Influenza (II): A Persistent Disease May Yield to New Vaccines

The next influenza season will not begin for another 6 months, but pharmaceutical manufacturers have already completed production of vaccines for it and are now in the process of distributing them. For most of the companies, these vaccines are the second batch produced by methods based on the most recent knowledge about the molecular biology of the influenza virus. This knowledge has enabled the manufacturers to respond somewhat more rapidly to the emergence of new influenza variants, to increase the rate of production of vaccines, and to produce the vaccines at a lower unit cost.

But these advances in vaccine production are relatively modest and leave much to be desired. It has become readily apparent that final control of influenza will almost certainly rest with new vaccines that are now in the early stages of development.

Influenza vaccines now in use are produced with killed viruses, but many scientists believe that attenuated live viruses not only may be more effective in stimulating immunity, but also may have less potential for provoking undesired side effects. Other investigators argue that the best approach, particularly in vaccines for those at highest risk—children, the elderly, and the chronically ill—is stimulation of immunity with purified antigens isolated from the surface of the virus. Nearly everyone agrees, however, that one of the most important goals of influenza research is to reduce further the time that lapses between emergence of new influenza variants and the production of new vaccines, and perhaps even to anticipate that emergence.

Influenza is a truly unique infectious disease. Most other virus diseases, such as measles or smallpox, are caused by stable viruses whose continued existence is sustained by passage through animals or by infection of newborn susceptible infants. Influenza, in contrast, is caused by a highly mutable virus whose continued existence is made possible only by frequent changes in its antigenic identity. These changes, termed antigenic shifts, enable new variants partially or wholly to circumvent existing immunity, and thus to reinfect the same population repeatedly.

The antigenic shifts are manifested in two glycoproteins, hemagglutinin and neuraminidase, found on the surface of the influenza virion. Hemagglutinin binds the virus to the target cell; if the hemagglutinin function is inhibited, as by an antibody, the virus is no longer infective. Neuraminidase cleaves a glycosidic bond in the host cell membrane to free the newly formed virus from the cell; inhibition of the neuraminidase has little effect on the infectivity of the virus, but inhibits the spread of virus particles to other cells.

The hemagglutinin and neuraminidase moieties are the antigens by which the influenza virus is recognized in the host organism, and formation of antibodies specific for these glycoproteins is the primary response of an organism subjected to virus attack or to vaccination. The antigens, in turn, undergo two types of change in response to rising antibody concentrations: frequent (once every year or two) minor mutations that give the mutant a somewhat increased resistance to antibody attack, and less frequent (once every 10 to 12 years) major changes that circumvent nearly all existing immunities.

A Gross Change in Composition

The minor changes result from point mutations, the simple substitution of one amino acid for another at one or more sites on the polypeptide chain. Major changes, however, involve a gross alteration in the amino acid composition of one or both antigens, and are thought to result from an interchange of genes between two different subtypes of influenza virus.

This interchange is possible because the RNA of influenza virus, unlike the RNA or DNA of virtually all other human viruses, is found in five to seven discrete pieces. Each piece is an individual gene that codes for one or more proteins of the virus. If a host cell is infected by two different subtypes of influenza virus, the genes from these subtypes undergo a random reassortment or recombination to produce not only the two original subtypes, but also one or more hybrid subtypes. Each of these hybrids has a different, but complete, set of genes and inherits characteristics from each parent.

Since only one influenza subtype is observed in humans at any one time, some scientists believe that this recombination takes place in animal hosts with influenza subtypes that have become adapted to growth in animals. In this manner, the virus can acquire either one or two new antigens for which there are no specific antibodies in the population and initiate a new pandemic (global epidemic). Recombination may thus be the source of influenza's persistence. It may also, however, be a valuable tool that can be employed in the control of influenza.

Recombination has already been put to use to speed the production of new influenza vaccines. In the past, the emergence of each new influenza variant has initiated the time-consuming process of adapting the human virus for optimal growth in chick embryos for production of vaccines. Influenza spreads so rapidly, though, that an epidemic or pandemic initiated by the new variant would typically have run its course before sufficient quantities of vaccine were available for its control.

