

The HI test modified by ether treatment in the sero-epidemiological surveillance of influenza B

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SUMMARY

Ether-treated influenza B haemagglutination inhibition (HI) antigen was used in a study of serum collections from three different epidemic seasons.

For diagnostic purposes, ether treatment increased the efficacy of the HI test by about 50% over the conventional HI technique, raising it to the same level of sensitivity as the complement fixation (CF) test. The treatment reduced the specificity of the HI test, but its reliability in the diagnosis of influenza B infections was only slightly diminished. With regard to evaluation of the immune status of a given population, an HI test using ether-treated antigen from the epidemic influenza B strain seems to give more relevant information about the antibody level associated with protection than a conventional HI test using untreated virus antigen.

INTRODUCTION

The conventional haemagglutination inhibition (HI) test is widely used in the serodiagnosis of influenza infection and the determination of response to influenza vaccination. Antibodies to the surface antigens of the virus, especially the HI antibodies in sera, have repeatedly been shown to be associated with protection against influenza infection, whereas no such clear correlation has been shown with antibodies to the internal antigens. The strain-specific class of HI antibodies is expected to be of greater importance to resistance (Haaheim & Schild, 1980; Oxford *et al.* 1981). It follows that the evaluation of pre-epidemic HI antibody status against prevalent viruses is an index of immunity in the population; in influenza surveillance it is also the most popular method for predicting the intensity of a potential outbreak.

One disadvantage of the HI test is that it is not sufficiently sensitive to reveal small amounts of antibody against certain 'poorly reactive' influenza strains, including the influenza B strains epidemic in recent years. This problem has been approached by developing or applying new test methods (Schild, Pereira & Chakraverty, 1975; Chakraverty, 1980; Turner *et al.* 1982; Oxford, Yetts & Schild, 1982; Julkunen, Pyhälä & Hovi, 1985) and by modifying the HI test with ether treatment of the influenza B virus antigen (Monto & Maassab, 1981). Kendal & Cate (1983) stated that this treatment increased the sensitivity but reduced the specificity of the test.

The present serological survey is a study of the effect of the ether treatment of influenza B virus antigen on the results obtained using the HI test. Particular attention is paid to the protective level of pre-epidemic HI antibodies determined by the modified method and to the influence of the reduction of specificity on the usefulness of the test as a tool in sero-epidemiological surveillance.

MATERIALS AND METHODS

Serum collections

Four collections of sera were examined:

(1) The initial sample of the first collection consisted of acute and convalescent-phase sera sent to the National Public Health Institute for routine antibody testing from patients who had contracted an influenza-like illness or other acute respiratory infection during the epidemic season 1981/82. The sera were examined using HI for antibodies to certain influenza A and B viruses and using complement fixation (CF) for antibodies against a variety of virus antigens and *Mycoplasma pneumoniae*. The influenza strains used as antigens are listed below.

The final sample included 44 patients who had a fourfold or greater increase in influenza B virus antibodies as determined by CF and/or HI. The efficiency of the two tests in revealing diagnostically significant increases in the final sample is shown in the results: the HI test was either modified using ether treatment (HI(ET+)) or not modified (HI(ET-)).

The majority (30) of the 44 patients were civilians; the sera were sent by general hospitals from different parts of the country. The other 14 patients were conscripts hospitalized in the training centres of the Finnish Defence Forces. The sera were tested immediately or after a maximum of one month at +4 °C.

(2) The second collection was like the first, but the sera were taken during the epidemic season 1982/83. The final sample contained paired sera from 102 subjects who had a fourfold or greater increase in influenza B virus antibodies in CF and/or HI tests; there were 53 civilians and 49 conscripts.

(3) Paired sera were taken from a total of 135 conscripts at two military training centres, the pre-epidemic specimens in the middle of October 1982 and the post-epidemic specimens from the same subjects at the beginning of May 1983. The sera were stored at -20 °C until tested for antibodies against influenza A and B viruses in summer and autumn 1983.

