INFLUENZA, A GENERAL REVIEW OF RECENT DEVELOPMENTS*

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Influenza is a febrile illness of Man caused by a virus, *Myxovirus influenzae*, of which there are at least 3 distinct immunological types. This virus has a predilection for the epithelium of the respiratory tract. The infected cells undergo necrosis, which is followed by inflammation involving the various parts of the tract in a varying degree in different cases. The resulting illness is characterized by a sudden onset, headache, prostration, and fever, and symptoms and signs of involvement of the respiratory tract, including coryza, sore throat, slight cough, and in some cases pneumonitis. The death rate of uncomplicated influenza is very low, but pneumonia complicating influenza is one of the commonest causes of death, especially of the old and frail.

RECENT HISTORY

The cause of the influenza epidemics of the late 19th century and of the 1918-19 pandemic was not ascertained. The influenza virus was first isolated in 1933 by Wilson Smith, Andrewes, and Laidlaw[1]. They transferred the infection from human cases by instilling their throat washings intranasally into ferrets, which then developed a febrile condition associated with coryza and sneezing. The first strain isolated was passed serially from ferret to ferret. On recovery the ferret was immune to challenge with the same virus, which was also neutralized by sera from convalescent patients. Later the virus was transmitted from ferrets to white mice, which greatly simplified its study. This was still further facilitated by its adaption to embryonated eggs[2] and the subsequent finding that it caused red cells to agglutinate.[3] This property of causing haemagglutination has been of the greatest value in the study of the influenza virus and has had many important practical applications.

A second immunologically distinct type of virus was isolated independently in 1940 by Francis and by Magill,[4] from throat washings of patients with influenza. This virus was thus called Influenza B, and the type isolated previously, Influenza A.

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A third immunologically distinct type was isolated by Taylor in 1947,[5] and a similar strain was isolated in 1949 by Francis, Quilligan and Minuse[6] from cases of illness indistinguishable from mild influenza. This virus has been named Influenza C.

In 1952 Kuroya N. *et al.*[7] investigated an outbreak of pneumonitis among newborn infants in a hospital at Sendai in Japan. The onset was sudden, with fever, cyanosis, and marked dyspnoea; 12 of 17 patients died. From 5 of the fatal cases a virus was isolated by the intranasal instillation of mice with lung extracts. It was adapted to grow in embryonated eggs and was later found to cause haemagglutination of fowl red cells. It is now known as the Sendai virus. Its characteristics place it in the same group as the influenza viruses. Evidence of infection by the Sendai virus has been found in the United States[8] and more recently in Britain.[9] The illnesses in the patients studied varied from a mild influenza-like illness to severe pneumonia.

In studies on the aetiology of cases of acute laryngo-tracheo-bronchitis in Toronto in 1953-54, Morgan *et al.*[10] found that there was no evidence to incriminate a specific bacterial cause in the majority of cases, but from 5 cases an agent, producing cytopathogenic changes in tissue culture of human embryonic lung and monkey kidney cells, was isolated.

More recently Chanock[11] has described the isolation of a virus from cases of infantile croup in Cincinnati. The infants from whom this virus was isolated, as well as some others with the same condition, developed significant increases in neutralizing antibody, or haemagglutination-inhibiting and complement-fixing antibody, during convalescence—findings clearly suggesting that this virus was responsible for their illness. Its size has been estimated to be 90-135mμ in diameter. It was not ether-resistant and it agglutinated chick red cells and human O red cells. These properties are those required for placing it in the myxovirus group.

