International Journal of Medical Microbiology 314 (2024) 151607

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.com/locate/ijmm

# Vaccine-induced neutralizing antibodies bind to the H protein of a historical measles virus

Anne Zemella [a,1], Kerstin Beer [b,1], Franziska Ramm [a], Dana Wenzel [a], Ariane Düx [c,f], Kevin Merkel [c], Sebastien Calvignac-Spencer [c,f,g], Daniel Stern [d], Martin B. Dorner [d], Brigitte G. Dorner [d], Navena Widulin [e], Thomas Schnalke [e], Cornelia Walter [b], Anne Wolbert [b], Bernhard G. Schmid [b], Annette Mankertz [b], Sabine Santibanez [b,*]

[a] Fraunhofer Institute for Cell Therapy and Immunology (IZI), Branch Bioanalytics and Bioprocesses (IZI-BB), Am Mühlenberg 13, D-14476 Potsdam, Germany
[b] WHO Measles/Rubella European RRL and NRC Measles, Mumps, Rubella, Robert Koch-Institut, Seestr. 10, D-13353 Berlin, Germany
[c] Viral Evolution, Robert Koch-Institut, Seestr. 10, D-13353 Berlin, Germany
[d] Biological Toxins (ZBS3), Centre for Biological Threats and Special Pathogens, Robert Koch-Institut, Seestr. 10, D-13353 Berlin, Germany
[e] Berlin Museum of Medical History, Charité, Berlin
[f] Helmholtz Institute for One Health, Helmholtz-Centre for Infection Research (HZI), 17489 Greifswald, Germany
[g] Faculty of Mathematics and Natural Sciences, University of Greifswald, 17489 Greifswald, Germany

ARTICLE INFO

*Keywords:*
Measles virus hemagglutinin
Cell-free protein synthesis
Microsomal membranes
Neutralizing antibodies
Monoclonal antibodies
Neutralizing epitopes

ABSTRACT

Measles is a highly contagious airborne viral disease. It can lead to serious complications and death and is preventable by vaccination. The live-attenuated measles vaccine (LAMV) derived from a measles virus (MV) isolated in 1954 has been in use globally for six decades and protects effectively by providing a durable humoral and cell-mediated immunity. Our study addresses the temporal stability of epitopes on the viral surface glycoprotein hemagglutinin (H) which is the major target of MV-neutralizing antibodies. We investigated the binding of seven vaccine-induced MV-H-specific monoclonal antibodies (mAbs) to cell-free synthesized MV-H proteins derived from the H gene sequences obtained from a lung specimen of a fatal case of measles pneumonia in 1912 and an isolate from a current case. The binding of four out of seven mAbs to the H protein of both MV strains provides evidence of epitopes that are stable for more than 100 years. The binding of the universally neutralizing mAbs RKI-MV-12b and RKI-MV-34c to the H protein of the 1912 MV suggests the long-term stability of highly conserved epitopes on the MV surface.

## 1. Introduction

Measles is a highly contagious airborne viral disease that can lead to serious complications and death. The disease is preventable by immunization using the live-attenuated measles vaccine (LAMV) that induces a durable immunity. Infection with the wild-type measles virus (wt-MV) as well as vaccination induces both antibody and cellular immune responses, but the antibody quantity is lower in vaccinated persons (Lin et al., 2020). Protection against measles correlates best with the quality and quantity of MV-neutralizing antibodies directed against the MV membrane glycoproteins, the tetrameric attachment protein hemagglutinin (H) and the trimeric fusion protein (F) (Griffin, 2018; Brindley et al., 2012). Neutralizing antibodies inhibit MV infection of host cells by preventing the interaction with the cellular receptors (the signaling lymphocytic activation molecule (SLAM) on immune cells and nectin-4 on epithelial cells) and the fusion of viral and cellular membranes (Bouche et al., 2002; Tatsuo et al., 2000; Mühlebach et al., 2011). The H protein is the major target of neutralizing antibodies (de Swart et al., 2005) and therefore determines the antigenic properties of MV.

For the purpose of surveillance, wild-type MV are divided into 24 genotypes. Extensive vaccination has resulted in a decreasing number of MV genotypes from 13 in 2002 to two (B3 and D8) in 2021 and 2022 (Williams et al., 2022; Minta et al., 2023). The commonly used LAMV are derived from the Edmonston strain isolated in 1954 (Katz et al., 1962) and are assigned to genotype A. Global measles surveillance data indicate that the vaccine protects universally against all genotypes

* Corresponding author.
*E-mail address:* SantibanezS@rki.de (S. Santibanez).
[1] Contributed equally to this work.

https://doi.org/10.1016/j.ijmm.2024.151607
Received 20 December 2023; Received in revised form 18 January 2024; Accepted 18 January 2024
Available online 20 January 2024

(Bankamp et al., 2011). MV is hence considered serologically monotypic. However, neutralization studies using vaccine-induced H-specific monoclonal antibodies (mAbs) have shown that most of these mAbs distinguish between MV-H variants whereas only a few mAbs neutralize all variants (Santibanez et al., 2005; Finsterbusch et al., 2009; Beer, 2012).

This study addresses the temporal stability of neutralizing epitopes on the MV-H protein. We investigated the binding of seven mAbs raised against the Edmonston Zagreb vaccine (vacc. Edm. Z.) to cell-free synthesized MV-H proteins derived from the H gene sequences obtained from a lung specimen of a fatal case of measles pneumonia in 1912 that was preserved in a museum of medical history (Düx et al. 2020) and an isolate from a current case detected in 2019. The focal question is whether the epitopes defined by mAbs that universally neutralize the recently circulating MV variants show long-term evolutionary stability.

Cell-free protein synthesis (CFPS) based on translationally active eukaryotic lysates was selected to synthesize the MV strain-specific H proteins. The crude cell lysate contains the necessary components for protein synthesis, including ribosomes, amino acids, and energy sources. This allows the synthesis of "difficult-to-express proteins" such as membrane proteins or cytotoxic proteins (Henrich et al., 2015; Ramm et al., 2022; Thoring et al., 2017). In particular, the endogenously present membrane vesicles in eukaryotic systems which are based on the endoplasmic reticulum are favored for the synthesis of membrane proteins such as the MV-H protein since an incorporation into the natural membrane is possible. Moreover, these so-called microsomes enable posttranslational modifications such as disulfide bridging and glycosylation (Thoring et al., 2019). In this study, a CFPS system based on Chinese Hamster ovary (CHO) cells was used to synthesize MV strain-specific H proteins integrated into microsomes. Subsequently the binding of the vaccine-induced mAbs to the synthesized H proteins was evaluated in an in-solution enzyme-linked immunosorbent assay (ELISA).

## 2. Methods

### 2.1. MV-H-specific monoclonal antibodies

Previously generated MV-H-specific chimeric mAbs (cmAbs) were used (Beer, 2012). Briefly, two Balb/c mice were immunized and boosted by intraperitoneal injection with whole virus particles of the vacc. Edm. Z. The virions were ß-propiolactone-inactivated, purified using sucrose gradient centrifugation, suspended in PBS-buffer and mixed with Complete Freunds Adjuvant (final booster injections without adjuvant). After cell fusion, a semi-quantitative foci of infection reduction neutralization test (FRNT) with the homologous challenge virus was used to select hybridoma clones secreting MV-neutralizing antibodies. For the secreted mAbs, the binding to the MV-H protein was detected by immunoprecipitation. Sequencing of the genes encoding the light and heavy chains of the mAbs and generation of recombinant chimeric mouse/human IgG1 antibodies (cmAbs) was conducted by Absolute Antibody, Redcar, UK.

### 2.2. MV-H sequences and plasmids

#### 2.2.1. MV-H sequence data

For the wt-MV 1912 (MVs/Berlin.DEU/1912; GenBank: ERS4249335), novel high-throughput sequencing libraries were sequenced, doubling the initial number of libraries (n = 18) and of high-quality reads (n = 36,660,231). Based on these data, we reconstructed a completely resolved H gene sequence (doi: 10.5281/zenodo.10519771). For the vacc. Edm. Z., the H sequence data published in GenBank were used (GenBank: AF266290). For the wt-MV 2019 (WHO MV strain name: MVi/Offenburg.DEU/10.19), viral RNA extracted from supernatant of MV-infected Vero/hSLAM cells (3rd passage) was reverse transcribed and the amplified cDNA was sequenced as previously described (Finsterbusch et al., 2009; Beer, 2012). The H gene sequence is deposited in the MeaNS2 database (https://who-gmrln.org/means2) and is identifiable via the WHO MV strain name. The MV genotype was assigned by phylogenetic analysis of N and H genes according to WHO nomenclature (Williams et al., 2022) using the MEGA 11 software (Tamura et al., 2021).

#### 2.2.2. DNA templates for cell-free protein synthesis

Gene sequences encoding the MV strain-specific H proteins were adapted for CFPS as previously described (Brödel et al., 2013) and a cleavable melittin signal peptide to support translocation into endogenous membrane vesicle was added upstream of the MV-H gene sequence. The sequences were manufactured by *de novo* gene synthesis (Biocat GmbH) and cloned into the pUC57–1.8 K vector backbone.

### 2.3. Cell-free synthesis of H protein

Cell-free synthesis reactions were performed using translationally active lysates derived from Chinese hamster ovary cells (CHO-K1, DSMZ) as previously described (Thoring et al., 2016). Protein synthesis was carried out in a coupled transcription / translation mode. Reactions were incubated in a thermomixer (Eppendorf) for 3 h at 30 °C and 500 rpm.

#### 2.3.1. Quantitative analysis

The incorporation of $^{14}$C-leucine allowed for quantitative analysis by hot trichloro acetic acid (TCA) precipitation as previously described (Ramm et al., 2020). Total protein yield was determined by liquid scintillation counting (Hidex 600SL, Hidex).

#### 2.3.2. Qualitative analysis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using precast gels (NuPAGE, 10% Bis-Tris, Life technologies) was performed to determine molecular weight of radiolabeled cell-free synthesized MV-H proteins as described previously (Ramm et al., 2020). Protein N-glycosylation was analyzed using Endo H (endoglycosidase H, NEB, USA) on 14 C-labeled wt-MV-H protein. The assay was performed according to the manufacturer's protocol.

### 2.4. Quantification of antibody binding to MV-H protein

#### 2.4.1. In-solution ELISA

For the determination of binding of cmAbs an in-solution ELISA was performed as previously described (Haueis et al., 2023). First, the protein yield in the microsomal fraction (MF) was determined as described and for each MV-H protein the same amount of total H protein, usually 2 pmol in 200 µl binding buffer containing PBS (1x, VWR) and 0.05% (v/v) Tween20 (Sigma-Aldrich) and 1% BSA (VWR) was applied to the SpeedBead Sera-Mag Neutravidin magnetic particles (GE Healthcare). The microsomes were bound via hydrophobic interactions to the beads. As no-template control (NTC), a cell-free reaction without DNA template was used in an equivalent volume and treated in the same way. The individual cmAbs (2 mg/ml) were diluted 1:2000 in PBS containing 2% BSA and incubated with the beads for 2 h at room temperature. After three washing steps, a goat-anti-mouse-horseradish peroxidase linked secondary antibody (1 mg/ml, Biozym, diluted 1:1000 in PBS containing 2% BSA) was incubated with the beads for 1 h at room temperature. After three additional washing steps, the sample was incubated with substrate TMB for 60 s. The color reaction was stopped with $H_2SO_4$ and the absorption of the samples was measured by multi-mode microplate reader FLUOstar Omega (BMG Labtech) at 450 nm and 620 nm as reference.

## 3. Results

### 3.1. MV-H sequence comparison

The amino acid (aa) sequences predicted for the H protein (617 aa) of the wt-MV 1912, the wt-MV 2019 and the vacc. Edm. Z. indicate that the SLAM-interacting aa residues (D505, R533, Hashiguchi et al., 2011; L185, S548, P549, Seki et al., 2020), the nectin-4-interacting aa residues (F483, Y541 and Y543, Zhang et al., 2013), five potential N-linked glycosylation sites located at positions 168, 187, 200, 215 and 238, and the sequence motif $P_{309}$YQGSGK identified for binding of mAb 34c (Beer, 2012) are conserved between these MV strains. The wt-MV 2019 was assigned to genotype D8 that currently still circulates worldwide. The wt-MV 1912 cannot be assigned to any genotype of the WHO nomenclature since in phylogenetic analyses it is positioned as a sister lineage to the lineage comprising all modern MV genotypes. The H protein aa sequence identity between the wt-MV 1912 and each of the genotype reference strains is high, ranging from 95.46% (reference strain of genotype D11) to 97.73% (reference strain of genotype C1). A high H protein sequence identity was also found when the wt-MV 1912 was compared to the vacc. Edm. Z. (96.92%) and the wt-MV 2019 (96.43%).

### 3.2. MV-H-specific monoclonal antibodies

Hybridoma clones secreting MV-neutralizing H-specific IgG antibodies were obtained from Mouse 1 used on day 41 post 1st immunization (clones/mAbs RKI-MV-1d, RKI-MV-9c, RKI-MV-12b and RKI-MV-13a) and Mouse 2 used on day 216 post 1st immunization (clones/mAbs RKI-MV-32c, RKI-MV-33a and RKI-MV-34c) for hybridoma fusion. The sequence data obtained from the individual hybridoma clones revealed deviating aa sequences for the variable part of both the light and the heavy chains of IgG.

### 3.3. Cell-free synthesis of MV-H proteins

The CHO cell-free system was chosen for the synthesis of H proteins from different MV strains (Fig. 1a). For the wt-MV 2019, the wt-MV 1912 and the vacc. Edm. Z. H protein, a defined protein band at a molecular weight above 60 kDa was detected (Fig. 1b) and thus corresponds to the predicted molecular weight of 69 kDa. Additional bands are visible at approximately 140 kDa, 200 kDa and above 200 kDa that

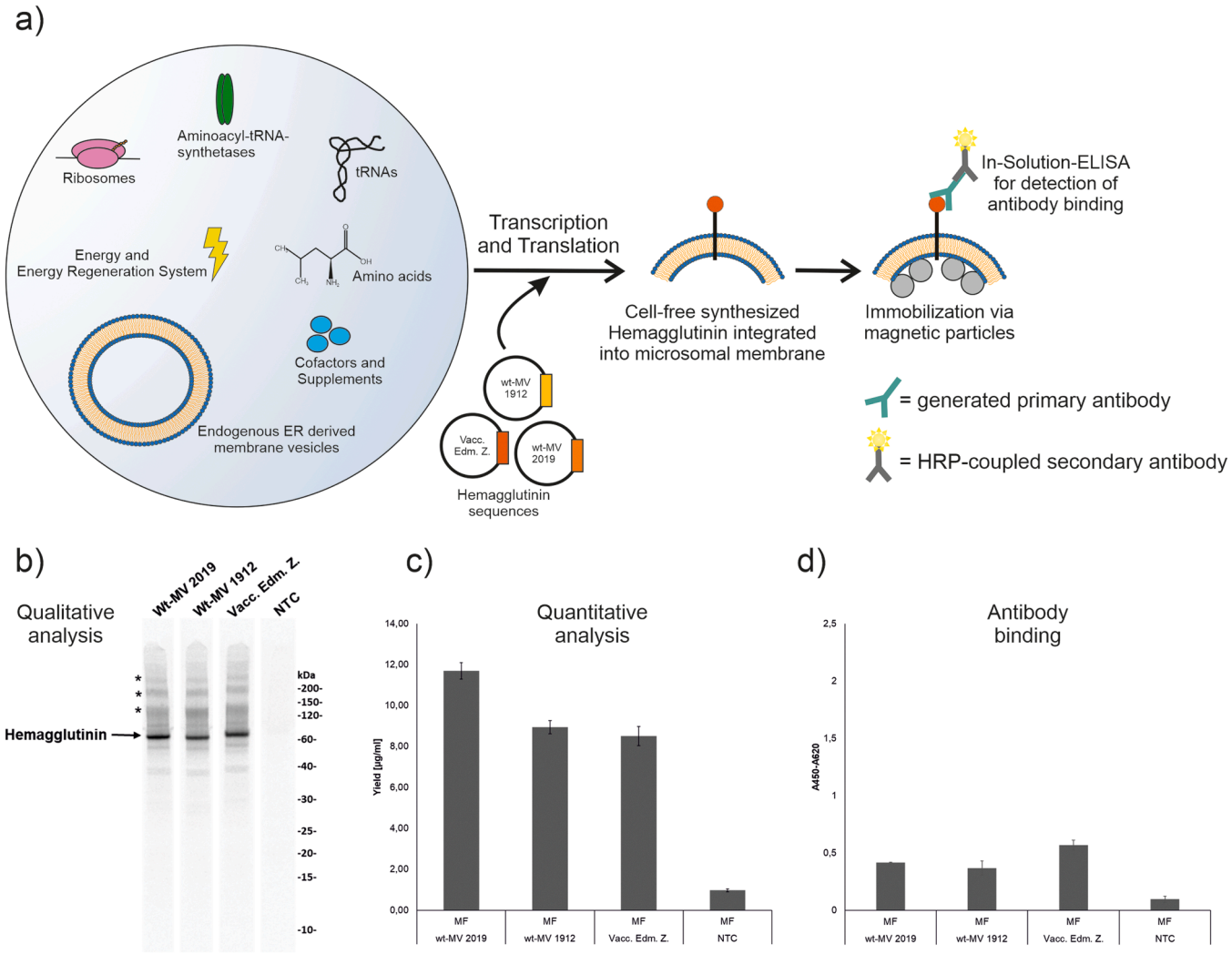


**Fig. 1.** Schematic overview of the study outline. a) Cell-free protein synthesis of the H proteins from the different MV strains with subsequent immobilization on magnetic particles and in-solution ELISA for detection of antibody binding. b) Qualitative analysis of cell-free synthesized H proteins by autoradiography. The specific band of hemagglutinin (H protein) is marked by an arrow. Putative multimeric structures are marked with an asterisk. c) Quantitative analysis of cell-free synthesized H proteins by liquid scintillation counting in triplicates. Depicted is the protein yield in the microsomal fraction (MF) which was afterwards used in d) for the evaluation of the binding of cmAb 34 by an in-solution ELISA. Shown are the results of the absorbance measurement resulting from triplicates. NTC = no template control.

might correlate to dimeric, trimeric and tetrameric structures. Interestingly, no bands indicate glycosylation even though the MV-H protein has five putative glycosylation sites and the CHO system is capable of performing glycosylation (Supplementary Fig. 1). The no-template control, a cell-free synthesis without the addition of any templates showed no specific band in the autoradiogram. The quantitative analysis revealed that for the wt-MV 2019 approximately 12 μg/ml ended up in the microsomal fraction (MF), whereas for wt-MV 1912 and vacc. Edm. Z. approximately 9 μg/ml were present in the MF (Fig. 1c).

### 3.4. Evaluation of cmAbs binding to cell-free synthesized MV-H proteins

The MV strain-specific H proteins located in the microsomal membranes were immobilized tag-free on magnetic beads and used for an in-solution ELISA. The cmAbs were utilized as the primary antibody, specifically binding to the different MV-H proteins. The complex was detected by a horseradish peroxidase (HRP)-linked secondary antibody (Fig. 1a and d). For each of the seven antibodies, a specific binding pattern was observed (Table 1, Supplementary Fig. 2–8). As expected, all seven cmAbs showed binding to the homologous vacc. Edm. Z.-H protein that was therefore used as an internal positive control for the in-solution ELISA. The H protein of both the historical and the current wt-MV was recognized by the cmAbs 9c, 12b, 13a and 34c. The cmAb 12b showed however only a weak binding signal for each of the investigated MV. For cmAb 1d, selective recognition of the current wt-MV was seen whereas cmAb 32c showed no binding signal for both wt-MV. (Table 1 and Supplementary Information).

## 4. Discussion

The LAMV has been in use for six decades and is still highly effective in protecting against measles. An estimated 57 million measles deaths were averted worldwide by vaccination during 2000–2022 (Minta et al., 2023). The maintained high efficacy of the commonly used Edmonston-derived LAMV could be explained by a high temporal stability of virus surface epitopes targeted by vaccine-induced neutralizing antibodies. This study provides evidence for the long-term evolutionary stability of neutralizing epitopes on the MV-H protein. The binding of seven vaccine-induced cmAbs on cell-free synthesized H proteins of a historical MV detected in a lung specimen from a fatal case of measles pneumonia in 1912 and a current MV of genotype D8 isolated from an acute case in 2019 was quantified. The genomic sequence used to synthesize the wt-MV 1912-H protein represents the oldest ever detected MV and the only MV sequence from the pre-vaccination period that was obtained directly from a clinical specimen (Düx et al., 2020). The H protein sequence predicted for the wt-MV 1912 shows a high degree of aa identity with the wt-MV 2019 (96.43%). Characteristics that might determine the antigenic properties of the H protein, e.g. the aa residues interacting with the host cell receptors SLAM and nectin-4, as well as the five potential N-linked glycosylation sites, are shared between the historical and the current virus. The similarity in the antigenic structure can therefore be expected.

To date, different virus-related proteins were successfully synthesized and characterized using eukaryotic cell-free systems (Kubickova et al., 2021; Merk et al., 2015; Mikami et al., 2006; Ramm et al., 2022; Spearman and Ratner, 1996; Wang et al., 2008). Most of the studied proteins showed a specific behavior that was comparable to the in vivo synthesized counterpart. Studies combining the cell-free synthesis of viral proteins with the evaluation of binding of neutralizing antibodies are rare. To date, only one recent study used an E. coli-based cell-free system for the analysis of 119 anti-SARS-CoV-2 antibodies from a mouse immunized with the spike protein to identify neutralizing antibody candidates (Hunt et al., 2023). In our study, we have used eukaryotic CFPS to enable glycosylation. Interestingly, the glycosylation of the cell-free synthesized MV-H protein did not occur, despite previous reports of successful glycosylation of other proteins in CHO CFPS systems (Brödel et al., 2014; Thoring et al., 2019). The reason for the lack of MV glycosylation might be the orientation of the MV-H protein in the microsomal membranes. The putative N-linked glycosylation sites are located downstream of the transmembrane domain of the H protein and therefore are oriented to the outside of the microsomes where no glycosylation takes place. It has been concluded from the crystal structure that wide areas of the MV-H protein appear to be covered with N-linked sugars and only unshielded side areas of the protein are allowed to interact with antibodies (Hashiguchi et al., 2007). In our study, the binding of all seven cmAbs to the homologues cell-free synthesized non-glycosylated vacc. Edm. Z. H protein indicates that the respective epitopes were maintained in the absence of N-linked sugars.

The binding to the cell-free synthesized wt-MV 1912 H protein was detected for four of the seven mAbs tested (cmAbs 9c, 12b, 13a and 34c). A previously conducted MV neutralization study comprising the 1954 low passage Edmonston MV (genotype A) and recently circulating MV strains of eight frequently detected genotypes has shown that the mAbs 9c and 13a exhibit a differing neutralization pattern between MV variants whereas the mAbs 12b and 34c neutralize all MV variants (Beer, 2012). These data suggest that the mAbs 12b and 34c recognize epitopes that are conserved between MV variants whereas the mAbs 9c and 13a target epitopes that are present only in some variants. Distinct epitopes are assumed for recognition by mAbs 12b and 34c since a previous binding study using a peptide microarray that represented the globular head domain of the H protein revealed the binding of mAb 34c to the highly conserved sequence $P_{309}$YQGSGK whereas no binding signal was obtained for mAb 12b (Beer, 2012). Overall, these results suggest that the four vaccine-induced cmAbs that bind to the wt-MV 1912-H protein recognize four distinct epitopes. Hypothetically, the Edmonston vaccine could have prevented the measles death case in 1912. The cmAbs 9c, 12b, 13a and 34c bind to the H protein of both the historical wt-MV 1912 and the current wt-MV 2019 indicating the presence of four epitopes that have been stable for more than 100 years. For each of the analyzed MV strains (wt-MV 1912, wt-MV 2019 and vacc. Edm. Z. as reference strain) the strongest binding signal was obtained for cmAb 9c and the lowest for cmAb 12b. Neutralization data did not, however, show a different neutralizing activity between these mAbs (Beer, 2012; Santibanez, unpublished data) so that the strength of binding signal in the in-solution ELISA might not be considered as a correlate for the neutralizing capacity of the respective mAb. The most relevant finding of this study is that the epitopes defined by the mAbs 12b and 34c and previously characterized as being conserved between recently circulating MV-H variants exhibit long-term stability. The binding sequence for mAb 34c ($P_{309}$YQGSGK) is located in a large loop protruding from the β2 sheet of H protein (Beer, 2012; Tahara et al., 2016) and thus outside of the predicted binding site for the cellular receptors SLAM and nectin-4 in the ß4-ß5 groove (Hashiguchi et al., 2011). The receptor-binding site forms an effective conserved neutralizing epitope

**Table 1**
Binding of different cmAbs to cell-free synthesized H protein originating from wt-MV 2019, wt-MV 1912 and vacc. Edm. Z. (positive control). (+++) = strongest binding signal in ELISA, (++) = strong binding signal in ELISA (three times signal of negative control), (+) = weak binding signal in ELISA (two times signal of negative control), (-) = binding signal in ELISA is below defined thresholds.

| MV-strain | cmAb 1d | cmAb 9c | cmAb 12b | cmAb 13a | cmAb 32c | cmAb 33a | cmAb 34c |
|---|---|---|---|---|---|---|---|
| wt-MV 2019 (D8) | ++ | +++ | ++ | + | - | + | ++ |
| wt-MV 1912 | - | +++ | + | + | - | - | ++ |
| Vacc. Edm. Z. | ++ | +++ | ++ | ++ | ++ | + | ++ |

(Tahara et al., 2013) and it remains to be determined whether mAb 12b binds to aa residues that interact with the cellular receptors.

The experimental evidence for longevity of conserved epitopes on the MV-H protein that are recognized by vaccine-induced neutralizing antibodies explains the consistently high efficacy of the Edmonston-derived vaccines over the last six decades and underscores their continued use in achieving measles eradication. Furthermore, the presence of long-term stable conserved epitopes constitutes a necessary prerequisite for the development of a mAb-based alternative to the use of immunoglobulins for passive immunization.

## CRediT authorship contribution statement

**Walter Cornelia:** Methodology, Investigation, Formal analysis. **Beer Kerstin:** Methodology, Investigation, Formal analysis. **Schnalke Thomas:** Writing – review & editing, Resources, Project administration, Methodology, Conceptualization. **Zemella Anne:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Widulin Navena:** Resources, Methodology, Investigation. **Santibanez Sabine:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Data curation, Conceptualization. **Dorner Brigitte G.:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Dorner Martin B.:** Supervision, Methodology, Data curation, Conceptualization. **Stern Daniel:** Validation, Supervision, Methodology, Data curation, Conceptualization. **Calvignac-Spencer Sebastien:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Merkel Kevin:** Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Mankertz Annette:** Writing – review & editing, Resources, Project administration, Conceptualization. **Düx Ariane:** Validation, Methodology, Investigation, Formal analysis, Data curation. **Schmid Bernhard G.:** Writing – review & editing, Formal analysis, Data curation. **Wenzel Dana:** Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Wolbert Anne:** Validation, Methodology, Investigation, Formal analysis. **Ramm Franziska:** Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

## Acknowledgements

We would like to thank Prof. Dr. Martin Mielke and the virologists, immunologists and bioinformaticians at the RKI, as well as Dr. Mick N. Mulders, Dr. Kevin E. Brown, Prof. Dr. Claude P. Muller and all other scientists within the WHO Global Measles/Rubella Laboratory Network, for their expert advice and inspiring discussion. We thank the laboratory technicians of the NRC MMR at the RKI for their excellent technical assistance.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ijmm.2024.151607.

## References

Bankamp, B., Takeda, M., Zhang, Y., Xu, W., Rota, P.A., 2011. Genetic characterization of measles vaccine strains. J. Infect. Dis. 204 (Suppl 1), S533–S548. https://doi.org/10.1093/infdis/jir097. PMID: 21666210.

Beer, K., 2012. Charakterisierung neutralisierender Epitope des Hämagglutinins aktueller Masernwildviren im Vergleich zum Impfvirus. Doctoral dissertation. Freie Univ. Berl. https://doi.org/10.17169/refubium-16361.

Bouche, F.B., Ertl, O.T., Muller, C.P., 2002. Neutralizing B cell response in measles. Viral Immunol. 15, 451–471.

Brindley, M.A., Takeda, M., Plattet, P., Plemper, R.K., 2012. Triggering the measles virus membrane fusion machinery. Proc. Natl. Acad. Sci. USA 109 (44), E3018–E3027. https://doi.org/10.1073/pnas.1210925109. Epub 2012 Oct 1. PMID: 23027974; PMCID: PMC3497790.

Brödel, A.K., Sonnabend, A., Kubick, S., 2014. Cell-free protein expression based on extracts from CHO cells. Biotechnol. Bioeng. 111 (1), 25–36.

Brödel, A.K., Sonnabend, A., Roberts, L.O., Stech, M., Wüstenhagen, D.A., Kubick, S., 2013. IRES-mediated translation of membrane proteins and glycoproteins in eukaryotic cell-free systems. PloS One 8 (12), e82234.

de Swart, R.L., Yüksel, S., Osterhaus, A.D.M.E., 2005. Relative contributions of measles virus hemagglutinin- and fusion protein-specific serum antibodies to virus neutralization. J. Virol. 79 (17), 11547–11551. https://doi.org/10.1128/JVI.79.17.11547–11551.2005.

Düx, A., Lequime, S., Patrono, L.V., Vrancken, B., Boral, S., Gogarten, J.F., Hilbig, A., Horst, D., Merkel, K., Prepoint, B., