Increasing Hemagglutination Inhibition Antibodies against Two Lineages of Type B Influenza Virus in 2017-2018 Winter Season in Beijing, China

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Abstract

Background Type B influenza virus circulates every year with lower activity than that of influenza A virus in China. During 2017 to 2018 winter season, a sharp surge of influenza activity dominated by type B/Yamagata lineage virus caused unprecedented medical burden in Beijing. The research aimed to understand the underlying mechanism for this circulation and be prepared for epidemics in the future. Methods Sera samples collected from the patients in 2016-2017 and 2017-2018 flu seasons were tested for profiling hemagglutinin inhibition (HI) antibodies against both prevailing Victoria and Yamagata lineages of type B influenza viruses. Results It showed that the positivity of HI antibodies against both lineages of the virus in 2017-2018 winter was higher than that in 2016-2017, while no difference of the positivity of HI antibodies was observed between the two winter seasons. Meanwhile, significant elevated geometric mean titer (GMT) against both lineages of influenza B viruses was found in the specimens collected during 2017-2018 flu season than that from 2016-2017, suggesting the viruses might undergo antigenic changes. These results also suggested that lower GMT against both type B variants in 2016-2017 might serve as an immunological niche for the dominating of B/Yamagata virus in China during 2017-2018 winter season. Conclusions Our findings have implication that vaccines including both lineages of influenza B virus in formula might be a better preparation against influenza B epidemics.

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Increasing Antibodies against Flu B Viruses in 2017-2018

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Abstract

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Key words: Type B influenza virus; Hemagglutinin inhibition (HI) antibodies; Geometric mean titer (GMT)

INTRODUCTION

Since October 2017, information from the World Health Organization (WHO) has highlighted the increasing level of influenza activity compared with previous flu seasons[1]. It dramatically reached the peak and caused a huge medical burden in Beijing and such situation had been lasting till April, 2018[2]. Sharply increasing number of patients with influenza-like-illness (ILI) suggested that the circulating virus might have evaded the herd immunity or mutated to achieve a higher transmissibility[3]. This predication soon was confirmed by the evidence from China CDC that Yamagata lineage of type B influenza virus (Flu B) was the predominant strain resulting the epidemic, although few cases were reported to be type A influenza virus (Flu A) infection including pdmH1N1/2009 and H3N2[4].

Flu B virus firstly identified in 1940 was associated with considerable hospital admissions and deaths worldwide annually[5, 6]. During early 1980s, it evolved into two lineages, designated as B/Yamagata (B/Y) and B/Victoria (B/V), showing distinct antigenicity and transmission pattern[6-8]. Since then, Flu B viruses have co-circulated with Flu A virus during epidemic season and the dominant lineage has changed over years in different geographical locations[9, 10]. Several studies have pointed out the differences of the epidemiology between B/Y and B/V lineages, such as younger average ages of persons preferred being attacked by and higher transmissibility of B/V viruses, compared with B/Y viruses[8, 11, 12].

For a long time, much effort has been done on Flu A virus, whereas Flu B virus was relatively less investigated, resulting in poor lineage matches with recommended influenza vaccine strains and the control options require further improvement[13, 14]. Hemagglutination inhibition (HI) tests measure the presence of specific antibodies of IgG in sera that inhibit virus-mediated agglutination of erythrocytes[15]. This is a sensitive assay that is affected, in some level, by nonspecific hemagglutinin inhibitors in the sera. However, it is a particularly reliable method in for influenza surveillance, providing confirmative result for suspect individuals with successive sera samples, also profile the herd immunity against upcoming influenza variant, in which case tiers [?]1:40 are considered protective[16]. In the present study, serological assay against two lineages of Flu B viruses

circulating in China was determined to investigate the cross-sectional HI antibodies in defined samples. Our findings provided insights on low level herd immunity to influenza B virus prior to the B/Y lineages epidemic would in some level contribute to this epidemic.

