Nagoya J. Med. Sci. 47. 1 ~ 4, 1984

ANTIGENIC ANALYSIS OF INFLUENZA B VIRUS ISOLATED FROM THE EPIDEMIC IN 1973

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ABSTRACT

Antigenic relationships between an isolate (B/Aichi/1/73) from the epidemic of influenza B in 1973 and earlier strains of the same type were studied by hemagglutination-inhibition and neuraminidase-inhibition tests. The results showed that B/Aichi/1/73 underwent considerable antigenic variation in hemagglutinin but not in neuraminidase.

Keywords: Influenza B virus, Hemagglutinin, Neuraminidase, Epidemic, Antigenic variations.

INTRODUCTION

Influenza virus particles possess two morphologically and biologically distinct spikes on their surface, one being hemagglutinin and the other neuraminidase.[2] The hemagglutinin is responsible for the attachment of virus particles to the receptor sites on the surface of host cells at the initial stage of infection, and it also has an affinity for receptors on the surface of erythrocytes, causing hemagglutination.[9] Antiserum to hemagglutinin inhibits both virus infection and hemagglutination. The neuraminidase possesses the enzyme activity for hydrolysis of N-acetyl neuraminic acid residues from glycoproteins forming the receptor, and its function in replication is thought to be the release of virus particles from the plasma membrane of host cells.[1]

Antigenic variation in hemagglutinin and neuraminidase is one of the most important characteristics of influenza viruses. In influenza type A virus, two kinds of antigenic variations, antigenic shift and drift, occur. Antigenic shift, where a new subtype virus suddenly arises, is probably the result of genetic reassortment between human and other animal influenza viruses.[5,11] These new subtype viruses appear every 10-15 years and present a major problem in the control of influenza by vaccination. Antigenic drift, which occurs within the new subtype virus at intervals of two or three years, also creates problems in the control of influenza by vaccination, particularly when the drift gives rise to viruses differing considerably in antigenicity from their predecessors. This variation occurs by small changes in the amino acid sequence of the polypeptides of hemagglutinin and neuraminidase.[5,11] Antigenic drift in influenza type B virus has been shown to occur since the virus was first isolated in 1940,[7] but no antigenic shift has been reported.[10,11]

Influenza type B virus generally causes a low frequency and magnitude of epidemics of influenza,[3] possibly because the minor antigenic changes occur less frequently than those in influenza type A virus.[12] In 1973 widespread outbreaks of influenza B were documented in Japan as well as in many other countries and a number of influenza B strains were isolated.[8] It has been reported that the isolates from the epidemic in Japan underwent considerable antigenic variation in hemagglutinin, but their neuraminidase antigens were not characterized (ab-

久野有文，井上裕正
Received for Publication January 21, 1984

stracts for the 22nd annual meeting of the Society of Japanese Virologists, Sendai, Japan, 1974). We studied the antigenic relationships in neuraminidase between the B/Aichi/1/73 isolate from the 1973 epidemic and the earlier strains of the same type.

MATERIALS AND METHODS

The following influenza B strains were used in this study: Lee/40, Akishima/1/64, Osaka/1/70, Aichi/1/71 and Aichi/1/73. All of these strains were grown in the allantoic cavity of 10-day-old chick embryos and purified by sedimentation through a sucrose gradient as previously described.[6] Antisera were prepared in chicken by intravenous injection of the purified virus (1000 HA units). This immunization procedure was repeated twice at an interval of 7 days. Antisera used in hemagglutinination-inhibition (HI) tests were pretreated with receptor-destorying enzyme and then heated at 56°C for 30 min. Hemagglutinin titration and HI-test were previously described.[6] Neuraminidase assay and neuraminidase-inhibition (NI) test were performed with a fetuin substrate by a modification of Warren's thiobarbituric acid method.[4] One unit of neuraminidase was defined as the amount of enzyme required to yield sufficient N-acetylneuraminic acid (NANA) to produce an optical density (OD) of 0.1 at 549 nm. NI test was done by incubating 4 to 6 units of enzyme at room temperature for 1 hr with equal volumes of three-fold serial dilutions of antiserum or normal serum before assay of enzyme activity, and NI titer was expressed as the reciprocal of the final dilution of antiserum which causes 50% inhibition of enzyme activity.

