Memoranda
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Memoranda are statements concerning the conclusions or recommendations of certain WHO scientific meetings; they are signed by the participants in the meeting.

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Progress in the development of influenza vaccines: Memorandum from a WHO Meeting*

Although influenza remains an important disease causing high levels of morbidity in many countries, the impact of epidemics can be reduced by immunoprophylaxis with available inactivated or cold-adapted live attenuated influenza vaccines. In closed or semi-closed communities, maximum benefit from immunization is likely to be achieved when more than 75% of the population are immunized. Other strategies to reduce the impact of influenza include mass immunization of certain populations to protect them as well as to reduce illness in the overall community. Adequate studies have not yet been undertaken to confirm whether the desired reduction in community-wide illnesses results from such target mass immunization programmes. Well-controlled studies to assess further the efficacy of influenza vaccines in not only protecting the vaccinee but also reducing the impact of influenza epidemics in the community should be encouraged.

THE NEED FOR CONTROL OF INFLUENZA

Influenza has long been recognized as a problem in both developed and developing countries. Recurring epidemics are known to have many consequences among the different populations of the world; these include a high frequency of morbidity that disrupts work in schools, the public services and elsewhere, as well as other activities. During major epidemics it causes large numbers of persons to require medical treatment or hospitalization. In the developed countries influenza is important as a cause of severe morbidity and mortality. Excess mortality often accompanies influenza epidemics, the vast majority affected being the elderly. The fastest growing sector of the populations of many countries is the elderly, whose numbers are projected to double by early in the next century. These changing trends in modern society, especially in the developed nations, will lead to increased awareness of the impact of influenza.

Influenza also occurs frequently in developing and tropical countries where the prevalence of individual virus variants is not substantially different from that in the industrialized countries. However, the relative impact of influenza as a cause of morbidity and mortality may be lower in the developing countries because of the high prevalence of other infectious diseases. Nevertheless, as general health standards improve and longevity increases, the numbers of elderly persons and consequently the number of high-risk individuals will increase in these countries, creating an increased need for effective vaccines against influenza and a well-considered policy of prevention.

It is clear that the priorities of individual countries in developing a programme of immunization against influenza will depend on the impact of influenza in relation to the incidence of other disease problems. National policies on the use of influenza vaccines vary widely from country to country. It is important to stress that immunity to influenza can be both solid and long-lasting as shown by the resistance of the population to H1N1 infection after the reappearance

* This Memorandum summarizes the discussions and conclusions of the WHO Informal Consultation on progress in the development and study of inactivated and cold-adapted live attenuated influenza vaccines, which was held in Geneva on 5-7 November 1986. A list of the participants is given on page 293. Requests for reprints should be sent to Microbiology and Immunology Support Services, World Health Organization, 1211 Geneva 27, Switzerland. A French translation will appear in a later issue of the *Bulletin*.

of viruses of this subtype in 1977–78. As a consequence of antigenic drift, however, reinfection by antigenically different viruses frequently occurs and may result in epidemics of the disease. The currently available vaccines are of variable efficacy because of the unpredictable antigenic variability of influenza viruses and their epidemiological behaviour.

Two main objectives for influenza immunization strategies were identified by the participants at the meeting. These are (1) to protect individuals who are at particular risk of disease, e.g., elderly persons in nursing homes, and (2) to protect other defined subsets of the population, e.g., schoolchildren (as in Japan) and factory workers (in the USSR). In the latter cases, immunization may have a direct benefit for both the individuals involved and the community as a whole. It is not, however, realistic to expect to prevent the disease in the whole community as is feasible with vaccines against measles and poliomyelitis.

Influenza morbidity and mortality

Influenza is not a notifiable disease in most countries, and may be greatly underreported. Consequently, indirect methods are often used to assess influenza-related morbidity and mortality. One such method is the study of data on mortality from acute respiratory diseases which are available from several countries. The World Health Organization has been conducting a collaborative study on excess mortality from acute respiratory diseases with a small number of countries since 1970. Although this study has generated useful information in the past it is doubtful whether the continuation of the study in its present design will add any new information of value in the WHO influenza programme.

