JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1997, p. 2651–2653 Vol. 35, No. 10
0095-1137/97/$04.00+0

Molecular Epidemiology and Changing Distribution of Genotypes of Measles Virus Field Strains in Japan

YUKO KATAYAMA,[1] KATSUKI SHIBAHARA,[1] TOMOAKI KOHAMA,[2] MORIO HOMMA,[3] AND HAK HOTTA[1]*

Department of Microbiology, Kobe University School of Medicine, Chuo-ku, Kobe, Hyogo 650,[1] Division of Viral Disease and Vaccine Control, National Institute of Health, Murayama Annex, Musashi-Murayama, Tokyo 190-12,[2] and Faculty of Home Economics, Kobe Women's University, Suma-ku, Kobe, Hyogo 654,[3] Japan

Received 21 February 1997/Returned for modification 4 June 1997/Accepted 10 July 1997

Based on phylogenetic and restriction fragment length polymorphism analyses of the hemagglutinin and nucleoprotein gene sequences, measles virus strains obtained in western Japan were divided into two types. Type 1 isolates have largely replaced type 2 isolates during the last 10 years in the area surveyed.

Measles virus (MV) causes an acute systemic infection in infants. The virus is also known as the causative agent of a rare but incurable slow virus infection called subacute sclerosing panencephalitis (5, 8, 18). Recently, it was reported that B95a cells, an adherent subline of B95-8 cells (9), are highly susceptible to field strains of MV (7). By using B95-8 cells, we have obtained many MV isolates from measles patients and analyzed their virological features (16). Although MV has been thought to be a virus with a single serotype, heterogeneous features of MV such as varying reactivities to different monoclonal antibodies (4) and nucleotide sequence diversity (1, 10–13, 15, 17) have recently been demonstrated. In Japan, it was reported that on the basis of the relative molecular mass (M_r) of the hemagglutinin (H) protein, MV isolates obtained between 1983 and 1990 could be divided into three types (14). In the present paper, we demonstrate that MV strains obtained in western Japan can be divided into two types on the basis of phylogenetic positions and restriction fragment length polymorphism (RFLP) of the H and nucleoprotein (NP) gene sequences, as well as the M_r of the H protein. We also demonstrate that genotype distribution among MV field strains in the above area has changed during the last 10 years.

Throat swabs and/or sera were obtained from a total of 87 patients at the acute stage of typical measles infection in the Hyogo and Osaka prefectures in three separate time periods (see below). MV isolation, radioimmunoprecipitation analysis (RIPA) of the H protein, and sequence analysis of the H and NP genes were performed as described previously (2, 6, 16). RFLP analysis of partial H gene sequences was done by using *Pst*I and *Sau*3A. These genetic analyses have an advantage over RIPA since they can be performed by amplifying the viral sequences directly from clinical specimens without isolating the virus in cultures.

We noticed that whereas the laboratory standard Edmonston strain had an 80-kDa H protein (data not shown), some field isolates of MV had an 82-kDa H protein (type 1) and others had an 84-kDa H protein (type 2) (see below). The entire H gene sequences were determined for type 1 and 2 isolates, and phylogenetic analysis was performed (Fig. 1). Our field isolates were divided into two phylogenetic groups, with the classification completely matching the typing results on the basis of the M_r of the H protein. The type 1 isolates were almost identical to some of the isolates obtained in the United States in 1993 to 1994, and the type 2 isolates were closely related to the isolates obtained in the United States in 1989 to 1992. Phylogenetic analysis of partial NP gene sequences (nucleotides [nt] 1389 to 1629) revealed practically the same relationship as that observed with the H gene sequences (data not shown).