RESULTS

Table 1 shows serological cross-reactions in HI test among influenza B strains isolated between 1964 and 1973 and B/Lee/40. The hemagglutinins of Akishima/1/64, Osaka/1/70, and Aichi/1/71 were antigenically closely related to one another but differed significantly from that of Lee/40. The hemagglutinin of B/Aichi/1/73 was antigenically different from those of all of the earlier isolates listed above. Such antigenic changes are thought to be antigenic drift, since cross-reactions were found at low levels among hemagglutinins of all strains tested. Similar findings were also observed with epidemic strains isolated not only in other areas of Japan but also in foreign countries including the United Kingdom and the U.S.A.[8]

Table 1. Serological cross-reactions among hemagglutinins of influenza B strains in HI tests

Virus strains	Antisera				
	Lee/40	Akishima/64	Osaka/70	Aichi/71	Aichi/73
Lee/40	2048	32	16	16	32
Akishima/64	32	1024	1024	1024	128
Osaka/70	32	512	2048	2048	32
Aichi/71	<16	256	2048	2048	64
Aichi/73	32	<16	16	<16	2048

It has been demonstrated that antibody directed against the hemagglutinin of influenza virus inhibits neuraminidase activity of the intact virus particle and this is thought to be due to steric

hindrance mediated through the reaction of anti-HA antibody in the antiserum with hemagglutinin of the virus particles.[6] Thus, we attempted to separate the active enzyme from the virions by trypsin treatment. The purified virus suspensions were treated with trypsin (1 mg/ml) at 4°C for 60 min, after which soybean trypsin inhibitor (1 mg/ml) was added to the mixtures. The virus particles were pelleted by centrifugation at 20,000 rpm for 60 min in a Beckman SW 27 roter and resuspended in the original volume of phosphate-buffered saline. As shown in Table 2, the majority of hemagglutinating activity of each strain was associated with the sedimenting fraction and more than half of the original enzyme activity was detected in the supernatant. Table 3 shows serological cross-reactions in NI test among neuraminidases of Lee/40, Akishima/1/64, and Aichi/1/73 strains, using soluble enzymes. The enzyme activities of Akishima/1/64 and Aichi/1/73 were both inhibited by antiserum to either virus to the same levels but poorly by antiserum to Lee/40, while the enzyme activity of Lee/40 was suppressed only by homologous antiserum, indicating that the neuraminidases of Akishima/1/64 and Aichi/1/73 are antigenically related to each other but differ significantly from that of Lee/40.

DISCUSSION

Antibody to hemagglutinin of influenza virus neutralizes homologous virus and contributes to host defence against influenza. Antibody to neuraminidase does not neutralize virus infectivity but does slow down the release of virus from infected cells,[1] and probably plays an important role in reducing viral replication in vivo and in preventing the spread of infection. Influenza virus is unique among infectious agents in its capacity to change its antigenicity so remarkably that the virus causes epidemic disease in man. Epidemic strains of influenza are currently believed to emerge by selection of naturally occurring variants which are little or imperfectly neutralized by the host's immune mechanisms.[12] We studied the surface antigens of one epidemic strain isolated from the large outbreaks of influenza B in 1973 and showed that antigenic variation was confined to the hemagglutinin. This result implies that the occurrence of antigenic variation in hemagglutinin confers the survival advantage on the virus and that variation in neuraminidase antigen is of lesser importance in the epidemiology of influenza.

Table 2. Virion-associated and soluble biological activities after centrifugation of trypsin-treated influenza B virus

Virus	HA activity per 0.25 ml		Enzyme activity OD_{549} per 0.1 ml	
	Pellet	Supernatant	Pellet	Supernatant
Lee/40	12800	160	4.15	5.80
Akishima/64	6400	320	1.08	5.52
Aichi/73	12800	80	1.59	6.54

Table 3. Serological cross-reactions among neuraminidases of Influenza B strains in NI tests

Soluble enzyme	Antisera		
	Lee/40	Akishima/64	Aichi/73
Lee/40	90	30	270
Akishima/64	10	2430	2430
Aichi/73	10	2430	2430

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