DEVELOPMENT OF VACCINES

Antigenic structure of influenza haemagglutinin

In recent years the amino acid sequence and three-dimensional structure of the most important viral antigen, the haemagglutinin (HA), have been determined. Changes in the structure of the haemagglutinin, which result in changes in antigenicity as new epidemic strains appear, involve surface residues in the region of the molecule furthest from the viral envelope. The available information does not permit prediction of future variations because the mechanism of selection of antigenic variants, which occurs during antigenic drift, is not known and multiple evolutionary pathways appear possible. Antigenic shift (i.e., the appearance of epidemic strains with a new haemagglutinin subtype) is also unpredictable.

In addition to antigenic drift and shift, evidence has recently been obtained for another aspect of variation among influenza viruses. This is the preferential growth of certain virus subpopulations in different host cells in which the virus is cultivated. Influenza viruses grown in embryonated eggs often exhibit minor antigenic and biological differences from viruses isolated and maintained in MDCK (canine kidney) cells. Sequence analysis of the HA gene of such variants has shown that, typically, only a single amino acid substitution in the HA molecule occurs between virus grown in mammalian cells and virus from the same source cultivated in eggs. For recent type B influenza viruses, this substitution resulted in the loss of a glycosylation site at the distal tip of the HA molecule of viruses adapted to growth in eggs. The phenomenon of host cell selection has also been demonstrated for isolates of H1N1 and H3N2 viruses from man. The implications of these findings for considerations of the design and efficacy of inactivated or live attenuated vaccines remain to be established.

Immune responses to influenza

In experimental systems (mainly murine), antibodies against the native HA are effective in neutralizing virus infectivity. Other viral antigens may be important in stimulating the formation of helper T-cells which facilitate anti-HA antibody production. Intranasal inoculation of infectious virus has been shown to induce the formation of specific antibody-secreting cells (ASC) of IgM, IgA and IgG isotypes in the mouse lung and these cells may persist for long periods. Infectious influenza virus, in contrast to inactivated virus, induces the formation of cytotoxic T-cells which are important in clearance of infectious virus from the mouse lung. In experimental animals, recognition of the surface glycoproteins appears to account for only a minor fraction of T-cell activity, which is mostly directed to the internal viral antigens, especially the nucleoprotein. There may, however, be considerable variation between members of a population in the extent to which an individual protein is recognized. T-cell recognition of individual epitopes can vary markedly between inbred mouse strains and may need to be taken into account in development of future vaccines.

Inactivated vaccine

Inactivated vaccines are in routine use in many countries and WHO every year makes recommendations on the antigenic composition of these vaccines which usually contain whole virus or disrupted virus.

Purified surface antigen (haemagglutinin and neuraminidase) vaccines are also available. The latter forms of vaccine were developed to reduce reactogenicity, particularly in children.

Inactivated vaccines are considered to be of low reactogenicity; a sore arm is noted in about 15% of persons and systemic symptoms in about 2% for a day or so after immunization. These vaccines vary in effectiveness but generally prevent about 70% of illnesses when virus causing epidemics are closely related to the vaccine virus. Because of falls in antibody titres early after immunization and frequent changes in the antigenic nature of circulating viruses, annual immunizations are generally recommended — in most countries for persons with chronic disease and other risk groups, but sometimes for healthy adults (USSR) or children (Japan) as well.

Since the early 1960s Japan has followed a policy of offering inactivated vaccine annually to all children aged 3–18 years. Immunization rates in individual classrooms have been reported to range from under 20% to over 90% of children. Surveillance of schools has indicated that there is a reduction in absenteeism and closure of school classes in those schools with the highest immunization rates; however, there have been no controlled studies of the effectiveness of this policy in reducing the spread of influenza to the general population.

The efficacy of inactivated vaccines against influenza virus type A in elderly persons in nursing homes has been found to range from 0% to 80%. It was suggested that the occasionally rather low efficacy of vaccine might be related to a number of alternative explanations including the relatively low antibody responses to immunization that have been observed in elderly persons. Induction of herd immunity in institutions can apparently be achieved when the proportion of residents immunized is high (e.g., more than 70%).

