Antigenic analysis of wild-type measles viruses currently isolated in Turkey

Gülay Korukluoğlu[1], Pınar Zarakolu[2]

[1]Department of Virology, Refik Saydam Hygiene Center, and [2]Section of Infectious Diseases, Department of Internal Medicine, Hacettepe University Faculty of Medicine, Ankara, Turkey

SUMMARY: Korukluoğlu G, Zarakolu P. Antigenic analysis of wild-type measles viruses currently isolated in Turkey. Turk J Pediatr 2006; 48: 105-108.

The aim of this study was to determine the genetic and antigenic diversity of vaccine-type and wild-type measles viruses by using different antisera in a neutralization assay. Virus strains included genotype A, D3 and D6 clades, and five different groups of antibodies were used. Wild-type measles viruses isolated in Turkey have been classified as D6 and the others isolated in Japan as D3. Antisera used in the assay were against genotype A, D3 and D6. Regardless of the antigenic diversity, protective antibody level (≥1/16) was determined against different genotypes of measles virus.

Key words: measles virus, antigenic analysis.

Despite the availability of an effective vaccine, measles continues to be one of the leading causes of childhood mortality and morbidity in many regions of the world[1]. In 1999, it caused nearly one million deaths, about 10% of global mortality among children under five years of age. In Turkey, the number of measles cases (mainly children) was 30,509 in 2001, and approximately 10% of them died due to the complications of the disease[2].

To reduce measles morbidity and mortality in Turkey, the Ministry of Health launched a National Measles Elimination Program in 2002. In parallel with the strategic plan of the European Regional Office of the World Health Organization (WHO), the Turkish national plan targets elimination of measles by 2010[3]. According to this plan, a "catch-up" vaccination campaign aims to reach nearly 20 million children 9 months to 14 years of age. The first phase of this supplemental vaccination targeting school-aged children was conducted in December 2003[4]. The second phase, targeting preschool-children and school-aged children not attending schools will be conducted in the fall of 2004.

However, countries that have achieved high levels of measles vaccination coverage have frequently witnessed large outbreaks of measles due to primary and secondary vaccine faiIure resulting in incomplete protection against disease[5]. Evaluation of the vaccination campaign is very important and, generally, seroepidemiological studies are conducted for this purpose. However, these are insufficient in evaluating the results of vaccination campaigns. Molecular epidemiological studies take an important part in monitoring the success of disease control and elimination programs. In Turkey, in addition to epidemiological data, viral genotype (D6) will be compared with the genotypes identified following the catch-up campaigns to monitor success of measles elimination[6]. The investigation of genetic and antigenic diversity of measles virus has an important function in assessing the efficiency of measles vaccine. Although measles virus is an antigenically stable virus, several different genotypes of wild measles virus are currently circulating worldwide. However, the contribution of antigenic and genetic diversity among wild-type measles viruses to the epidemiology of the infection is still unclear[7].

The aim of this study was to determine the genetic and antigenic diversity of wild-type and vaccine-type measles viruses by using different antisera in neutralization assay.

Material and Methods

The antigenic reactivity of the recently isolated strains in Turkey and Japan were compared with that of the 'Edmonston' vaccine strain using three sets of antibodies.

Antigens: Three different genotypes were used as antigens: genotype A (Edmonston vaccine strain), genotype D3 (two strains isolated in Japan) and genotype D6 (two strains in Turkey in 2001). All strains except the vaccine strain were isolated from children diagnosed clinically and serologically as measles. Vaccine-type and wild-type (genotype D3) strains isolated in Japan were kindly provided by Dr. Fumio Kobune from the National Institute of Infectious Disease, Tokyo. Wild-type strains were isolated in the National Measles Laboratory of Refik Saydam Hygiene Center and classified as D6 clade in Centers for Disease Control and Prevention (CDC)[6]. The characteristics of the strains used as antigens are presented in Table I.

Table I. Characteristics of the Strains Used as Antigens

Strain	Genotype	CCID50*
Edmonston	A	$10^{4.75}$CCID50/ml
Strain 1 (Japan)	D3	$10^{5.5}$CCID50/ml
Strain 2 (Japan)	D3	$10^{4.75}$CCID50/ml
Strain 1 (Turkey)	D6	$10^{5.5}$CCID50/ml
Strain 2 (Turkey)	D6	$10^{4.75}$CCID50/ml

*CCID50: Cell culture infectivity dose.

Antibodies: Three sets of antibodies were used after being diluted twice.

Set 1: This group of antibodies was provided by Dr. Fumio Kobune from the National Institute of Infectious Disease, Tokyo, Japan and consisted of three different antisera.

Antibody (Ab)1. Monospecific rabbit antisera against purified H protein (genotype A); its neutralizing antibody titer was detected as ≥1024.

Ab2. Immunized rabbit antisera against wild type whole measles virus (genotype D3); its neutralizing antibody titer was detected as ≥512.

Ab3. Immunized monkey antisera against vaccine strain (genotype A); its neutralizing antibody titer was detected as ≥512.

Set 2: This set included five different antisera (Ab4, Ab5, Ab6, Ab7, Ab8) obtained from children who were diagnosed as measles clinically (all had fever and generalized maculopapular rash) and serologically (all were Ig M positive, Ig G negative). All serum specimens were obtained within one week after the onset of rash. Measles virus was isolated and identified as D6 clade in three of five cases[8] (Table II).

Set 3: This set of antibodies (Ab9, Ab10, Ab11, Ab12, Ab13) were obtained from healthy adults (aged between 23-36 years) with unknown vaccination history. All sera were Ig G positive, Ig M negative.

Cell line: COBL cell line ($2x10^5$ cells/ml).

Medium: Growth medium was RPMI 1640 consisting of 10% fetal calf serum (FCS), 1% antibiotic supplement (penicillin, streptomycin, amphotericin B) and 2% $NaHCO_3$. Maintenance medium was RPMI 1640 consisting of 4% FCS, 1% antibiotic supplement (penicillin, streptomycin, amphotericin B) and 2% $NaHCO_3$.

Method: Antibody solutions were dispensed into 48 wells flat-bottom microplates serially starting from ½ titer to 1/256 titer (100 µl). One plate was prepared for each antibody solution (13 plates). Antigen solution equal to

Table II. Clinical and Serological Characteristics of Antisera in Set 2

				Serology			
Set 2	Clinical symptoms	Age (years)	Vaccination	Ig M	Ig G	Clinical specimen	Genotype
Ab4	Fever and rash	7	Unvaccinated	+	+	Urine	D6
Ab5	Fever and rash	2.5	Unvaccinated	+	–	Urine, blood	D6
Ab6	Fever and rash	4.5	Single dose	+	–	Throat swab, blood	D6
Ab7	Fever and rash	1.5	Single dose	+	–	–	UK
Ab8	Fever and rash	UK*	UK	+	–	–	UK

*Unknown. Ab: Antibody.

100 cell culture infectivity dose (CCID)50 was added into each well (100 µl) and incubated in 5% CO_2 incubator at 37°C for 90 minutes. After adding the cell suspension prepared in growth medium into each well (100 µl), plates were put into 5% CO_2 incubator at 37°C for overnight incubation. The next day 0.5 ml maintenance medium was added into each well and examined for cytopathic effect every day for five days. The highest dilution where no cytopathic effect was observed was accepted as neutralizing antibody titer. Back titration was performed to control each virus solution. Further, control of cell suspension and medium was performed separately in each plate[9].

Results

Antibodies against different virus antigens in Set 1 (Ab1, Ab2, Ab3) neutralized the different genotypic virus strains at titers >1/512. The neutralizing antibody titers observed in Set 2 (Ab5, Ab6 and Ab8) were similar, whereas in Set 3, the difference was more than two-fold (Table III).