(4) Collection and preliminary testing of the initial sample were like those of the first two collections, but the sera were taken during the epidemic season 1983/84. The final sample contained paired sera from 155 patients (120 conscripts and 35 civilians) who had a fourfold or greater increase in HI antibodies against H1N1 subtype viruses. The sera were accepted regardless of possible increases in antibody against other viruses, including the influenza B strains in the HI and CF tests.

Screening of antibodies

The initial sample of the first collection of sera was studied for HI antibodies against the following influenza viruses: B/HongKong/5/72, A/Finland/1/79 (H1N1), A/Finland/26/81 (H1N1), A/Finland/31/80 (H3N2) and A/Finland/34/80 (H3N2). The same viruses supplemented with A/Finland/1/82 (H1N1) served as

antigens in the HI tests of the second serum collection. The strains used for the third collection were B/HongKong/5/72 and B/Finland/6/83 (a B/Singapore/222/79-like isolate). Seven strains were used in the fourth collection: B/HongKong/5/72, B/Singapore/222/79, A/Finland/1/79(H1N1), A/Finland/1/82(H1N1), A/Finland/31/80(H3N2), A/Finland/1/83(H3N2) and A/Finland/3/83(H3N2).

The principles presented by Robinson & Dowdle (1969) were followed in the HI tests. The sera were pretreated with cholera filtrate (Philips-Duphar B.V., Holland) at a dilution of 1 in 6 to remove nonspecific inhibitors. Infected allantoic fluids from embryonated eggs were used as antigens; they were diluted in phosphate-buffered saline to contain four haemagglutinating units of virus. The treatment with ether was performed as described (Monto & Maassab, 1981). The paired sera were always studied simultaneously.

Sera from the first, second and fourth collections were studied for CF antibodies to S antigen preparates from influenza A and B viruses (A/Finland/23/75(H3N2) and B/HongKong/5/72). The test was performed using conventional methods (Casey, 1965).

The influenza outbreaks

Diagnostic findings of the National Public Health Institute, Helsinki, indicate that in the winter season 1981/82 three viruses were responsible for the influenza outbreaks in Finland. An influenza B virus related to B/Singapore/222/79 was the most prevalent in the general population. The 1982 outbreak started at the end of March and did not cease until the end of June. Influenza B activity was also recorded among conscripts in the Finnish Defence Forces, but findings of H1N1 subtype viruses related to A/England/333/80 were more frequent. Evidence of H3N2 subtype influenza A viruses was found mainly among conscripts.

In the epidemic season 1982/83 there was again an influenza B outbreak caused by a virus similar to B/Singapore/222/79. The outbreak began in January and ended in April in the military training centres and in May in the civilian population. The most prevalent viruses in the general population and among conscripts were, however, H3N2 subtype viruses; strains related to A/Belgium/2/81, A/Philippines/2/82 and A/Bangkok/1/79 were isolated. H1N1 subtype viruses were only responsible for some local outbreaks.

In 1983/84 the outbreak was almost exclusively due to H1N1 subtype viruses; it began in February and ceased at the end of April. Influenza B viruses were diagnosed, but their epidemic activity was low. Only sporadic cases of H3N2 influenza were recorded. The H1N1 strains isolated were heterogeneous, representing four antigenic variants: A/Victoria/7/83, A/Chile/1/83, A/Dunedin/27/83 and A/England/333/80.