Live attenuated vaccines

Although live attenuated vaccines are being studied in several countries, WHO has not formulated recommendations on their routine use as only a few countries employ such vaccines. Clinical studies in man suggest that live influenza A vaccines can afford protection, similar to that of inactivated vaccines, in healthy children and young adults. There have, however, been few studies in elderly high-risk persons and evidence of their value for protection against pandemic influenza is not available.

Progress on the development and clinical evaluation of cold-adapted (*ca*) strains of influenza A virus as an attenuated vaccine was discussed by the participants. Information was available from studies in the USA and USSR which resulted in basically similar findings. The biological markers associated with attenuation could be transferred reliably from a master attenuated influenza strain by genetic reassortment. Candidate attenuated vaccine strains are generally selected when the two genes coding for haemagglutinin (HA) and neuraminidase (NA) are derived from the variant virus and the remaining six genes of the reassortant are derived from the attenuated master strain. A similar approach to development of influenza B viruses has been less well studied.

In the studies reported, attenuated influenza A vaccine strains of the *ca* type caused mild respiratory symptoms (common cold-like illness) in 5–15% of susceptible vaccinees. Immune responses were detected in a high proportion of subjects with high doses of vaccine administered intranasally. Comparable vaccine properties have been demonstrated for some 15 different reassortant vaccine strains based on the master strain A/Ann Arbor/60 (USA) and about 20 vaccine strains derived from A/Leningrad/134/57 (USSR).

Serum antibody responses following a single dose of vaccine were variable and generally infrequent in children of one to 12 years of age but in some studies were found to be increased following a second dose of the vaccine. Transmission of *ca* vaccine viruses from vaccinees to susceptible contacts was not observed. Bivalent vaccines were studied (e.g., H3N2 and H1N1 viruses) and were apparently safe and as immunogenic as monovalent vaccines.

Studies of the protective efficacy of *ca* vaccines have been described, which involved either artificial challenge of human volunteers with the natural variant or natural challenge. The degree of protection varied somewhat between studies but ranged overall from 50% to 80%. In children two doses of vaccine are apparently required to give the higher levels of protection. Estimates of protection against illness were higher, ranging from 60% to 100% in adults, but were lower in children given a single dose of vaccine.

The levels of serum antibody to haemagglutinin induced by *ca* vaccines were generally lower than those induced by conventional inactivated vaccines, while the levels of IgA antibody in respiratory secretions were generally higher. Antibody in secretions is accompanied by reduced virus shedding, a finding that may reflect reduced transmissions. Antibody is apparently persistent but the duration of immunity is unknown.

The genetic basis for attenuation of *ca* influenza viruses is being sought. There is evidence for a limited number of mutations in the different segments of the *ca* viruses. Thus far, no reversions to virulence have been identified, although it is important to continue

to monitor the genetic stability of *ca* vaccines carefully.

A direct comparative study, initiated by WHO, of the reactogenicity and immunogenicity of two *ca* vaccines prepared from wild-type A/Korea/1/82 (H3N2) using the two master strains, A/Ann Arbor/6/60 (H2N2) and A/Leningrad/134/57 (H2N2), has been reported. The vaccines, which were studied in young adult volunteers in the United Kingdom, had similar characteristics; neither produced serious reactions although some 7–10% of recipients of each vaccine developed mild common-cold-like responses. Antibody responses to HA occurred after both vaccines but were higher for the A/Leningrad/134/57-derived vaccine.

The participants agreed that live vaccine strains with properties suitable for clinical evaluation could be produced reliably from *ca* master attenuated strains. These properties include failure to revert to virulence in clinical studies and satisfactory immunogenicity and infectivity. As experience in the development and evaluation of *ca* vaccines developed from given *ca* master strains increases, there will be a progressively reduced need to carry out extensive clinical studies to establish the safety and acceptability of each new vaccine. Nevertheless, at present there remains a need for clinical evaluation of every new vaccine before it can be recommended for wide use in the population.

