2	2
Headache	7	4	3	2
Sore throat	11	9	8	3
Cough	9	8	1	2
Fever (≥ 38.5 °C)	2	4	0	2
Fever (≥ 37.5 °C) + 3 or more symptoms	8	7	4	4
Any symptom	12	11	8	4

not given vaccine since they were apparently ill. Retrospective serological study showed that 5 out of these 6 individuals were affected by influenza A/Victoria virus infection. During the period between the first and the second administration of vaccines or placebo, 35 individuals among the 496 participants in the trial complained of one or more of the symptoms indicated in Table 1. Clinical signs taken as indicative for influenza (i.e. fever lasting 2 days or more, plus three or more of the following symptoms: malaise, myalgia, headache, sore throat, cough) were reported by 8, 7 and 4 volunteers who received live oral or nasal or inactivated intramuscular vaccine, respectively, and by 4 participants in the placebo group. The symptoms started 1 to 3 days after vaccination in 1 volunteer of either group of recipients of live oral or nasal vaccines, and four to ten days after vaccination in 7 and 6 recipients of live oral or nasal and in the 4 recipients of inactivated vaccine. The disease lasted three days or more in these cases. Among the volunteers in the placebo group, the four cases of illness became clinically apparent 2-5 days after treatment. Serological study showed that all the volunteers complaining of influenza-like disease, in vaccines and placebo groups, developed a significant immunity response (fourfold or greater increase of HI serum antibody titre) to A/Victoria virus.

Diseases resembling influenza were not reported coincident with, or following the second administration of vaccine or placebo, nor afterwards. Antibody titration performed on sera taken before and five weeks after vaccination showed that nearly 39% of the volunteers who received placebo experienced influenza A infection (fig. 1*d*). Among the vaccinees, significant antibody response to A/Victoria/3/75 virus occurred in about 59%, 77% and 94% of recipients of live attenuated oral or nasal, and of inactivated intramuscular vaccine, respectively (fig. 1, *a-c*). HI serum antibody GMTs, ranging from 1/19.6 to 1/27.0 in the 4 groups of volunteers before vaccination, rose, five weeks after, up to 1/132.9, 1/149.5, 1/612.4 and 1/71.9 for live oral and nasal vaccine, for inactivated intramuscular vaccine and for placebo groups, respectively.

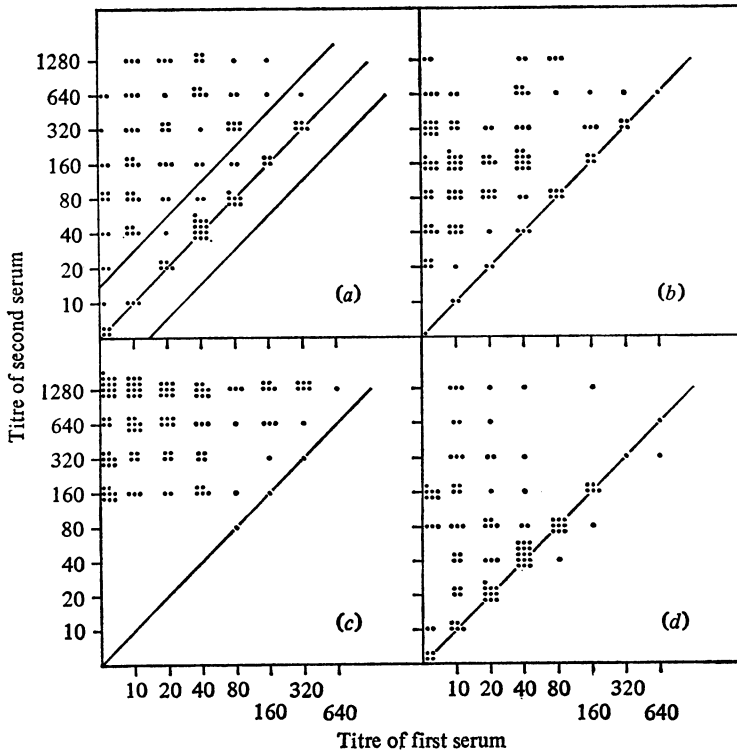


Fig. 1. Hemagglutination inhibition (HI) antibody titration on sera from volunteers who were vaccinated with A/Victoria/3/75 vaccines or treated with placebo, during an epidemic due to an antigenically related virus strain. HI reactivity against A/Victoria/3/75 virus was tested in four groups of volunteers before and five weeks after administration of: (a) live attenuated virus oral vaccine; (b) live attenuated virus nasal vaccine; (c) inactivated virus intramuscular vaccine; (d) placebo.

In the third serum sample, four months later, no further significant antibody titre rise was detected; GMTs were then 1/92.2, 1/110.5, 1/421.3 and 1/44.2 in the four groups, respectively.