Table III. Neutralizing Antibody Titers Obtained Against Five Different Measles Strains

	Antibody (Ab)												
Antigen	Ab1	Ab2	Ab3	Ab4	Ab5	Ab6	Ab7	Ab8	Ab9	Ab10	Ab11	Ab12	Ab13
Vaccine strain (Edmonston)	>512	>512	>512	>512	64	128	256	512	>512	128	256	512	256
Wild-type strain 1 (Japan)	>512	>512	>512	256	32	32	256	64	>512	128	>512	512	>512
Wild-type strain 2 (Japan)	>512	>512	>512	256	64	256	256	512	>512	256	512	>512	>512
Wild-type strain 1 (Turkey)	>512	>512	>512	>512	256	512	512	512	>512	256	>512	>512	>512
Wild-type strain 2 (Turkey)	>512	>512	>512	512	64	128	64	512	>512	512	512	256	>512

The neutralizing antibody titer against various virus genotypes (D3, D6 and vaccine strain) was ≥1/16 for all three sets of sera consisting of different virus clades (genotype A, D3 and vaccine).

Discussion

In 1990, the World Health Assembly and World Summit for Children set some global goals for measles control. These goals were to reach 95% reduction in measles deaths and 90% reduction in cases in the beginning of the 21st century compared to preimmunization levels by 1995, and this was planned to be achieved via an increase in the rate of vaccination coverage[10].

Measles outbreaks in cohorts with a high rate of vaccination with a single dose of measles vaccine have been frequently reported. Reduced vaccine effectiveness has been explained by primary or secondary vaccine failure (PVF, SVF). PVF, seen at a rate of 2-10%, represents a failure of immediate seroconversion with a documented lack of detectable specific antibody. One of the most important reasons for PVF is maternal antibodies. Others include technical mistakes during the storage and implementation of the vaccine or the usage of immunoglobulin at the same time as vaccination. On the other hand, SVF indicates infection in an individual following initial documented seroconversion, and represents a loss of protection. However, it is not possible to distinguish these cases from each other when an individual is seronegative[11].

Failure to maintain adequate vaccination coverage is considered the most significant reason for sporadic outbreaks in countries with high rates of vaccination[12]. According to certain other researchers' view, another potential factor is genetic and antigenic variability among wild-type measles virus. The H and F proteins are responsible for the induction of a neutralizing antibody response to measles virus. Therefore, the antigenic differences are most likely due to variation in these surface glycoproteins. Although measles virus is a stable virus, sequence analysis of the N, H, P and M genes has identified differences between wild virus strains as well as differences between wild and vaccine viruses. Evolutionary drift in measles viruses in the past 40 years has led to the development of an epidemiological tool to trace measles virus transmission by genetic analysis. The biological significance of these differences is not known because the immune response generated through vaccination appears to protect against all strains[7,13].

On the other hand, Hilleman[14] suggests that genomic identification or the molecular epidemiology of the virus has an important effect on the elimination and eradication programs. It

is emphasized that genetic variation in measles is not reflected in divergence of the antigenic specificity on which immunity depends and in which a single vaccine protects against all.

The contribution of antigenic changes to the epidemiology of measles virus infections is still unclear. The recent wild-type viruses are still neutralized by vaccine-induced antibody and the vaccine is still highly efficacious in disease prevention.

The most commonly used measles vaccine throughout the world is the derivative of Edmonston strain. The protection rates vary not only according to the derivative but also the vaccination schedule. It has been observed that the production rate of protective antibodies against measles is 85%, 95% and 98% in 9, 12 and 15 months, respectively. WHO recommends first-dose vaccination to be applied at nine months in developing countries[7,15]. In Turkey, the vaccination schedule was performed as a single dose at nine months until 1998 with the coverage rate of approximately 80%. After 1998, second- dose vaccination was started for children aged six years[2].

According to our results, the cause of reinfection associated with reduced antibody levels in individuals who received single-dose vaccination was not due to genotypic variation of vaccine and wild-type virus strains. Therefore, application of the second-dose measles vaccine was very important for maintenance of high level immunity in the population. The importance of the two-dose vaccination schedule started in 1998 in our country must be emphasized.

Acknowledgement

We thank Dr. Fumio Kobune for his guidance and technical support and biologist Omur Altınsoy for technical assistance.

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