About 12 years ago, Edwin D. Kilbourne of the Mount Sinai School of Medicine of the City University of New York demonstrated that the new variants could be mated with an older influenza subtype (PR-8) that grows very well in chick embryos. Recombination produces a hybrid that possesses both the external antigens of the new variant (or wild type) and the growth characteristics of PR-8. This process, which has now been used commercially for 2 years, reduces the minimum time for production of vaccines against new variants from about 6 months to less than 4 weeks. It also increases the yield of virus particles by as much as a factor of 10.

Vaccines based on these hybrids are still prepared in the conventional manner. The virus particles are first inactivated with an agent such as formalin so they are no longer infective. Either the intact, inactivated virus particles or virus particles whose lipid layer has been disrupted with ether (to expose the internal proteins) can then be administered parenterally.

Some studies suggest, however, that the systemic antibodies stimulated by the killed virus vaccines are no more than 70 to 80 percent effective in producing immunity to influenza. A much higher rate of protection, many investigators believe, can be achieved when attenuated live viruses are used to stimulate production of secretory antibodies in the localized immune system of the respiratory tract.

Influenza vaccines made with live viruses have been used in the Soviet Union for many years, but they have never been licensed in the United States, in part because of the risks of over- or underattenuation of the virus. These risks have been overcome in vaccines for other infectious diseases, but for influenza they are compounded because of the need for continual change in the vaccine formula to combat new variants. Accordingly, at least three different groups of investigators are pursuing lines of research that capitalize on the recombinational ability of influenza viruses to meet these problems.

One of the more promising approaches to a live virus vaccine is that of Robert M. Chanock, Brian R. Murphy, and their colleagues at the National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, who are investigating a site attenuation. Normal influenza virus subtypes grow readily only between 30° and 40°C. By exposing a 1965 A_2 influenza subtype to a chemical mutagen, 5-fluorouracil, Chanock and Murphy obtained temperature sensitive (ts) mutants that stop growing at temperatures between 37° and 40°C. They adopted this approach because the respiratory tract—the target of the influenza virus—is the only body system that exhibits a temperature differential. The upper respiratory tract has a temperature of about 32° to 34°C, whereas in the lower respiratory tract and lungs the temperature is 37°C. They reasoned that a ts mutant might be able to produce a mild infection in the upper respiratory tract and stimulate immunity, but would be attenuated in the lower respiratory tract, the site of most significant pathology.

This reasoning apparently proved correct, although confirmation required them to transfer the ts trait to a more current Hong Kong A_3 variant because they couldn't find any subjects who didn't already have antibodies to the A_2 variant. The best variant they found, called ts-1-E, stops functioning at 38°C. This mutant was able to infect all volunteers to whom it was administered intranasally; less than 25 percent of the volunteers developed acceptable, mild symptoms in the upper respiratory tract, while the remainder had no symptoms. This infection stimulated production of moderate concentrations of systemic antibodies and high concentrations of secretory antibodies in the respiratory tract. These concentrations provided 100 percent protection against infection when those vaccinated were exposed to the wild-type virus.

In subsequent studies, Chanock and Murphy have also demonstrated that ts-1-E is stable—it doesn't revert to the wild-type virus—and that it is not transmissible. In replication in man, Chanock points out, the ts mutant produces only 0.2 to 1.0 percent as many new virus particles as the wild type, so that there are probably not enough viral particles shed by an infected individual to provide the minimum density necessary for infection of other persons. Preliminary results obtained in association with other investigators, including R. Gordon Douglas, Jr., of the University of Rochester, Rochester, New York, Robert Parrott of Children's Hospital, Washington, D.C., and A. F. Beare of Harvard Hospital, Salisbury, England, indicate that the ts-1-E vaccine is also safe for use in the elderly and, unlike many killed virus vaccines, in young children.

Cold-Adapted Variants

A closely related approach, originally described by Anatoly A. Smorodintsev of the Soviet Union, has been adopted by Fred M. Davenport, H. F. Maassab, and their associates at the University of Michigan, Ann Arbor. Maassab cultured an older, mouse-adapted influenza subtype in chick kidney tissue cultures at progressively lower temperatures, and was able to produce a variant that grows efficiently at 25°C, but not above 41°C, and that had lost its virulence for mice.