DISCUSSION

The scattered cases of influenza that were observed in the military camp one week before the beginning of the vaccination trial and during the two subsequent weeks indicate that a small influenza epidemic, due to A/Victoria/3/75-like virus, prevailed among the volunteers at the time when the vaccination trial was started. An epidemic from the same virus was also documented through serology and virus isolation study in the same region in Italy at that time (Rocchi *et al.* 1979). The incidence of apparent disease among the infected individuals in the camp was rather low, since only four cases of clinical influenza were recognized among 43 volunteers experiencing A/Victoria virus infection in the placebo group. It is conceivable that the cases of influenza which were observed in the placebo group were due to natural infection rather than to spread of vaccine-virus from vaccinees since, (a) there was little occasion of contact among recruits belonging to the four

companies, (b) the disease cases appeared, as a rule, earlier among the recipients of placebo than among vaccinees and, (c) at least for one of the live attenuated vaccine preparations which were used in this trial, i.e. for the nasal vaccine, the occurrence of transmission to susceptible contacts has been ruled out (Lobmann, Delem & Jovanovic, 1977). As for the volunteers of the inactivated vaccine group, who experienced clinical influenza, the considerations which were made at the point (a) and (c) for the patients in the placebo group could apply, indicating natural infection as the possible cause of the disease. As for the live-virus vaccinees, the possibility that the scattered cases of influenza which were observed during the ten days following administration of the vaccines could be due to the vaccine itself cannot definitely be excluded. However, at least two facts stand out in support of the possibility that natural influenza infection was the cause of the disease in the patients belonging to these 2 groups, i.e. the lack of reactogenicity of the live attenuated virus vaccine preparation which was used in the trial (Lobmann *et al.* 1977; De Barbieri *et al.* 1977) and the late occurrence of the majority of the cases, after the fourth day after vaccination.

It can be concluded that in this study no protection from natural infection became appreciable with any of the virus-vaccine preparations which were used during the epidemic. This observation seems to be rather in contrast with data reported by Russian workers (Shvetshova *et al.* 1971; Alexieva *et al.* 1971; Shvetshova *et al.* 1973) indicating that protection from natural infection can be achieved soon after administration of the first oral vaccine dose, possibly through viral interference mechanisms. Indeed, it is generally accepted that a second dose of a live-virus vaccine, given one to two weeks after the first one, is able to increase immunization, possibly because of virus growth in the respiratory tract. This consideration does not support the hypothesis that a first vaccine dose is able to confer appreciable interferon and/or immunity against natural influenza virus infection before fifteen days, when the immunogenic effect of it becomes evident in terms of local and serum antibody activity rises (Crifò *et al.* 1978), even though it seems that a reduction of the incidence of influenza with symptoms could be achieved when live virus vaccine is given as late as five days after recipients become exposed to infection (Elveback *et al.* 1976).

Although in this trial the vaccination procedure performed during a small epidemic outbreak did not apparently produce any appreciable protective effect against clinical influenza, the immunizing effect of the procedure appeared rather clear at least for two of the vaccines used. In fact, for the intramuscular inactivated vaccine and for the nasal live-attenuated vaccine preparations the GMTs of specific HI antibody activity and the percentage incidence of individuals showing 'protective' serum antibody titres ($\geq 1/40$) appeared to be significantly increased after these two immunization procedures.