CONCLUSIONS AND RECOMMENDATIONS

Influenza continues to be an important disease, which is known to cause high levels of morbidity in developed countries at unpredictable intervals, usually every few years. Complications are most common in certain high-risk groups of persons. The extent of influenza in developing countries is less clear, but in pandemics all countries are involved. While prevention of epidemics is not feasible, largely due to the emergence of antigenic variants, the impact of epidemics can be reduced to some extent by immunoprophylaxis with available vaccines. Killed (inactivated) vaccines to influenza A and B viruses are used for this purpose in many countries, and live attenuated intranasal vaccines against influenza A are widely used in a few countries. Both types of vaccines can be prepared with current technologies so as to cause few, usually mild, side-effects, and to have high levels of immunogenicity in healthy children (above the age of about one year) and healthy adults. Two doses of either vaccine are generally required in unprimed individuals to achieve satisfactory immunogenicity. In primed individuals one dose of inactivated vaccine is considered adequate, but two doses of live vaccine may be desirable. Owing to higher levels of background immunity in older persons, they may not react to live vaccine, and it is unknown at present whether such persons are susceptible to severe illness in a natural epidemic. Inactivated vaccines may be less immunogenic in the very old, but data exist to show that even in these cases protection against influenza complications is produced. Based on available data, it is apparent that personal protection against influenza A virus can usually be safely induced by either type of vaccine in children or young, healthy adults, but present knowledge favours the use of inactivated vaccine in older persons, or in others for whom live vaccine might have unusual complications (e.g., immunocompromised persons, or persons with certain respiratory diseases). In closed or semi-closed communities, maximum benefit from immunization is likely to be achieved when more than about three-quarters of the population are immunized so that the benefits of herd immunity can be exploited. Other strategies to reduce the impact of influenza include mass immunization of certain populations, e.g., schoolchildren or working adults, not only to protect such persons but also to reduce illnesses in the overall community. Adequate studies have not yet been undertaken to conclude one way or the other whether the desired reduction in community-wide illnesses results from such targeted mass immunization programmes.

National health authorities are encouraged to review their influenza control recommendations and to take account of newer findings, while WHO will assist in the undertaking of controlled studies to answer questions about the optimum use of influenza vaccines and will encourage continued research to improve vaccine composition and manufacture.

Recommendations

1. Studies that are designed to assess vaccine efficacy should include their ability not only to protect the vaccine but also to reduce the impact of influenza epidemics in the community.

2. Well-controlled studies to assess further the protective efficacy of vaccines should be encouraged. Investigations in children, healthy adult populations, and elderly persons in residential communities would be of value. Studies should include, where appropriate, direct comparison between *ca* live and inactivated vaccines which should provide useful information on the relative efficacies of the two vaccine types. Further definition of the humoral and cellular immune responses to different viral antigens following immunization with live and inactivated vaccines should be undertaken.

3. Studies of the effectiveness of influenza vaccines should include detailed epidemiological surveillance

including characterization of prevalent influenza virus strains, full characterization of the vaccines used, and detailed analysis of the antibody responses in vaccinees. When required, WHO should play a major coordinating role in these studies (including defining protocols and arrangements).

4. Further development work on *ca* live attenuated vaccines should include the study of the genetic and molecular basis of attenuation of vaccine strains as well as the number and location of attenuating mutations and their stability. Of particular importance in the studies of live vaccines will be studies of safety in high-risk adults and in infants, investigations of the duration of vaccine immunity induced by one and two doses of the vaccine, and the ability of live vaccines to prevent the spread of the virus in the community.

5. For inactivated vaccines, improved methods of presenting influenza antigens so as to enhance their antigenic and protective properties should be studied, including the use of medically acceptable adjuvants and the use of antigen-presenting aggregates, e.g., liposomes and related structures.

6. Alternative strategies for developing influenza vaccines, other than the conventional inactivated and live vaccines, should be explored, e.g., expression of antigens by recombinant DNA methods or the use of genetically modified viruses including live vectors.

7. Further work is needed on the development of vaccines for influenza B. This should include improvement of inactivated vaccines and development of live attenuated vaccines.

8. The requirements for the manufacture, standardization and control of both inactivated and live attenuated vaccines should be reviewed and, where necessary, revised with WHO's support.

9. There is a great need for information on influenza epidemiology which will help in the selection (from among new influenza virus variants) of those that should be included in the annual recommendations on influenza vaccine composition. Such studies should aim at collecting data on influenza morbidity and mortality linked with data on virus isolation, strain characterization and seroprevalence. The WHO-recognized national institutions for influenza, as well as selected centres in developing countries with access to epidemiological and virological information, could be approached for collecting such data.

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