After demonstrating that this cold-adapted variant stimulated immunity to the wild-type virus in mice and ferrets, Davenport and Maassab transferred the trait to current A_3 variants by recombination. In tests on more than 1200 human volunteers, they subsequently showed that intranasal administration of the cold-adapted variant stimulates production of systemic and secretory antibodies without provoking symptoms of influenza. They also demonstrated that the variant is stable and is not transmitted under natural conditions, but they have no direct evidence that it protects against the wild-type virus. They hope to obtain such evidence in on-going clinical trials.

A very different approach that depends upon both live and killed viruses has been employed by Kilbourne at Mount Sinai. Most other investigators have, either directly or indirectly, focused their attention on the hemagglutinin moiety, since antibodies against hemagglutinin inhibit the infection of target cells and are thus the primary determinant of influenza immunity. But several lines of evidence indicate that antibodies against the neuraminidase moiety greatly limit the severity of an influenza infection by inhibiting spread of the virus within the host.

Kilbourne has produced vaccines specific for the neuraminidase antigen by mating the prevailing A_3 influenza variant with an equine influenza subtype whose hemagglutinin moiety is antigenically unrelated to that of the wild-type virus. Selective recombination produces a hybrid that incorporates the neuraminidase from the wild subtype and the "irrelevant" hemagglutinin from the equine subtype. Parenteral administration of the formalin-inactivated hybrid then stimulates production of antibodies against both antigens, although only the antibody specific to the neuraminidase participates in the immunization.

Kilbourne terms this method "infection-permissive" immunization because the complete realization of immunity requires either natural or planned infection of the vaccinated individual with the live wild-type virus or with a virus that has been only mildly attenuated. Because of the neuraminidase antibodies, however, this infection produces either no symptoms of influenza or only mild, acceptable symptoms. The combination of vaccination and infection provides the complete immunity. Because the hybrid is administered in an inactivated form, there is no problem with stability or transmissibility of the virus, but the method is subject to the previously discussed limitations of killed virus vaccines. The efficacy of this technique has been demonstrated in mice and in human volunteers, and clinical trials are now beginning.

All three vaccines are still in the early stages of development, and a conservative estimate is that it will be

at least 3 or 4 years before sufficient clinical data can be accumulated to permit licensing of any. Most investigators are hopeful, but not optimistic, that at least one will be licensed in time to help combat the influenza pandemic that is expected in the late 1970's. Meanwhile, investigators are also looking at other means of immunizing the population.

One problem with either killed or live virus vaccines is the finite probability of nonspecific pathologic reactions to parts of the virus that are not involved in stimulating immunity. Many investigators, including Kilbourne, Davenport, Edwin A. Eckart at the University of Michigan, Purnell W. Choppin of Rockefeller University, New York City, and Geoffrey Schild of the National Institute for Medical Research, London, England, are therefore studying the possibility of using purified antigens obtained from disrupted virus particles. Results from their experiments have been mixed.

Work with influenza and a variety of other virus diseases has shown that the ability of purified antigens to stimulate the production of antibodies is significantly reduced when they are separated from the virion. The origin of this phenomenon is unknown, but many scientists assume that the small size of the isolated antigen makes it nonimmunogenic. There have been some attempts to increase the immunogenicity of isolated antigens by polymerizing them into larger molecules, but these attempts have met with only limited success, suggesting either that the structure of the antigen is changed during polymerization or that its small size is not the only cause of its ineffectiveness.

The immunogenicity of antigens or of killed viruses has been increased by administering them in conjunction with adjuvants—such as alum or emulsified organic liquids—that potentiate the effects of weakly antigenic agents. The mechanism of adjuvant action is unknown. But whatever the mechanism, adjuvants apparently do work. Maurice R. Hilleman and his associates at the Merck Sharp & Dohme Research Laboratories in West Point, Pennsylvania, for example, recently reported that a killed virus vaccine prepared to combat a 1957 A_2 influenza subtype provides protection against a 1968 A_3 subtype when administered with an emulsified peanut oil adjuvant.