RESULTS

In the final sample of the first collection from the epidemic season 1981/82, a fourfold or greater rise in antibodies to influenza B viruses was detected by CF in 41 patients (93%), by HI(ET-) in 23 patients (52%) and by HI(ET+) in 38 patients (86%). The respective figures for the second collection in 1982/83 were

Table 1. *The effect of ether treatment of the antigen on the HI test in the serodiagnosis of influenza B: significant antibody increases detected in the final samples of the 1981/82 and 1982/83 collections*

CF	Anti-B increases in the test system indicated		Number (%) of anti-B increases*	Number of anti-A increases* among anti-B response cases	
	HI(ET+)	HI(ET-)		Anti-A(H1N1) increases by HI with (+) and without (-) anti-A increases by CF	Anti-A(H3N2) increases by HI with (+) and without (-) anti-A increases by CF
+	-	-	17 (12)	+	-
+	+	+	77 (53)	0	0
+	+	-	38 (26)	0	14
+	-	+	0	0	7
-	+	+	6 (4)	0	0
-	+	-	8 (5)	0	1
-	-	+	0	0	1
-	-	-	0	0	0

* \geq fourfold increase in titre.

Table 2. *Serological influenza B infections in a follow-up material from the 1982/83 season containing pre-epidemic and post-epidemic sera*

Pre-epidemic titre	Infections* occurring in relation to the titre of pre-epidemic antibodies using the following test systems and antigens			
	HI(ET-)		HI(ET+)	
	B/Fin/6/83†	B/HongKong/5/72	B/Fin/6/83†	B/HongKong/5/72
< 12	34/130 (26%)	25/72 (35%)	22/57 (39%)	6/17 (35%)
12	0/4	5/18 (28%)	8/26 (31%)	5/12 (42%)
24	0/1	3/24 (13%)	4/28 (14%)	11/32 (34%)
48	-	0/11 (0%)	0/9 (0%)	9/35 (26%)
96	-	1/6	0/10	1/14
192	-	0/3	0/4	1/17
\geq 384	-	0/1	0/1	1/8

* \geq fourfold increase in titre in antibodies to one or more of the four influenza B strains used as antigens.

† A strain similar to B/Singapore/222/79.

91 (89%), 60 (59%) and 91 (89%). Thus, the ether treatment enhanced the diagnostic efficiency of the HI test to the level typical of the CF test.

In the combined final samples of the two collections (146 subjects) a significant rise in influenza B virus antibodies was detected by CF and HI in 115 (79%) cases, by CF only in 17 (12%) cases and by HI only in 14 (10%) cases. A more detailed comparison of the tests is given in Table 1. It can be seen, e.g. that at least fourfold increases in HI antibodies to H1N1 subtype strains of influenza A viruses were frequently (23 cases, 16%) associated with anti-B rises, both among patients whose anti-B response was detected by CF and HI (21 of 115, 18%) and among those in whom it was detected only by HI (2 of 14, 14%). No significant rise in CF antibodies to influenza A viruses could be detected in any of these cases with HI responses to the H1N1 viruses. Thus, the aetiology of influenza A infection was not confirmed. On the other hand, three subjects (2%) in the combined final samples demonstrated a significant increase in antibodies to H3N2 subtype viruses; in two of them the influenza A infection was confirmed by CF.

In the initial samples of the first two collections 33 patients exhibited simultaneous minimum fourfold rise in both influenza A(H1N1) antibodies detected by HI and influenza A antibodies detected by CF. No significant response to influenza B viruses could be detected in any of these patients, not even by HI(ET+). All these cases were therefore eliminated from the final samples and are not included in the values given in Table 1. Among these patients there were, however, two subjects (6%) who showed a twofold increase in antibodies to influenza B/HongKong/5/72 detected using the HI(ET+) test.

Among the 155 patients of the fourth collection who exhibited a fourfold or greater rise in HI antibodies to H1N1 viruses during the season 1983/84 there were only four cases who showed a simultaneous significant rise in antibodies to influenza B virus antigens. In two of the cases the rise could be detected using both CF and HI, thus indicating an aetiology of influenza B infection. In one of the two remaining cases the influenza B response was detected by HI(ET+) and HI(ET-), and in the other only by HI(ET+).

