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Measles viruses of genotype H1 evade recognition by vaccine-induced neutralizing antibodies targeting the linear haemagglutinin noose epitope

Tim Finsterbusch[1], Anne Wolbert[1], Ingrid Deitemeier[1], Kerstin Meyer[1], Maria Mar Mosquera[2], Annette Mankertz[1] and Sabine Santibanez[1]

[1]*National Reference Centre Measles, Mumps, Rubella, Division of Viral Infections (FG12), Robert Koch-Institute, Nordufer 20, 13353 Berlin, Germany*

[2]*Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, Spain*

Correspondence: Sabine Santibanez (santibanezs@rki.de)

Abstract

The linear haemagglutinin noose epitope (HNE; aa 379–410) is a protective B-cell epitope and considered to be highly conserved in both the vaccine and the wild-type measles virus (MeV) haemagglutinin (H) proteins. Vaccine virus-derived monoclonal antibodies (mAbs) BH6 and BH216, which target the HNE, neutralized MeVs of genotypes B3, C2, D4, D5, D6, D7 and D8, and the vaccine strain Edmonston Zagreb. In the case of genotype H1, only strain Berlin.DEU/44.01 *was neutralized by these mAbs, whereas strains* Shenyang.CHN/22.99 *and* Sofia.BGR/19.05 *were not. The* H *gene sequences of these two strains showed an exchange of proline 397 (P397) to leucine (L397). Mutated H proteins, with P397 exchanged to L and vice versa, were compared with original H proteins by indirect fluorescence assay. H proteins exhibiting P397 but not those with L397 were recognized by BH6 and BH216. This indicates that L397 leads to the loss of the neutralizing HNE. In contrast, human sera obtained from vaccinees (*n*=10) did not discriminate between genotype H1 variants P397 and L397. This concurs with the epidemiological observation that the live-attenuated vaccine protects against both H1 variants. Furthermore, we demonstrated that MeVs of genotype H1 also lack the neutralizing epitopes defined by the vaccine virus-induced mAbs BH15, BH125 and BH47. The loss of several neutralizing epitopes, as shown for H1 viruses currently circulating endemically in Asia, implies that epitope monitoring should be considered to be included in measles surveillance.*

Introduction

Vaccination with a live-attenuated measles vaccine elicits long-lasting immunity that involves humoral, cellular and mucosal responses. Protection from (re)infection is mediated by both antibodies specific for measles virus (MeV) and circulating MeV-specific T cells (Duke & Mgone, 2003). Neutralizing antibodies are directed against the two viral surface glycoproteins, the haemagglutinin (H) and the fusion (F) protein. These antibodies block the interaction of the H protein with its cellular receptors and the fusion activity (Bouche *et al.*, 2002). It has recently been demonstrated that H-specific antibodies are the main link to vaccine-induced MeV neutralization (de Swart *et al.*, 2005). They are mainly directed against conformational epitopes of the H protein (Bouche *et al.*, 2002). However, vaccine virus-derived monoclonal antibodies (mAbs) BH6 and BH216 neutralize MeVs of various genotypes efficiently by recognizing the linear haemagglutinin noose epitope (HNE; aa 379–410) (Ertl *et al.*, 2003; Santibanez *et al.*, 2005; Ziegler *et al.*, 1996). The HNE domain contains three cysteine residues (C381, C386 and C394) forming a surface-exposed loop (Ziegler *et al.*, 1996). Binding studies with a panel of mutated peptides representing the HNE sequence of several wild-type MeVs suggest that MeV-neutralizing and protective mAbs bind to the motif X7C[KR]GX[AINQ]QX2CEX5 (aa 379–400) (Putz *et al.*, 2003). Peptides mimicking the HNE induced high levels of antibodies neutralizing MeVs of all genotypes in mice. Therefore, the HNE was proposed as a promising peptide vaccine for infants that could cover the interval between waning maternal immunity and protection by the live-attenuated measles, mumps, rubella (MMR) vaccine (Bouche *et al.*, 2003, 2005; El Kasmi *et al.*, 2000; Ziegler *et al.*, 1996). The HNE is highly conserved among wild-type MeVs, although some field isolates with mutations have been reported. This study addresses the question of whether these variants are still recognized by HNE-specific mAbs.