The Toronto virus was found to have similar properties and to be indistinguishable by haemagglutination-inhibition tests from one of the croup-associated viruses isolated in Cincinnati. No antigenic relationship with influenza virus A, A^1, B or C, Newcastle-disease virus or Sendai virus was found. It appears, then, that another immunologically distinct virus of this group has been uncovered. Thus, up to the present, the following influenza viruses or influenza-like viruses have been recognized:

Myxovirus influenzae	A
" "	A^1
" "	B
" "	C
" "	D (Sendai virus)
" "	E (The virus associated with infant croup in Toronto and Cincinnati)

THE VIRUSES OF INFLUENZA

These viruses are all members of the Myxovirus group, which also includes, among others, the virus of mumps and the virus of Newcastle disease of fowls. The viruses of this group are characterized by being moderately large viruses, occurring as spheres varying from 80 to 150 mμ in diameter, or as filaments of about the same diameter and up to several microns in length. They are stable at —76°C but less so at —10°C, and their infectivity is readily destroyed by treatment with 20% ethyl ether. They also have the property of causing red cells to agglutinate, apparently resulting from adsorption of the virus onto receptors on the surface of the red cell. Haemagglutination and its inhibition by the corresponding specific antiserum has been extensively used in the study of this group of viruses, particularly of their immunological relationships.

These studies have shown that the influenza A viruses have been undergoing a progressive series of antigenic changes with the frequent appearance of new varieties. The WS subtype was found in 1933. From 1934 to 1945 the strains were of the PR8 subtype. The strains isolated from 1946 and subsequently have been so different antigenically from the A strains isolated earlier that they have been allotted to a new subtype, the A prime (A^1) subtype. Strains of this subtype isolated in one epidemic tend to be serologically uniform, but those isolated in different epidemic years show definable differences. All influenza A viruses share a common soluble antigen which may be demonstrated by the complement-fixation test.

There is also evidence that the influenza B viruses include a number of different subtypes, but generally the influenza B Strains are more uniform serologically than the A viruses.

The antigenic variations within the influenza C type, the Sendai type, and the myxovirus associated with infantile croup, have not yet been defined.

'Asian' Influenza. Viruses having the characteristics of the Myxovirus group have been isolated from cases of 'Asian' influenza occurring in Hong Kong, Malaya, the Phillipines, India and, more recently in Holland and the United States of America. These viruses are apparently immunologically similar, and by complement-fixation tests have been shown to be strains of influenza A type. However, the haemagglutination-inhibition tests have revealed that these strains are antigenically distinct from all previously isolated strains of this type. Preliminary studies of the sera of persons of different ages carried out in Holland suggest that many people over the age of 70 have antibodies against this virus, but almost none under this age have immunity. This interesting finding needs further investigation but, if confirmed, it suggests that the virus responsible for this epidemic is immunoligally related to the viruses responsible for the pandemic of 1890-91.

PATHOGENESIS

The influenza viruses have a predilection for the epithelium of the respiratory tract. The adsorption onto these cells is mediated through receptors on the surface of the cell. The virus multiplies within the cells and brings about their destruction. Large areas of the epithelial lining may thus be damaged.

CLINICAL PICTURE

The incubation period is 1-4 days, usually 1-2 days. The onset is characteristically sudden, with headache, muscle aches, and shivering. The illness is characterized by headache, anorexia, prostration, drowsiness, and muscle aching, especially of the limbs and the small of the back. The temperature rapidly rises to its maximum and the fever on the average lasts 3 days, but in many cases is shorter.

In the early stage of the illness the constitutional signs and symptoms are outstanding; later those arising from involvement of the respiratory tract become more prominent.

The nose becomes blocked and there is usually coryza and cough, but little sputum is produced. The throat is sore and dry, and the voice is usually changed, but marked hoarseness is not often noted. The appetite is decreased and there may be slight nausea, but vomiting and diarrhoea are not a feature of the illness.

On examination the patient has a flushed, weary face, and his eyes are congested. The throat may show congestion and enlargement of the tonsils and adenoids. The chest may be clear, but a few rales and rhonchi may develop, especially in the later stages of the illness. During the fever the pulse rate is increased in proportion to the temperature. The spleen and liver are not enlarged or tender and there are no other abnormalities of the abdomen.

In convalescence some patients complain of debility and depression, and often a bradycardia is found, but most cases recover rapidly and completely.