Since the H gene sequences of the field isolates showed extremely high degrees of homology within the same type but not with the other type, we tried to develop a typing method on the basis of the RFLP of the sequences. All of the type 1 isolates sequenced were shown to have the *Pst*I recognition site in a partial H gene sequence (nt 1 to 597) at residues 220 to 225 and the *Sau*3A recognition site in another partial sequence of the H gene (nt 552 to 997) at residues 797 to 800. On the other hand, none of the type 2 isolates sequenced had either recognition site. By using those restriction enzymes, we performed RFLP analysis and compared the results with those obtained by RIPA (Fig. 2). All of the H gene sequences (nt 1 to 597) amplified from type 1 isolates, but not type 2 isolates, were cleaved by *Pst*I into two fragments of the expected sizes (Fig. 2b). Similarly, the other amplified H gene sequences (nt 552 to 997) of type 1 isolates, but not type 2 isolates, were shown to possess the *Sau*3A recognition site at the expected position, being cleaved into two fragments (Fig. 2c). Thus, the results of typing of the H gene sequences on the basis of RFLP completely matched those of typing on the basis of the M_r of the H protein and those of typing on the basis of phylogenetic analysis of the H and NP gene sequences. It should also be noted that the RFLP patterns of the H gene sequences of other MV strains are expected to correlate well with their phylogenetic positions (right side of Fig. 1). Therefore, this method would be useful for classification of MV strains for certain purposes although it does not distinguish all of the phylogenetic branches.

We then determined the genotypes of MV strains obtained during the last 10 years. In 1985, type 2 strains were prevalent, representing about 80% of the total, as determined by phylogenetic analysis of partial NP gene sequences (Table 1). In 1989 to 1990, on the other hand, the majority of MV strains were determined to be type 1 by RFLP analysis of the partial H gene sequences. Type 1 strains appeared to increase further, and in 1993 to 1995, all of the MV strains were type 1 as determined by RFLP analysis. The type determination on the

* Corresponding author. Mailing address: Department of Microbiology, Kobe University School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe, Hyogo 650, Japan. Phone: 81-78-341-7451, ext. 3300. Fax: 81-78-351-6347. E-mail: hotta@kobe-u.ac.jp.