Elimination of measles has been achieved in highly vaccinated populations irrespective of the molecular genetic characteristics of imported MeVs. This indicates that the immune responses induced by the live-attenuated vaccine virus protect against current wild-type MeVs. In an earlier study we reported that most neutralizing epitopes of the H protein were shared between vaccine and wild-type MeVs (Santibanez *et al.*, 2005), although some were lost from wild-type MeVs isolated in Europe between 2000 and 2002. In this study, MeVs currently imported from Africa and Asia and causing large outbreaks in Europe were investigated with respect to the conservation of the immunodominant HNE. Our results demonstrate that the HNE of several genotype H1 MeVs was not recognized by mAbs derived from vaccine virus, and that other neutralizing epitopes of H1 viruses were lost.

Methods

Nucleotide sequence determination of the MeV H coding region.

Viral RNA was reverse transcribed using primer MeH1 (5′-CCTCTGGCCGAACAATATCG) and reverse transcriptase Superscript III (Invitrogen). A fragment containing the coding region of the H protein was amplified from cDNA by nested PCR using the Phusion high-fidelity PCR kit (New England Biolabs). Primers MeH1 and MeH6 (5′-CAGATAGCGAGTCCATAACG) were used for the first round and MeH3 (5′-CTTAGGGTGCAAGATCATCC) and MeH8 (5′-GGTATGCCTGATGTCTGG) for the second amplification round. Amplified DNA was sequenced with an ABI Prism Big Dye Terminator cycle sequencing kit (PerkinElmer) using forward primers MeH3 and MeH5 (5′-TTCCCTATCAGGGATCAGGG) and reverse primers MeH2 (5′-AGCCTGTCTATCACTGGATC) and MeH8.

Plasmid construction.

The cDNA of the *H* gene of MeV isolates *Berlin.DEU/44.01* and *Sofia.BGR/19.05* and the Edmonston Zagreb vaccine was cloned into vector pCMV-HA (Clontech) and pcDNA3.1 (Invitrogen). All cloning steps were performed according to standard techniques (Sambrook & Russell, 2001). DNA fragments were amplified via standard PCR techniques using the Phusion high-fidelity PCR kit and restriction site-tagged primers. The H protein coding region of MeV H1 strains were amplified with primer pair Me-76 (5′-CGGAATTCGGATGTCACCACAACGAGACCGGAT) and Me-79 (5′-CGGGGTACCCTATCTGTGATTGGTTCCATCTTC), whereas that of Edmonston Zagreb was amplified with primers Me-76 and Me-78 (5′-CGGGGTACCCTATCTGTGATTGGTTCCATCTTC). Cycling parameters were 94 °C, 2 min; 30× (94 °C, 15 s; 60 °C, 30 s; 72 °C, 45 s); 72 °C, 7 min. PCR products were inserted via the introduced *Eco*RI and *Kpn*I restriction sites into pCMV-HA and sequenced with the plasmid-specific primers 1219 (5′-GGATGTTGCCTTTACTTCTAG) and 1270 (5′-ATAAAGCATTTTTTTCACTGCATTC). *H* genes were subcloned into pcDNA3.1 via *Eco*RI and *Not*I and subsequently mutated with the QuikChange II site-directed mutagenesis kit (Stratagene). To mutate the *H* genes of the respective MeVs, we used primer pair Ma-80 (5′-AGCACTCTGCGAGAATCTCGAGTGGGCACCATTGA) and Ma-81 (5′-TCAATGGTGCCCACTCGAGATTCTCGCAGAGTGCT) for strain Edmonston Zagreb; primer pair Ma-82 (5′-AGCACTCTGTGAAAATCTCGAGTGGGCACCGTTGA) and Ma-83 (5′-TCAACGGTGCCCACTCGAGATTTTCACAGAGTGCT) for strain *Berlin.DEU/44.01* and primer pair Ma-84 (5′-AGCACTCTGTGAAAATCCCGAGTGGGCACCGTTGA) and Ma-85 (5′-TCAACGGTGCCCACTCGGGATTTTCACAGAGTGCT) for isolate *Sofia.BGR/19.05*. All pcDNA3.1 constructs were sequenced with the plasmid-specific primers T7 (5′-TAATACGACTCACTATAGGG) and BGH (5′-TAGAAGGCACAGTCGAGG).

Immunofluorescence analysis (IFA).