Effective adjuvants have never been licensed for use in the United States, however, primarily because the most commonly examined emulsified mineral oil adjuvants have produced tumors when injected into experimental animals. Despite their promise, then, the use of adjuvants will, at the minimum, require the accumulation of a very large amount of new information about their safety and efficacy, and possibly the development of new adjuvants. It is thus likely that live virus vaccines will be introduced first.

In all of the approaches discussed so far, the ability of the influenza virus to undergo antigenic changes remains a major problem. All approaches require the isolation and identification of each newly emerged variant before any start can be made toward vaccine production, so that each new variant is able to infect a large number of people before vaccines are available to control it. Some method must thus be found to anticipate such antigenic shifts before they occur, but only one approach has so far met with any success—acceleration of the pace of the minor mutations that occur within any subtype of influenza virus.

Using a method conceived by S. Fazekas de St. Groth, then at the Commonwealth Scientific and Industrial Research Organization, Epping, New South Wales, Australia, Claude Hannoun and his associates at the Pasteur Institute in Paris have tried to speed those mutations by growing a recent variant of the A_3 influenza subtype in the presence of antibodies specific for that variant. In a manner analogous to that occurring in nature, point mutations in the viral antigens

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Gravity Waves: Correlation with Geomagnetic Storms

Exactly 4 years ago the first report of gravitational radiation was published, and a new field of research was established. Since then many observers have built devices for detecting gravity waves, many have sought to explain what astronomical bodies could produce such waves, and a few have asked what the signals could be, if not gravitational waves from space. The original report was made by Joseph Weber at the University of Maryland, College Park, and he is still detecting coincident signals between detectors at College Park and Argonne National Laboratory outside Chicago.

About 1 year ago a team of Russian researchers found a correlation between some of the events reported by Weber and the planetary geomagnetic activity index K_p (*1*). This index is the mean of measurements of fluctuations of the earth's magnetic field at 12 locations and is primarily an indicator of auroral activity. The Russian paper suggested the possibility that pulsations of the earth's magnetic field could have caused the signals reported as gravitational radiation, but the analysis was based on a very small sample of data, namely, the 17 events originally reported by the group at Maryland.

Last month J. A. Tyson, C. G. Maclennan, and L. J. Lanzerotti at the Bell Laboratories, Murray Hill, New Jersey, evaluated the correlation of a much larger sample of Weber's data with various geophysical, meteorological, and other phenomena (*2*). The sample they analyzed consisted of 262 gravitational radiation events observed over a 4-month period ending 22 December 1969, and is much larger than any sample of raw data Weber and his colleagues have published.

The geomagnetic correlation did not disappear when more data were studied. The Scientists at Bell Laboratories found a relatively high correlation, at 2.7 standard deviations, with the geomagnetic index D_{st}, that measures changes in the ring currents circling the earth in the magnetosphere, and a lower correlation, at 2 standard deviations, with the geomagnetic activity index (K_{FR}) at Fredericksburg, Virginia. Correlations at 2 standard deviations were also found with sunspots and earthquakes (Fig. 1). Whereas K is an index of the activity of components of the magnetic fields in three directions, D_{st} is a measure only of the component parallel to the earth's magnetic axis. If the global ring currents—westward drifts of protons and eastward drifts of electrons—are altered by solar winds, the change is reflected in a variation of the component of

10 Columbus Circle, New York 10019)

14–21. International Congr. on **Tropical Medicine and Malaria**, 9th, Hellenic Ministry of Social Services and Hellenic Ministry of Culture and Sciences, Athens, Greece. (J. Papavassilious, ICTMM, P.O. Box 1373, Athens)

15–16. **Environmental Geologic Mapping · Colloquium**, Austin, Tex. (E. G. Wermund, Bureau of Economic Geology, Univ. of Texas, Box X, University Station, Austin 78712)

15–17. National **Electronics** Conf., Inst. of Electrical and Electronics Engineers, Chicago, Ill. (IEEE, 345 E. 47 St., New York 10017)

15–17. **Energy Resources** Symp., Royal Soc. of Canada, Ottawa, Ont. (Executive Secretary, RSC, 395 Wellington, Ottawa, K1A0 N4)