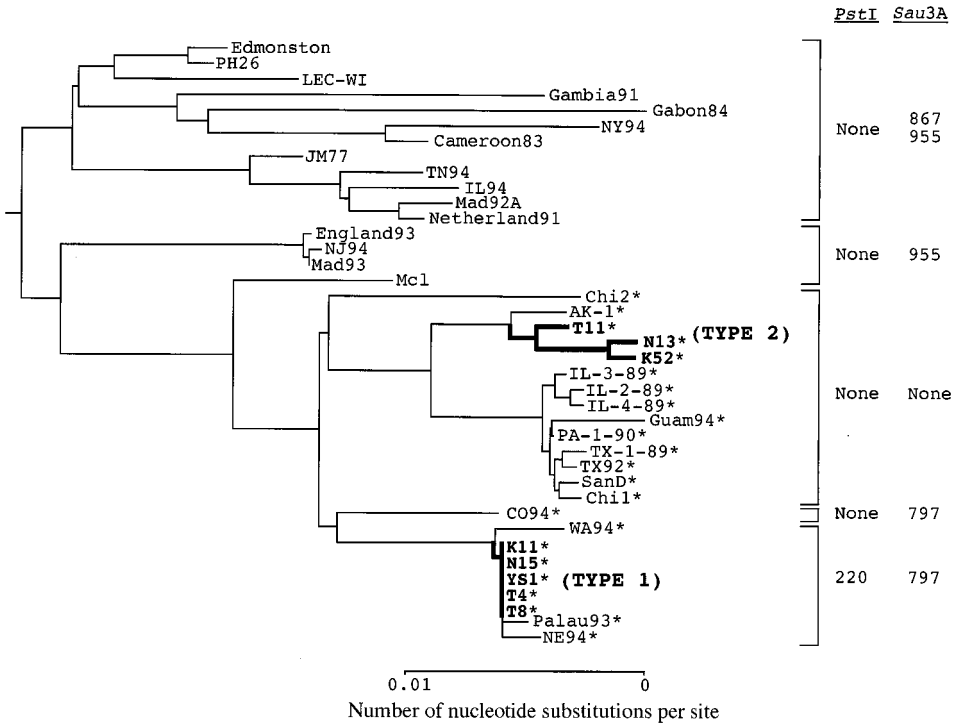

FIG. 1. Phylogenetic analysis of the entire coding region of H gene sequences. The field isolates obtained in this study are in boldface. Asterisks indicate MV isolates that have the N-glycosylation site at position 416 of the H protein. Shown at the right are positions of the most 5′ nucleotides of the expected restriction sites for *Pst*I and *Sau*3A in partial H gene sequences of nt 1 to 597 and 552 to 997, respectively. The GSDB/DDBJ/EMBL/NCBI accession numbers of the sequence data for the MV isolates in the phylogenetic tree are as follows: Edmonston, U03669; PH26, U08417; LEC-WI, X68043; Gambia91, L46732; Gabon84, L46731; NY94, L46752; Cameroon83, L46726; JM77, M81898; TN94, L46759; IL94, L46739; Mad92A, L46741; Netherland91, L46747; England93, L46729; NJ94, L46749; Mad93, L46743; Mcl, M81897; Chi2, M81896; AK-1, S73869; T11, D28946; N13, D28942; K52, D87759; IL-3-89, L46737; IL-2-89, L46736; IL-4-89, L46738; Guam94, L46734; PA-1-90, L46751; TX-1-89, L46761; TX92, L46763; SanD, M81902; Chi1, M81895; CO94, L46727; WA94, L46766; K11, D87761; N15, D87763; YS1, D87760; T4, D87762; T8, D28949; Palau93, L46757; NE94, L46745.

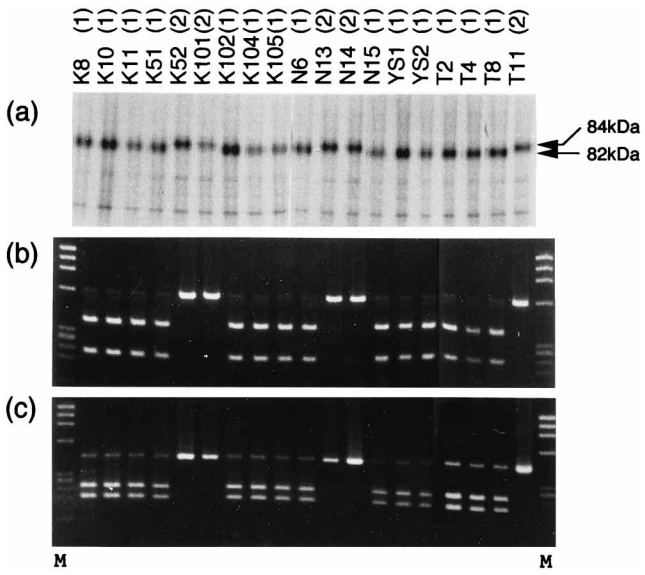

FIG. 2. Comparison of results based on the M_r of the H protein (a) and those based on RFLP analysis of amplified H gene sequences using *Pst*I (b) and *Sau*3A (c). The names of the MV isolates are at the top. The types of the MV isolates are in parentheses. M, size marker. Note that small amounts of uncleaved fragments are still observed with the type 1 isolates due to incomplete digestion by *Sau*3A.

basis of RFLP was verified by NP gene sequence analysis (data not shown).

Besides types 1 and 2, a number of different phylogenetic groups of MV isolates have been identified in other regions of the world (Fig. 1). Nevertheless, such MV strains were not found in the present study. It appears that only a few types or less prevail in an area at a time, and genotype distribution may change over time. It is possible that the other types of MV will be, or may have already been, introduced to some areas in Japan to replace the existing types. The occurrence of MV vaccine failure that has recently been reported (3) could have been caused by a variant(s) of MV. Genetic analysis of MV field strains may provide a clue to the mechanism(s) of vaccine failure and also provide information useful in the development of a more effective vaccine.

Nucleotide sequence accession numbers. The nucleotide sequence data reported in this paper will appear in the GSDB/DDBJ/NCBI DNA databases under accession numbers

TABLE 1. Changing distribution of genotypes of MV field strains during the last 10 years in western Japan

Yr(s)	No. of samples tested	No. of strains (%)	
		Type 1	Type 2
1985	29	6 (21)	23 (79)
1989–90	36	31 (86)	5 (14)
1993–95	22	22 (100)	0 (0)

D28942, D28946, D28949, D87759 to D87763, and D87770 to D87777.

We are grateful to H. Negishi, Takatsuki Hospital; T. Nagai, Hyogo Prefectural Nishinomiya Hospital; A. Hayakawa, Hyogo Prefectural Tsukaguchi Hospital; and Y. Yamada, Rokko Island Hospital, for providing clinical specimens from measles patients.

This work was supported in part by a Research Program for Slow Virus Infection from the Ministry of Health and Welfare, Japan, and a research grant from Yakult Co., Ltd.

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