MATERIALS AND METHODS

Sera samples and ethics approval

Two batches of serum samples from Beijing Tsinghua Changgung Hospital (BTCH) and Beijing Children Hospital (BCH), respectively, were used in the present study. Batch one was 190 sera samples collected during Nov, 2016 to Apr, 2017 (2016-2017) from outpatients in BTCH with the age ranging from 0.5 to 94 years old. Batch two was 283 sera samples collected from outpatients presenting acute respiratory infections (ARI) from BCH during the Dec, 2017 to Mar, 2018 (2017-2018), with the age ranging from 0.5 to 17 years old. Samples in Batch one was subdivided into 3 groups according to ages and diagnosis, group 1 from 69 adults aged from 18 to 94 years old and excluded respiratory diseases, group 2 from 75 patients aged from 0.5 to 17 years old present other than respiratory symptoms, group 3 from 46 patients aged from 0.5 to 17 years old and presenting ARI. All sera samples were remaining from routine clinical tests and were stored at -40degC till use. The studies did not involve any health-related patient information and were approved by the Ethics Committee of both Beijing Children's Hospital and Beijing Tsinghua Changgung Hospital (Approval No. 2018-k-130 / 17120-0-01).

Representative influenza viruses for HI assay

Type B influenza viruses isolated from China during the two winter seasons were selected for HI assay on the basis of data collected by the World Health Organization (WHO) as well as our own study[17]. For Victoria lineage, B/Jiangxi_yushui/11102/2014 which was phylogenetically and antigenically resemble to the recommended vaccine strainB/Brisbane/60/2008, belonging to V1A sublineage, was selected as the antigen for HI. For Yamagata lineages, the representative virus B/Beijing/BTCH_71/2018, belonging to Y3 sublineage isolated in early 2018 in Beijing was used[17]. The phylogenetic relationship of the selected virus was presented (Figure1). Viruses were amplified in 9-11-day-old specific pathogen free (SPF) chicken embryonated eggs at 35degC for 2 days. The allantoic fluid were collected and cleared by low-speed centrifugation. The titer of the virus was determined by HA assay and viral antigen aliquots was stored at -80degC till use. All experiments associated with live viruses were performed in the biosafety level 2 plus laboratory.

Figure 1. Phylogenetic tree of the HA gene of type B influenza viruses used in the study.

Phylogenic tree was constructed using the partial HA genes (370-610NT covering the most important positions distinguishing the Victoria and Yamagata lineages) from the clinic isolates and full-length HA sequences from the GenBank as well as GISAID. Strains labeled with black triangle represented as B/Victoria and B/Yamagata lineage viruses were used as antigens in the present study.

Hemagglutinationinhibition (HI) assay

Serum samples were examined for the presence of antibodies against the hemagglutinin of the selected type B influenza viruses by HI assay as described previously[18, 19]. Briefly, serum samples were heat inactivated at 56degC for 1 hour and then were absorbed by 20% (v/w) turkey red blood cell (TRBC) to reduce the non-specific binding. 2-fold serial dilutions with 1:10 staring dilution of pretreated serum samples were subsequently incubated with 8 hemagglutination units of influenza virus or phosphate-buffered saline (PBS) for 30 min at 37degC, and subsequently, 1% TRBC was added. HI pattern were read after incubation for 30 mins at 22degC. The highest dilution of serum that still gave complete inhibition of the hemagglutination was recorded as the titer. Serum samples were considered negative when they failed completely to inhibit agglutination of TRBC by any of the selected viruses. Serum samples collected from mice before and after immunization with each of the type B influenza viruses were used as negative and positive controls, respectively. Considering previous exposures to Flu B viruses or vaccinations in individual's life would have interference on HI antibody profile, we took [?]1:80 as cut-off limit for "positive reaction" of recently infection.

Statistics

Data analyses were performed using SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA). Nonparametric test was used for comparing the age difference of children and GMT of the sera samples. Correlation of HI titers against two lineages of Flu B virus was employed Spearman tests. The Pearson Chi-square test or Fisher's exact tests were performed to evaluate HI antibodies seroprevalence to Yamagata and Victoria lineages of Flu B virus, with cut-off value as HI titers [?]1:80. Two-sided at the 5% level of significance was considered statistically remarkable in all tests.