Complications are common in influenza and most often involve the respiratory system. Bronchitis and bronchiolitis occur frequently, and influenzal pneumonia is not uncommon. The occurrence of these complications is suggested by a prolongation of the fever, and they are revealed by the characteristic signs on examination of the chest.

In most epidemics an occasional case of an acute fulminating pneumonia is seen. In the 1918-19 pandemic such cases were common. Early in the illness the patient developed dyspnoea associated with sweating and tachycardia, followed soon by cyanosis, which rapidly deepened as the difficulty in breathing increased. Many such cases ended fatally within 24-48 hours of the apparent onset. This fulminating type of influenzal pneumonia may be caused by the action of the virus alone.

More commonly pneumonia complicating influenza is due to a secondary bacterial infection with pneumococci, streptococci, staphylococci, or *Haemophilus influenzae*.

DIFFERENTIAL DIAGNOSIS

Influenza has to be differentiated from a number of acute febrile illnesses, especially those involving the respiratory tract. Amongst the latter are particularly those infections caused by the recently discovered group of Adenoviruses. Often the differentiation of influenza, which may present as a mild coryza or as a rapidly fatal fulminating pneumonia, from a number of other infections which may present in similar fashion, is impossible on clinical grounds alone.

The features favouring a diagnosis of influenza are: its occurrence in epidemic form; its sudden onset, with at first a predominance of constitutional symptoms over respiratory symptoms; and fever which unless complicated by secondary bacterial infections is of short duration. In the absence of such complications a feverish illness lasting longer than 1 week is not likely to be influenza, and other causes of the illness should be sought.

LABORATORY DIAGNOSIS

The clinical picture of a fully developed case of influenza is fairly characteristic, but there are many gradations in its severity, and in the mild, less typical, cases, as in the severe fulminating cases, it may be necessary to appeal to the laboratory for help in the diagnosis.

For the isolation of influenza virus from a suspected case, the patient should be asked to gargle with nutrient broth for about 30 seconds. These throat washings should then be sent under refrigeration to the laboratory, by putting the bottle in a watertight plastic bag and wrapping well in cotton wool before placing in a thermos flask containing ice. At the same time a specimen of blood—the acute-phase specimen—should be collected and also sent to the laboratory. About 2 weeks later a second specimen of blood—the convalescent-phase blood—should be collected. The sera separated from these two specimens are tested for the presence of, and the titre of antibodies against, known influenza viruses, and also against the patient's own strain.

Isolation of the virus may be attempted by instilling ferrets intranasally with a suspension prepared from the patient's throat washings. If infected, the ferrets will develop a febrile illness associated with coryza. This may be apparent on the original inoculation. More often several ferret-to-ferret passages are necessary before the characteristic picture is developed.

Today the usual method of isolating a strain of virus is to inoculate a suspension of throat washings from the patient into the amniotic cavity of chick embryos. After one or two passages it is adapted to grow in the allantoic cavity. Its serological relationships can then be studied by the application of the haemagglutination technique. The influenza virus causes human O, guinea-pig, and chick red cells to agglutinate. The inhibition of this haemagglutination by specific antisera enables the type and subtype and strain of the virus to be identified.

The antibody contents of the acute- and convalescent-phase sera are measured by the complement- fixation test, or more commonly by the haemagglutination-inhibition test. A fourfold rise in the titre is significant and indicative of an infection by the corresponding virus.

TREATMENT

A patient with influenza should stay at home in bed. If possible he should be isolated in his own room separate from the rest of the family to diminish the risk of passing on a heavy infection. It is particularly important to ensure this for the protection of very young infants and the old and frail. The patient should be given a light diet with plenty of liquid, including fresh fruit drinks.

No drug or antibiotic at present available is of specific value in controlling the influenza virus infection. They should be reserved for the treatment of complications, in which, of course, their use is clearly indicated and may be life-saving.

PREVENTION

It is almost impossible to prevent the spread of influenza. In pandemics the only communities which have escaped have been those living on isolated islands. Much, however, can be done to minimize the severity of the illness by insisting that patients should stay at home and that they should not for any reason become over-fatigued, or over-stressed in other ways, during the illness.

Vaccines which have been shown to be effective have been produced. Those in general use are of the killed or inactivated type. The virus suspensions from which these vaccines are prepared are harvested from chick embryos. The method used in preparing these vaccines is briefly as

follows: Hen eggs are incubated for 10-12 days. They are then candled. The infertile and dead eggs are discarded. The live eggs are inoculated into the allantoic cavity with the virus through a hole punched in the blunt end. After this hole has been sealed, the eggs are re-incubated for 2 days. They are then candled again. The dead ones are discarded. The live eggs are then opened at the blunt end by burning round with a fine oxyacetylene flame. The shell membrane is lifted off. The allantoic membrane is torn and the vessels allowed to bleed into the allantoic fluid, which is then harvested. This fluid contains high titre of influenza virus, but it is further concentrated and purified either by spinning the virus down in a Sharples centrifuge or by allowing the red cells to settle in the cold, and then taking off the supernatant fluid and adding 1/10th its volume of saline. The red cells are then re-suspended by shaking gently and then the suspension is incubated for 2-3 hours at 37°C. During this incubation the virus elutes from the red cells, which can then be separated by centrifuging. The supernatant fluid, now containing virus in high concentration, is inactivated by the addition of formalin in appropriate concentration.

The vaccine so prepared is controlled bacteriologically to ensure its sterility. Its potency is determined by the inoculation of animals, usually mice, guinea-pigs or chickens, with 2 doses of vaccine with an interval of 14 days between inoculations. The animals are bled 1 week later and the haemagglutination-inhibition antibody content is determined. There should be a significant rise in titre, at least fourfold, in the post-inoculation specimen as compared with the pre-inoculation specimen.

In human trials vaccines so prepared have been shown to be effective in stimulating significant rises in antibody titre in the majority of individuals inoculated. In field trials it has also been found that, provided the vaccine contains the strain responsible for the current epidemic, it confers a significant amount of protection on those inoculated. This has emerged particularly from studies in schoolchildren in England. In one such trial there were 10 times as many cases in the unvaccinated children as in the adequately vaccinated children.

Unfortunately vaccines prepared from strains isolated in previous outbreaks are usually of much less protective value. To have maximum efficacy the vaccine must be prepared from the strain responsible for the current epidemic. To make this possible is one of the objectives of the World Influenza Centre set up in London by the World Health Organization. Regional Centres have been established at strategic centres in various parts of the world. In April 1957 an extensive outbreak of influenza began in Hong Kong. This epidemic then spread to other countries in the Far East and from there has spread widely in the Northern Hemisphere. Virus isolated in Malaya was identified as belonging to the A type by complement fixation, but was found to be different from the recent strains of previous epidemics. This finding was confirmed by the London Centre. Ampoules of virus were then flown by airmail to several centres, including institutions engaged in the preparation of influenza vaccine. Although it will be possible in many of these countries to prepare vaccine before the epidemic begins, unfortunately the amount that can be prepared is very limited. Reliance can therefore not be placed on prophylactic vaccination to prevent the spread of influenza in a country. Its use will be limited to the inoculation of key personnel so as to reduce the disorganization of a community which may result from an extensive epidemic of influenza.

Two inoculations of vaccine at an interval of 6 weeks or longer are recommended. In most instances, therefore, it may be possible to give only one before the epidemic begins. However, even one injection, particularly of patients who have had previous experience of influenza, may be of value in stimulating a certain degree of immunity.

As it will not be possible to prevent the spread of influenza by vaccination, the authorities concerned have to make arrangements to ensure that essential services are kept going, and that special arrangements which may be necessary are made to provide extra hospital accommodation to treat those cases whose condition requires hospital treatment. It is to be emphasized that uncomplicated cases of influenza should be treated at home. It is not necessary to send them to hospital. In providing such services the help of voluntary services such as the Noodhulpliga, the Red Cross, and St. John Ambulance Association will be essential.

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