The frequencies of serologically determined influenza B infections in the third collection, containing pre-epidemic and post-epidemic sera taken in 1982/83, can be seen in Table 2. The following points are emphasized: (1) the pre-epidemic titres of HI(ET-) antibody to the epidemic virus (B/Finland/6/83) were rather low and probably not useful for the evaluation of resistance to infection; (2) higher titres of HI(ET-) antibody to the virus which circulated in the community in previous years (B/HongKong/5/72) was depicted - 50% protective titres was of the order of 12-24, but in one case even a titre of 96 was not protective; (3) the poorest correlation between pre-epidemic antibody level and resistance against serologically confirmed infection was recorded by HI(ET+) with B/HongKong/5/72 strain as antigen; (4) the best information about the immune status was obtained using HI(ET+) with the epidemic virus B/Finland/6/83.

DISCUSSION

The HI test has been less efficient than the CF test in detecting significant antibody increases in the serodiagnosis of influenza B infections, which contrasts with the diagnosis of infections with the recently epidemic H3N2 subtype (Pyhälä & Kleemola, 1976) and H1N1 subtype (Hammond, Smith & Noble, 1980) influenza A viruses. The sensitivity of the HI test employing influenza B virus antigen has been described as more (Kendal & Cate, 1983) or less (Mancini *et al.* 1983) increased by ether treatment of the antigen. A clear increase in the diagnostic efficiency up to the level typical of the CF test was recorded in the present study.

In influenza B infections, increases of HI antibodies directed against H1N1 subtype influenza A viruses have been found during the last few years (Hall *et al.* 1981; Kendal & Cate, 1983) and were shown to be common in the present study, too. Significant antibody rises to H1N1 subtype viruses were frequently detected in influenza B infections also in both the 1981/82 season and during the season of 1982/83, when the H1N1 viruses did not circulate in the general population. Thus, it is unlikely that persons were infected with both influenza A and influenza B viruses.

On the other hand, it has been proposed that ether treatment of influenza B virus antigen reduces the specificity of the HI test in such a way that the test becomes capable of measuring antibody responses in infections by H1N1 subtype viruses, too (Kendal & Cate, 1983). The phenomenon was described in experimental and natural influenza A(H1N1) infections, and the authors emphasized that caution is needed in interpretation of the HI test for serodiagnosis.

Some evidence of this loss of specificity was also found in the present study. The reliability of the HI(ET+) test in the diagnosis of influenza B infections was not, however, substantially diminished by H1N1 subtype influenza A infections. Similar observations have been described with the HI test employing Tween-ether treated antigens (Profeta & Ballerini, 1981). Nevertheless, caution is still needed and further work is necessary to standardize the method and to clarify the basis of the heterotypic responses and reactions.

The protective titre of pre-epidemic HI antibodies to epidemic viruses varies greatly from host to host, from outbreak to outbreak and from virus to virus (Pyhälä & Aho, 1981; Fox *et al.* 1982). A titre of ≥ 40 –48 has usually been regarded as a useful level in estimating the susceptibility of a population to influenza A viruses. In another common approach, attention has been paid to the frequency of seropositive subjects (a titre of ≥ 10 or ≥ 12 , as a rule).

In the present study it appears that better information about the immunity of a population to influenza B viruses may be inferred from data obtained using the HI test modified by ether treatment of the antigen. The modified method was useful, however, only if an epidemic virus was used as antigen. Besides, low levels of HI(ET+) antibodies (actually a titre of 12) did not confer protection against serologically determined infection. It is probable that these disadvantages are partly due to the reduced specificity of the HI test by ether treatment.

The pre-epidemic titre of ≥ 48 in HI(ET+) antibodies to the epidemic virus was associated with complete protection in conditions where the overall rate of serologically determined infections was 25%, but the rate of conscripts exhibiting

this protective level was not higher than 18%. Under these circumstances this level was a poor indicator of the immune status. A more satisfactory level may be a titre of ≥ 24 , which conferred better than 50 per cent protection as compared to the lower titres. It is necessary to emphasize, however, that the follow-up material of the present study contained a narrow age group and institutional conditions. Thus it may be incorrect to extend the conclusion to the general population.

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