RESULTS

HI titers against two lineages of Flu B viruses in sera from 2016-2017

The HI titers to the two lineages of Flu B viruses in sera from 2016-2017 were compared. Result indicated that the frequency with HI titers over the cut-off value in the 3 subgroups did not show difference, either to B/Y, or to B/V lineage virus (Table 1). HI titers in group 3, the sera from children present ARI did not increase as compared to the group 2, sera from children without ARI (P =0.47 for BY and P =0.78 for BV). Hence, we put group 2 and group 3 together (n=121) to represent HI value in children (CHI) from 2016-2017. The HI titers in CHI group were also compared to subgroup 1 of adults and no difference was found (P =0.65 for BY and P =0.49 for BV). The results indicated a lower epidemic of Flu B in 2016-2017 winter, with the HI titers in children and adult showing no difference previous the B/Y epidemic year.

Table1. Summary and comparing of HI titers to B/Y and B/V lineages in 2016-2017

Sera cohorts	Group1(N=69) No. (%)	Group2 (N=75) No. (%)	Group3 (N=46) No. (%)	P value (3 groups)	P value (group 2 to 3)	P value (CHI to adults^{+})
Sera cohorts Group1(N=69) No. (%) Group2 (N=75) No. (%) Group3 (N=46) No. (%) P value (3 groups) P value (group 2 to 3) P value (CHI to adults^{+})						
B/Yamagata^{++}	14(20.29)	19(25.33)	9(19.57)	0.69	0.47	0.65
B/Victoria§	13(18.84)	18(24.00)	10(21.74)	0.76	0.78	0.49
P value	1.00	1.00	1.00	/	/	/

Table 2. Comparing prevalence of HI titers to B/V and B/Y lineages in 2016-2017 and 2017-2018

Sera cohort/Antigen	2016-201(N=121) No. (%)	2017-2018(N=283) No. (%)	p value
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B/Yamagata^{+}	28(23.14%)	187(66.08%)	<0.01
B/Victoria^{++}	28(23.14%)	169(59.72%)	<0.01 P value 1.00 0.054 /

Figure 2. Comparison of HI titer distributions against two lineages of Flu B viruses by Spearman tests.

We can see categories of HI titer to both lineages increased significantly in 2017-2018 than in 2016-2017. Moderate correlation can be found between B/Y and B/V lineage as the HI titers in 2016-2017 (a) and 2017-2018 (b).

Antigenic drift of Flu B viruses from 2016-2017 to the 2017-2018

Geometric mean titer (GMT) was usually used for evaluating the influenza vaccine efficacy and potency in defined population. In the study, the GMT of HI titer against both lineages of Flu B viruses in sera collected in 2017-2018 (74.16 against B/Y and 67.57 against B/V, respectively) was much higher than that collected in 2016-2017 (31.87 against B/Y and 26.44 against B/V, respectively). Significant increase in the GMT to both lineages could be seen in 2017-2018, as compared to 2016-2017. However, we did not find difference as the GMT to B/Y and B/V either in 2016-2017 (P =0.14), or in 2017-2018 (P =0.11). Given the same antigens used in the HI assay for sera samples from two different years, we predict that Flu B viruses might undergo antigenic drift and it is the reason for the B/Y lineage virus evades herd immunity. Collectively, these data suggested wake HI response in 2016-2017 to both Flu B viruses may contribute to the severe epidemic of Flu B in winter of 2017 (Table 3), and the antigenic drift produce a transition and higher HI titers against most recent/homologues infection.

Table 3. GMT of HI titers against two lineages of Flu B viruses in sera from children in 2016-2017 and 2017-2018.

Sera cohorts	B/Yamagata^{+} (95% CI§)	B/Victoria^{++} (95% CI§)	P value
Sera cohorts			
B/Yamagata^{+} (95% CI§) B/Victoria^{++} (95% CI^{SS}) P value			
2016-2017 (N=121)			
31.87 (27.56-36.86)			
26.44 (21.54-32.46) 0.14			
2017-2018 (N=283)			
74.16 (66.39-82.83)			
67.57 (60.51-75.44) 0.11			
P value **<0.01 <0.01**