REFERENCES

- ALEXANDROVA, G. I., SMORODINTSEV, A. A., BELJAEVA, N. M., VASIL'EV, B., GEFT, R. A., PANTELEEV, V. G., SEIFER, M. A. & SELIVANOV, A. A. (1970). Testing the safety and effectiveness of oral administration of a live influenza vaccine. *Bulletin of the World Health Organization* **42**, 429.
- ALEXEIEVA, A. K., ANGIAPARIDZE, O. G., AKOPOVA, I. I., PORUBEL, L. A., IAKOVLEVA, L. A., ALEXANDROVA, A. H., VASILIEVA, I. N., ERMAKOVA, M. N., SIDOROVA, L. A., RUKAVISHNIKOVA, G. E., MELNIKOVA, S. K. & GRACEVA, E. N. (1978). Impiego dei virus dell'influenza coltivati nei tessuti nella produzione dei vaccini vivi dell'influenza. Symposium on live influenza vaccines, Florence, 5 November 1977. *Giornale di malattie infettive e parassitarie* (in press).
- ALEXIEVA, R. B., PETROVA, S. M. & JANCHEVA, B. N. (1971). Studies on some biological properties of vaccinal influenza strains cultivated at low temperatures. *Proc. Symposium on Live Influenza Vaccine*. Yugoslav Academy of Sciences and Arts, Zagreb, 43-49.
- BEARE, A. S., HABERSHON, R. B., TYRREL, D. A. J. & HALL, T. S. (1973). Recombinant live influenza vaccine virus-tests of transmissibility in man. *Journal of Biological Standardization* **1**, 233.
- CRIFÒ, S., FILLIACI, F., PELOSIO, A. & ROCCHI, G. (1978). Risposta secretoria e sierica dopo vaccinazione antiinfluenzale per via nasale con virus vivo attenuato A/Victoria/3/75. *Bollettino della Società Italiana di Biologia Sperimentale* **54**, 1021.
- DE BARBIERI, A., CROVARI, P., CUNEO-CROVARI, P. & GIACOMETTI, G. (1977). Further researches on live influenza virus vaccines. *Developments in Biological Standardization* **39**, 73.
- DONKIAN, M. A., MCKEE, J. & GREENE, L. C. (1977). Challenge versus natural infection as an index of protection after influenza immunization. *Developments in Biological Standardization* **39**, 149.
- ELVEBACK, L. R., FOX, J. P., ACKERMAN, E., LANGSWORTHY, A., BOYD, M. & GATEWOOD, L. (1976). An influenza simulation model for immunization studies. *American Journal of Epidemiology* **103**, 152.
- IKIĆ, D., PASINI, N., HEĆIMOVIĆ, M., JUZBAŠIĆ, M., ŠMERDEL, S., RAJNER, N. & JELAVIĆ, V. (1977). Attenuation of influenza A/New Jersey/76 virus strain, preparation of live vaccine and vaccine reactogenicity in volunteers. *Developments in Biological Standardization* **39**, 61.
- LOBMANN, M., DELEM, A. & JOVANOVIĆ, D. (1977). Properties of A/Victoria/3/75 recombinants: developments of an attenuated strain RIT 4050. *Developments in Biological Standardization* **39**, 43.
- MCDONALD, J. C., ZUCKERMAN, A. J., BEARE, A. S. & TYRREL, D. A. J. (1962). Trials of live influenza vaccine in the Royal Air Force. *British Medical Journal* **i**, 1036.
- MACKENZIE, J. S., MACKENZIE, I., LLOYD, J. & DENT, V. (1975). Comparative trials of live attenuated and detergent split influenza virus vaccines. *Journal of Hygiene* **75**, 425.
- MINOR, T. E., DICK, E. C., DICK, R. C. & INHORN, S. L. (1975). Attenuated influenza A vaccine (Alice) in an adult population: vaccine-related illness, serum and nasal antibody production and intrafamily transmission. *Journal of Clinical Microbiology* **2**, 403.
- MURPHY, B. R., CHALBUB, E. G., NUSINOFF, S. R. & CHANOCK, R. M. (1972). Temperature-sensitive mutants of influenza virus. II. Attenuation of ts recombinants for man. *Journal of Infectious Diseases* **126**, 170.
- NOBLE, G. R., COREY, L., ROSENBERG, R. L., HOKE, K., BROWN, W. J., KAYE, H. S., BREGMAN, D. J., GREGG, M. B. & DOWDLE, W. R. (1975). An open field trial of live attenuated influenza A/England/42/72 (H3N2) vaccine. II. Illness and infection rates during an epidemic of A/Port Chalmers/-like influenza. *15th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington D.C. (abstract).
- PRÉVOST, J. M., PEETERMANS, J., LAMY, F. & HUYGELEN, C. (1973). Immune response to vaccination with a live influenza virus (H3N2) vaccine ('Ann' strain). *Infection and Immunity* **8**, 420.
- ROCCHI, G., CARLIZZA, L., ANDREONI, M., RAGONA, G., PIGA, C., PELOSIO, A., VOLPI, A. & MUZZI, A. (1979). Protection from natural infection after live influenza virus immunization in an open population. *Journal of Hygiene* **82**, 231.

- RUBIN, R. G., NOBLE, G. R., COREY, L., BROWN, W. J., BRANDLING-BENNETT, D., KAYE, H. S., COLEMAN, M. T., GREGG, M. B. & DOWDLE, W. R. (1976). Live, attenuated influenza A/England/42/72 (H3N2) virus vaccine: a field trial. *Journal of Infectious Diseases* **133**, 603.
- RYTEL, M. W., JACKSON, L. J., FERSTENFELD, J. E. & ROSENKRANZ, M. A. (1975). New live attenuated influenza A/England/42/72 (H3N2) vaccine (Alice): reactogenicity, immunogenicity, and protection efficacy. *Journal of Infectious Diseases* **132**, 652.
- SEVER, J. L. (1962). Application of a microtechnique to viral serological investigations. *Journal of Immunology* **88**, 320.
- SHVETSHOVA, E. G., MALUSHEVA, A. M., KARAPAZ, N. M., TAROS, L. U. & SMORODINTSEV, A. A. (1971). Epidemiological efficacy of live influenza vaccine and its use together with interferon inductors and amantadine. *Proc. Symposium on Live Influenza Vaccine*. Yugoslav Academy of Sciences and Arts, Zagreb 143-155.
- SHVETSHOVA, E. G., MALUSHEVA, A. M., KARAPAZ, N. M., OLEYNIKOVA, E. V., VASILJEVA, R. I., GAGARINOVA, V. M., TAROS, L. U. & SMORODINTSEV, A. A. (1973). Epidemiological efficacy of live vaccine and other preparations against influenza. *Transactions of the All-Union Research Institute of Influenza*, Leningrad.