HEK-293 cells were grown on coverslips in 24-well plates. Transfection was performed with Effectene transfection reagent (Qiagen). For each transfection, a total of 200 ng plasmid DNA was used. At 48 h post-transfection (p.t.), cells were washed with PBS and fixed with 4% paraformaldehyde in PBS for 10 min at room temperature, afterwards cells were permeabilized with 0.1% Triton X-100 in PBS for 10 min. Cells were blocked for 1 h with blocking solution (PBS, 0.05% Tween 20, 1% BSA) and incubated with 10 µg ml^{-1} of the respective primary antibody for 1 h at room temperature. After three washing steps with PBS-T (0.05% v/v), cells were incubated with fluorescein isothiocyanate (FITC)-conjugated secondary antibody at 15 µg ml^{-1} (Dianova) for 1 h at room temperature. Coverslips were washed three times with PBS-T, mounted in Mowiol (Merck) and examined with a confocal laserscanning microscope (cLSM 510, Zeiss).

Focus of infection reduction neutralization test (FRNT).

H-specific mAbs were diluted by $f_d \times 2^n$ (f_d=mAb-specific dilution factor, *n*=1, 2, 3, 4, 5 and 6) in MEM alpha medium (Invitrogen) supplemented with 5% fetal calf serum (FCS). MeV suspensions containing 40–60 p.f.u. per 100 µl were incubated with serially diluted mAbs and incubated for 60 min at 37 °C. Aliquots (100 µl) were transferred onto a confluent monolayer of signalling lymphocytic activation molecule (SLAM)-transduced Chinese hamster ovary (CHO) cells and incubated at 37 °C for 60 min. The inoculum was removed and the cells were covered with an overlay containing 0.5% CM cellulose and 3% FCS and incubated for 3 days. MeV-infected cells were detected by the use of an indirect immunocolorimetric assay, which has been previously described for detection of rubella virus (Chen *et al.*, 2007). Cells were fixed with 2% paraformaldehyde in PBS and permeabilized with ice-cold methanol (at −20 °C). MeV-infected cells were detected using the mAb NP cl.120 directed against the MeV nucleocapsid (N) protein (hybridoma cells were kindly provided by T. F. Wild, Institute Pasteur de Lyon, France) as primary antibody, goat anti-mouse IgG peroxidase conjugate (Chemicon International) as secondary antibody and tetramethylbenzidine (Mikrogen) as precipitating peroxidase substrate. Foci of MeV infection are visible to the naked eye as dark blue spots, which were counted. For each MeV, the number of foci of infection was determined in the absence of antibody. The amount of IgG per well resulting in 50% reduction of the foci number was calculated. All tests were performed in triplicate and repeated at least twice. FRNT tests with human sera were performed as described for mAbs, except that sera were diluted by 5×2^n (*n*=1, 2, 3, 4, 5, 6 and 7). Sera obtained from 10 vaccinated individuals were chosen from a panel collected from 13–15-year-old students in the scope of the seroprevalence study SCARPOL which was recently performed in Switzerland (Tischer *et al.*, 2007). All serum donors had received two doses of MMR vaccine, the second dose had been administered more than 2 years prior to serum donation.

Results

The HNE is not universally conserved among MeVs

To study the presence of the HNE on contemporary MeVs, an FRNT was performed using the two HNE-specific mAbs BH6 and BH216. These mAbs blocked infection of SLAM-transduced CHO cells (Erlenhoefer *et al.*, 2001) by MeV isolates of the genotypes B3, C2, D4, D5, D6, D7 and D8 plus the vaccine virus Edmonston Zagreb (Table 1⇓). When MeVs of genotype H1 were investigated, BH6 and BH216 neutralized *Berlin.DEU/44.01* efficiently, while *Shenyang.CHN/22.99* and *Sofia.BGR/19.05* were able to infect the cells in presence of the mAbs. This result indicates that the HNE is not universally conserved and heterologous between the genotype H1 viruses.

The neutralizing capacity of the mAbs was determined by FRNT on SLAM-expressing CHO cells. Values indicate IgG concentration (ng per 100 µl) required for 50% reduction of number of foci of MeV infection.

MeVs of genotype H1 show diversity in the HNE sequence

The nucleotide sequence of the *H* gene of all MeVs used in this study was determined and correlated with the results of the FRNT. The sequence alignment revealed three amino acid exchanges, but only that at aa 397 was unique for *Shenyang.CHN/22.99* and *Sofia.BGR/19.05*. L397 was seen for *Shenyang.CHN/22.99* and *Sofia.BGR/19.05*, while P397 was found in *Berlin.DEU/44.01* and all other MeVs included in our study. This mutation is located within the HNE sequence (aa 379–410) (Table 2⇓) and our observation implied that P397L influenced recognition of H by mAbs BH6 and BH216. A search for MeV H protein sequences deposited in GenBank showed that MeVs of all genotypes exhibited P397 (data not shown), with the exception of one D6 (Ser397, derived from a subacute sclerosing panencephalitis case) and several H1 MeVs. Amongst genotype H1, both sequence variants P397 and L397 were seen: 23 sequences of the L397 variant were found, while 21 sequences carried the P397. Based on these findings, L397 in the HNE seems to be restricted to genotype H1 viruses.

The presence of P397 is critical for binding of vaccine virus-induced HNE-directed mAbs

H proteins from several genotypes were cloned into plasmid pCMV-HA. The constructs were transfected into HEK-293 cells. At 24 h p.t., cells were fixed and the expression of H protein was tested with mAbs BH17, BH6 and BH216. The findings corroborated the FRNT result, since BH6 and BH216 did not react with the H protein of *Sofia.BGR/19.05* (Fig. 1⇓) and *Shenyang.CHN/22.99* (data not shown). This approach was used to investigate the effect of P397L on the recognition of the HNE by mAbs BH6 and BH216. P was changed to L in H proteins of the Edmonston Zagreb vaccine and MeV *Berlin.DEU/44.01*, while the reciprocal exchange of L397P was introduced into the H protein of *Sofia.BGR/19.05*. The mutated H proteins were expressed in HEK-293 cells and tested by IFA. The mAb BH17 was used as a positive control and recognized all H variants. All H proteins carrying P397 were detected by BH6 and BH216 regardless of their genotype (Fig. 1⇓), while those displaying L397 were not.

Analysis of neutralizing epitopes shared by genotype H1 and vaccine MeVs

To identify further differences in neutralizing epitopes of the H protein between the H1 MeVs (*Berlin.DEU/44.01*, *Shenyang.CHN/22.99* and *Sofia.BGR/19.05)* and the Edmonston Zagreb vaccine, other vaccine virus-induced mAbs (BH15, BH17, BH47, BH67, BH81, BH125 and BH141) were used in an FRNT in addition to BH6 and BH216. These mAbs recognized additional epitopes of the H protein: mAbs BH17, BH67, BH81 and BH141 neutralized the vaccine as well as genotype H1 viruses with high efficiency (Table 3⇓). It was shown previously that BH17 and BH141, as well as BH67 and BH141, competed for binding sites on the H protein (Bouche *et al.*, 2002). All mAbs recognize conformational epitopes with the exception of mAb BH47 which binds to a linear epitope (aa 244–250) (Fournier *et al.*, 1997). BH47 neutralized all four viruses, except *Shenyang.CHN/22.99*, with a very low efficiency, indicating that the linear epitope defined by mAb BH47 is heterologous between the genotype H1 viruses. In contrast, mAb BH15 and BH215 neutralized the vaccine virus with variable efficiency but did not interfere with genotype H1 infection. In summary, our investigation identified two neutralizing epitopes of the H protein, which are shared by genotype H1 and vaccine MeVs. These epitopes are considered as conformational.

Sera from vaccinated individuals do not discriminate between H protein variants P397 and L397

Sera with low levels of MV-specific IgG of 10 vaccinated individuals were tested in FRNTs against the genotype H1 virus isolates *Berlin.DEU/44.01* (P397) and *Sofia.BGR/19.05* and vaccine virus Edmonston Zagreb (P397) to determine if their neutralizing capacity was affected by the absence of the HNE. The differences in neutralization titres measured for each serum were less than fourfold, which is below the level of significance (Table 4⇓). These data suggest that vaccine-induced polyclonal human antibodies are able to neutralize MeV regardless of the presence of the HNE.

Discussion

Surveillance data do not provide any indication for the emergence of escape mutants from vaccine-induced neutralizing antibodies in humans. However, the pattern of neutralizing epitopes on the H protein of currently circulating wild-type MeV differs between strains and compared with the vaccine virus.

For the HNE, though it is a surface-exposed target of strongly neutralizing antibodies and is thus assumed to be under a continuous pressure for change, previous studies reported a high degree of conservation between all genotypes (El Kasmi *et al.*, 2000; Putz *et al.*, 2003), corroborating the hypothesis that alterations in the HNE might interfere with the functionality of H protein. Neutralization assays showed that vaccine virus-induced mAbs BH6 and BH216, which target the HNE, were able to neutralize 14 wild-type MeVs of different genotypes (B3, C2 and D4–D8). In the case of genotype H1, *Berlin.DEU/44.01* was neutralized, but *Shenyang.CHN/22.99* and *Sofia.BGR/19.05* were not. Sequence analysis revealed a P397L change in these two strains. IFA demonstrated that BH6 and BH216 exclusively recognized H proteins containing P397, both original and mutant, indicating that this position is critical for recognition of the HNE. The implications of the L397 variant are not easy to assess, but results obtained for other viruses point at a certain risk potential regarding escape from antibodies provided by vaccination or previous infection. In the case of influenza virus type A, a proline-to-leucine/histidine point mutation in the ectodomain of the transmembrane protein M2 emerged that permitted escape from antibody recognition (Zharikova *et al.*, 2005).

Previous results obtained by a competition study between mAbs and human serum antibodies identified the HNE as one of two main targets of human antibodies. The second one, an epitope recognized by mAbs BH26, BH67 and BH81, might be associated with SLAM (Ertl *et al.*, 2003). Our study detected two epitopes that were still conserved between all genotypes tested, one that was recognized by the strongly neutralizing mAbs BH17 and BH141, and the supposedly SLAM-associated epitope mentioned above. The presence of conserved epitopes may be responsible for the continued protection provided by the measles vaccine, also against MeV variants that have lost the HNE epitope. This was confirmed on a serological level by FRNT tests we performed with a set of human sera from vaccinees, which did not discriminate between the genotypes used.

In contrast with our study employing the complete H protein, mAb BH216 bound with equal affinity to HNE peptides (aa 379–400) regardless of whether they exhibited P397 or L397 (Putz *et al.*, 2003). When mice were immunized with a polyepitope vaccine containing eight copies of the HNE, the antiserum neutralized not only MeV isolate *China94-1* encoding P397 but also MeV isolate *China93-2* (L397) (Bouche *et al.*, 2005). Thus, it is possible to speculate that P397 is critical for binding of BH216 and also that the nature of the antigen influences recognition by altering the folding of the polyepitope or the protein differentially. A database search in 2005 revealed that only 2% of MeV H sequences contained L397 (Putz *et al.*, 2003). Analysis of the H protein sequences currently published in GenBank revealed that L397 was restricted to genotype H1, the MeV genotype predominantly circulating in China. More than 109000 cases were reported from China in 2007, representing 39% of the global measles cases (Bian *et al.*, 2006; Liffick *et al.*, 2001; Xu *et al.*, 1998; Yu *et al.*, 2007; Zhang *et al.*, 2008). H1 has also been observed in Korea and Japan (Na *et al.*, 2003; Nakayama *et al.*, 2003; Tomita, 2006; Zhou *et al.*, 2003). In China, both H variants, P397 and L397, have been found since 1993 whereas in Korea and Japan, only the L397 variant has been detected in samples collected since 2000.

MeVs with a partial loss of immunodominant epitopes like the HNE are still effectively neutralized by vaccine-induced polyclonal human sera, but as additional losses might reduce the efficacy of vaccination, we suggest implementation of epitope monitoring into measles surveillance. In this context, the acceleration of the measles elimination process by vaccination is a principle strategy to minimize the risk of critical alteration of the epitope pattern.

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Tables and Figures

Table 1. Neutralizing capacity of HNE-specific mAbs

The neutralizing capacity of the mAbs was determined by FRNT on SLAM-expressing CHO cells. Values indicate IgG concentration (ng per 100 µl) required for 50% reduction of number of foci of MeV infection.

Strain/isolate	Genotype	GenBank accession no. (H protein)	Neutralizing capacity of mAb	
			BH6	BH216
Edmonston Zagreb vaccine	A-related	AF266290	<20	<20
MVi/Almeria.ESP/11.03/3	B3	FJ865561	<20	<20
MVi/Murcia.ESP/18.03	B3	FJ865562	<20	<20
MVi/Kempten.DEU/23.00	C2	AF480473	<20	<20
MVi/Tuebingen.DEU/24.00	C2	AF480468	<20	<20
MVi/Glasgow.GBR/14.94	D4	GQ331933	<20	<20
MVi/Palma de Mallorca.ESP/33.04	D4	FJ869874	<20	<20
MVi/Tuebingen.DEU/10.08	D5	GQ121274	30	<20
MVi/Berlin.DEU/47.00	D6	AF489474	<20	<20
MVi/Luxembourg.LUX/24.01	D6	FJ869876	<20	<20
MVi/Mainz.DEU/06.00	D7	AF480470	<20	<20
MVi/Duesseldorf.DEU/10.00	D7	AF480471	<20	<20
MVi/Alicante.ESP/19.03	D8	FJ869875	<20	<20
MVi/Duesseldorf.DEU/19.07	D8	FJ808738	<20	<20
MVi/Shenyang.CHN/22.99	H1	GQ338160	>2000	>2000
MVi/Berlin.DEU/44.01	H1	FJ808737	<20	<20
MVi/Sofia.BGR/19.05	H1	FJ808736	>2000	>2000

Table 2. Sequence comparison of the HNE (aa 379–410) found within the H protein of different MV genotypes used in this study

Some genotype H1 strains showed an amino acid exchange at position 397 from P to L.

Strain/isolate	Genotype	Sequence of the HNE (aa 379–410)
Edmonston Zagreb	A-related	ETCFQQACKGKIQALCENPEWAPLKDNRIPSY
Almeria.ESP/11.03/3	B3	..................P.......N.....
Murcia.ESP/18.03	B3	..................P.......N.....
Kempten.DEU/23.00	C2	..................P.......H.....
Tuebingen.DEU/24.00	C2	..................P.......H.....
Glasgow.GBR/14.94	D4	..................P.......N.....
Palma de Mallorca.ESP/33.04	D4	..................P.......N.....
Tuebingen.DEU/10.08	D5	..................P.......N.....
Berlin.DEU/47.00	D6	..................P.......S.....
Luxembourg.LUX/24.01	D6	..................P.......N.....
Mainz.DEU/06.00	D7	..................P.......N.....
Duesseldorf.DEU/10.00	D7	..................P.......N.....
Alicante.ESP/19.03	D8	.............T....P.......N.....
Duesseldorf.DEU/19.07	D8	..................P.......N.....
Shenyang.CHN/22.99	H1	..................L.......S.....
Berlin.DEU/44.01	H1	..................P.......S.....
Sofia.BGR/19.05	H1	..................L.......S.....

Table 3. Capacity of anti-H mAbs to neutralize MeV isolates of genotype H1 compared with the Edmonston Zagreb vaccine virus

The neutralizing capacity of the mAbs was determined by FRNT on SLAM-expressing CHO cells. Values indicate IgG concentration (ng per 100 µl) required for 50% reduction of number of foci of MeV infection.

Strain/isolate	Genotype	aa 397	BH6	BH15	BH17	BH47	BH67	BH81	BH125	BH141	BH216
Edmonston Zagreb vaccine	A-related	P397	<20	40	<20	200	<20	<20	400	60	<20
MVi/Shenyang.CHN/22.99	H1	L397	>2000	>2000	<20	1500	<20	<20	>2000	60	>2000
MVi/Sofia.BGR/19.05	H1	L397	>2000	>2000	<20	300	<20	<20	>2000	60	>2000
MVi/Berlin.DEU/44.01	H1	P397	<20	>2000	<20	600	<20	<20	>2000	40	<20

Table 4. Capacity of human sera to neutralize MeV isolates of the distinct H protein variants of genotype H1

Sera were collected from 10 vaccinees (V1–V10). Each serum was tested by FRNT on SLAM-expressing CHO cells against MeV isolates of genotype H1 representing the H protein variants P397 (*Berlin.DEU/44.01*) and L397 (*Sofia.BGR/19.05*), and the vaccine virus Edmonston Zagreb (P397). Values of neutralizing capacity are expressed as serum titre resulting in 50% reduction of number of foci of MeV infection. The genotype and amino acid found at position 397 are given in Table 3.

Strain/isolate	Serum donor:	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
	IgG (IU ml^{-1})*:	0.45	0.55	0.41	0.31	0.31	0.34	0.20	0.29	0.15	0.24
Edmonston Zagreb vaccine		83	22	38	43	33	39	35	38	28	37
MVi/Berlin.DEU/44.01		42	20	46	38	43	48	84	65	91	29
MVi/Sofia.BGR/19.05		58	53	65	106	81	50	85	82	86	47

*MeV-specific IgG was determined by enzyme immunoassay (Enzygnost, Dade Behring).

Figure 1. The H proteins of the vaccine strain Edmonston Zagreb (P397), *Berlin.DEU/44.01* (P397) and *Sofia.BGR/19.05* (L397) were cloned into pcDNA3.1. Additionally, point mutants of H were produced in which P397L and L397P were introduced. HEK-293 cells were transfected with the indicated plasmids. At 48 h p.t., cells were fixed and the reactivity of HNE-specific mAbs BH6 and BH216 was tested by IFA. mAb BH17, which is specific for all H protein variants, was used as a positive control.