15–17. National **Noise Control Engineering** Conf., Washington, D.C. (R. Cohen, Ray W. Herrick Labs., School of Mechanical Engineering, Purdue Univ., Lafayette, Ind. 47907)

15–17. **Soil Microcommunities** Conf., 3rd, Syracuse, N.Y. (D. L. Dindal, Dept. of Zoology, College of Environmental Sciences and Forestry, State Univ. of New York, Syracuse 13210)

15–18. **Estuarine Research** Federation, 2nd intern. conf., cosponsored by American Soc. of Limnology and Oceanography, Myrtle Beach, S.C. (A. B. Williams, Systematics Lab., Natl. Marine Fisheries Service, U.S. Natl. Museum, 10th and Constitution Ave., NW, Washington, D.C. 20560)

15–18. **Instrument** Soc. of America 28th mtg., Houston, Tex. (H. S. Kindler, ISA, 400 Stanwix St., Pittsburgh, Pa. 15222)

15–18. **Lubrication** Conf., American Soc. of Mechanical Engineers and American Soc. of Lubrication Engineers, Atlanta, Ga. (ASME, United Engineering Center, 345 E. 47 St., New York 10017)

15–18. American Inst. of **Ultrasound in Medicine**, 18th annual, Detroit, Mich. (M. Wainstock, Dept. of Ophthalmology, Univ. of Michigan Medical School, Ann Arbor)

15–19. American College of **Surgeons**, 59th annual clinical congr., Chicago, Ill. (E. W. Gerrish, ACS, 55 E. Erie St., Chicago 60611)

15–19. **Youth in a World of Change**, World Psychiatric Assoc. and Australian and New Zealand College of Psychiatrists, Sydney, Australia. (Congress Secretary, Box 475, G.P.O., Sydney, New South Wales 2001)

15–20. International Soc. of **Radiology** Congr., 13th, Madrid, Spain. (J. Bonmati, ISRC, Lagasca 27, Madrid 1)

16–18. Society of **Automotive Engineers**, aerospace engineering and manufacturing mtg., Los Angeles, Calif. (A. J. Favata, SAE, 2 Pennsylvania Plaza, New York 10001)

16–19. American **Chemical** Soc., rubber chemistry mtg., Denver, Colo. (F. M. O'Connor, Harwick Standard Chemical Co., 60 S. Seiberling St., Akron, Ohio 44305)

16–19. **Human Factors** Soc., Washington, D.C. (M. G. Knowles, HFS, P.O. Box 1369, Santa Monica, Calif. 90406)

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produced a variant with increased resistance to the antibodies. This new variant was isolated and grown in the presence of antibodies specific for it.

After several such cycles of growth and mutation, Hannoun isolated a variant that no longer mutated under the experimental conditions. This variant, he postulates, represents the end point of evolution within the A_3 subtype, and is thus a virus that would be expected to appear in the late 1970's. Support for this postulate was provided by the discovery that the London influenza variant first isolated in 1972 was antigenically quite like the first mutant Hannoun had produced in his laboratory a year earlier.

As a result of an only partially understood aspect of the mutation process, the Pasteur group believes, antibodies specific for any one influenza mutant also provide protection against all antecedent mutants within that subtype. Vaccines produced from Hannoun's final variant should thus provide protection against all A_3 variants that might appear within this decade—although the emergence of the next major variant will necessitate beginning all over again. Limited studies have already shown that a killed virus vaccine produced from the Pasteur variant is effective against current strains of influenza, and the French government has licensed it for use as soon as possible. It is unlikely that the vaccine will be licensed for use in the United States for at least another year, however, because of the need for more data on its efficacy.

Because the Pasteur vaccine is made with inactivated viruses, it is expected to be no more effective than current killed virus vaccines. If Hannoun's methodology is proved correct, then, the best approach might involve a combination of techniques. That is, the final variant isolated by Hannoun could be used to produce attenuated virus vaccines by the method of Chanock, Davenport, or Kilbourne. In that fashion, almost complete protection could be provided from shortly after the appearance of a major new subtype until the appearance of the next subtype. Given adequate funding for the development and application of these techniques, some investigators argue, there need never be another influenza pandemic.

—THOMAS H. MAUGH II