Recurrence of influenza with A_2/Hong Kong/68 within two months

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Abstract

A CASE REPORT of two influenza-like illnesses in the same person, within two months, where a diagnosis by virus isolation from throat swabs on both occasions was A_2/Hong Kong/68. Only the second illness was confirmed serologically by H.I. and C.F. as no blood was collected during the first illness. Previously, the earliest reported interval between natural reinfection with the same strain was two years.

Introduction

Repeated illness with influenza in any selected population is certainly not rare, but has nearly always been accounted for by a major or minor change in antigenic composition of the offending strain. (1) Natural reinfection of an individual with the same or closely related strain has been described but with minimum time intervals of at least two to three years between illnesses (1, 2, 3). It is the purpose of this article to report positive virus isolations (A_2/Hong Kong/68-like strains) from the same individual during the onset of two influenza-like episodes of illness with a time interval between them of only two months.

Case Report

First Illness

A 29-year-old female laboratory worker had a sudden onset of fever, malaise, chilliness, myalgia and arthralgia on 19th July, 1968. On examination the next day, the temperature was 103°F, throat congested, and conjunctivae – injected but there were no other significant findings. The fever subsided two days later but she now complained of cough, wheezing and retrosternal soreness. Because of a previous history of pneumonia following influenza in 1957, she was started on ampicillin 250 mg six hourly, together with bronchodilators. The wheezing subsided in a week but the cough persisted for another two weeks. A throat swab labelled F9/68 was collected on Day Two of the illness.

Second Illness

On 14th September 1968 – almost two months later – while she was working in the laboratory with influenza specimens collected during the 1968 influenza epidemic with the A_2/Hong Kong/68 strain, she again contracted a similar illness. She was now pregnant at six weeks gestation. This time the maximum temperature was 102° and cough and wheezing developed the next day. She developed status asthmaticus three days later. She was again

treated as before. Convalescence was prolonged and complicated by asthmatic bronchitis. She became asymptomatic, however, in about eight weeks. On 4th May, 1969, she was delivered of an apparently normal 6 lb. 9 oz. infant boy. A throat swab, labelled F221/68, and an acute specimen of blood was collected on Day One of the illness. A second blood sample was collected 17 days later and a third sample on 25th November 1968 – almost two months later.

Material & methods

Virus Isolations

Throat swabs were collected by touching the posterior pharynx with a dry swab immediately after asking the patient to cough. They were placed in sterile tubes containing 2 ml. of bacteriological broth and this was stored at –70°C till processed. Before inoculation into eggs, penicillin and streptomycin were added to the specimen to yield a final concentration of 625 units and 125 ugms per ml. respectively. Viruses were isolated in 9–11 day-old embryonated eggs following standard procedures (4). When the eggs were negative in a spot haemagglutination test two blind passages were carried out. F9/68 and F221/68 were processed on 20th July, 1968 and 14th September 1968 respectively. In both instances, agents were isolated and labelled as A_2/Malaysia/9/68 and A_2/Malaysia/221/68 respectively. Agents were reisolated successfully from the original specimens on the 8th and 14th April 1969.

Virus Identification

Identification of isolates was by means of cross-haemagglutination-inhibition tests (H.I.) employing allantoic fluid antigens of the isolates labelled A_2/Malaysia/9/68 and A_2/Malaysia/221/68 and the prototype strain A_2/Hong Kong/1/68. Other antigens included in the test were A_2/Taiwan/1/64, B/Switzerland/265/67 and B/Singapore/3/64. The antisera were A_2 Polyvalent, B Polyvalent, antisera against the isolates and A_2/Taiwan/1/64. The A_2/Taiwan/1/64 antigen and the A_2 and B polyvalent antisera were supplied in the W.H.O. influenza kit in 1969 by the Influenza Centre for the Americas, National Communicable Disease Centre, Atlanta, Georgia, U.S.A. The B/Singapore/3/64 virus was obtained from the Department of Bacteriology, University of Malaya, Singapore. The A_2/Hong Kong/1/68 prototype strain and A_2/Hong Kong/124/68 antisera were kindly sent to us by Dr. W.K. Chang, Government Virus Unit, Queen Mary's Hospital, Hongkong; the B/Switzerland/265/67 virus was sent by Dr. H.G. Pereira, WHO Influenza Reference Centre, Mill Hill, London.

The method of the HI test and chicken antisera preparation against our isolates and rabbit antisera against B/Singapore/3/64 A_2/Taiwan/1/64 was essentially that recommended by the subcommittee on diagnostic procedures for viral and rickettsial diseases (4) except that WHO perspex plates instead of tubes and eight HA units of antigen were used. Initial serum dilutions were 1:20 and the titre of H.I. antibody was expressed as the reciprocal of the highest dilution of serum which inhibited agglutination of red cells.

Serology

Blood was collected by venepuncture on Days 2 and 17, 2½ months and a year after the onset of the second illness and was allowed to clot at room temperature. Sera were separated and stored at –20°C till processed. They were tested simultaneously against the two isolates – A_2/Malaysia/9/68 and A_2/Malaysia/221/68 – A_2/Hong Kong/1/68, A_2/Taiwan/1/64 and B/Singapore/3/69 antigens by H.I. All sera tested by H.I. were treated by receptor-destroying-enzyme for removal of non-specific inhibitors. Initial serum dilutions were 1/10 for serology. The complement fixation test (C.F.) was done by the California State Health Department Virus Laboratory at San Francisco.

Result

Table 1 shows that both A_2/Malaysia/9/68 and A_2/Malaysia/221/68 belong to type A_2 because of high H.I. titres with A_2 polyvalent and negative reactions with B polyvalent antisera. They most closely resemble A_2/Hong Kong/68 giving identical titres with its antiserum. This was confirmed by Dr. Marion Coleman of the National Communicable Diseases Centre, Atlanta, Georgia, U.S.A. Cross-HI test with their antisera also confirm the close antigenic similarity of the two strains isolated from the patient. The formula of Archetti & Horsfall, which is a measure of antigenic similarity between two strains, is given as:

$$r = \sqrt{r_1 \times r_2} \text{ where}$$

$$r_1 = \frac{\text{heterologous titre of virus 2}}{\text{homologous titre of virus 1}}$$

$$r_2 = \frac{\text{heterologous titre of virus 1}}{\text{homologous titre of virus 2}}$$

When r = 1, it indicates no antigenic difference and any value of $\frac{1}{r}$ 2 is significant for antigenic dissimi-

Table 1

IDENTIFICATION OF ISOLATES BY H.I. TEST

ANTIGENS	ANTISERA					
	A_2/POLYVALENT	B POLYVALENT	A_2/HONG KONG/24/68	A_2/MALAYSIA/9/68	A_2/MALAYSIA/221/68	A_2/TAIWAN/1/64
A_2/HONG KONG/1/68	1280	< 20	1280	160	320	10
A_2/MALAYSIA/9/68	1280	<20	640	160	160	<10
A_2/MALAYSIA/221/68	1280	<20	640	160	320	< 10
A_2/TAIWAN/1/64	1280	<20	40	<20	<20	320
B/SWITZERLAND/265/67	<20	320	<20	<20	<20	–
B/SINGAPORE/3/64	<20	640	<20	<20	<20	–

larity (5). When applied to A_2/Malaysia/9/68 and A_2/Malaysia/221/68, $\frac{1}{r} = 1.4$. When either of the isolates are compared with A_2/Hong Kong/68, a value of $\frac{1}{r} = 1.4$ is also obtained. Serological results show an absence of HI antibody against either of the two isolates and A_2/Hong Kong/1/68 and minimal titre of ten for A_2/Taiwan/1/64 in the acute specimen for the second illness and a greater than four-fold rise against all four strains in the two convalescent serum samples with no significant lowering one year later (see Table 2). The CF test indicated a four-fold rise of antibodies for the second illness.

Discussion

Presented here are two episodes of influenza-like illness in the same individual within a remarkably short time of two months where the etiological diagnosis by virus isolation was A_2/Hong Kong/68. The second illnes was confirmed by a four-fold or greater rise of both CF and HI antibodies. A serological confirmation of the first illness could not be done as appropriate blood samples were not collected. Table 2, however, shows that two months later, at the onset of the second illness, there were no demonstrable HI antibodies against A_2/Malaysia/9/68. This could mean either that A_2/Malaysia/9/68 did not stimulate the production of any HI antibodies, (or that they could not be measured in the HI test) or they had not persisted for two months. Alternatively, A_2/Malaysia/9/68 might not be a valid isolate, and the first illness might not be influenza. It is probably valid because a throat-swab, from which virus was isolated, was collected during an influenza-like illness at the beginning of the 1968 influenza epidemic with A_2/Hong Kong/68 strains and it was reisolated from the original throat swab material in April 1969. Besides, there were reports in the literature when serology by H.I. or C.F. or both have been negative in cases with positive throat swabs (6, 7).

TABLE 2

SEROLOGY

HAEMAGGLUTINATION – INHIBITION TEST

Sera	Date of collection	ANTIGENS				
		A_2/HONG KONG/1/68	A_2/MALAYSIA/9/68	A_2/MALAYSIA/221/68	B/SINGAPORE/3/64	A_2/TAIWAN/1/64
VR 25152	14th September 1968	< 10	< 10	< 10	< 10	10
VR 25260	1st October, 1968	640	640	640	< 10	320
VR 25506	25th November, 1968	640	320	640	< 10	320
VR 26426	September, 1969	320	320	320	–	160

TABLE 3

SEROLOGY

COMPLEMENT FIXATION TEST WITH INFLUENZA-A

Sera	Date of Collection	Titre
VR 25152	14th September 1968	16
VR 25260	1st October, 1968	64
VR 25506	25th November, 1968	64

The chance that the second illness was a laboratory infection is great because the patient had been handling isolates from the 1968 epidemic in the week prior to the illness. The infecting dose of virus may thus have been larger than normal. In any case, the immunity was low in the face of challenge with the same virus. Except for the episode of pneumonia following Asian influenza in 1957, the patient has not exhibited unusual susceptibility to infection. Her Hb electrophoresis revealed Hb. A and results of total serum proteins and serum protein electrophoresis were normal. Her blood group is A. Tyrrell, Sparrow and Beare, who studied the relationship between blood groups and resistance to infection with influenza in human volunteers, found a greater proportion of those with blood group O became infected than those with blood group A.

Experimental studies in man have shown that four months after the induction of clinical influenza by inhalation of type B virus, when 24 of the same people were re-exposed to the same strain, they had clinical disease evidenced by fever in 21, symptoms in nine and serological responses in eight out of the 24 people. (9) Similar results were reported by Henle et al with influenza type A (10) in which inhalation of a second dose nine months following convalescence from the experimental disease by the same strain of virus resulted in symptoms and fever in five out of nine individuals. The anitbody titre of these five subjects had returned to the low pre-exposure level during the interval between the first and second exposure. A second natural illness with type A has also been described (2, 3) but the strains were at best only closely similar.

Studies of the effect of influenza on pregnancy and the unborn foetus have produced conflicting results (11, 12, 13, 14). In this patient, the pregnancy was uncomplicated and terminated spontaneously at term with the birth of a normal baby.

Summary

A case report of a reinfection with A /Hong Kong/68-like influenza virus within two months is presented. This occurred in a patient in the first trimester of pregnancy who delivered an apparently normal baby.

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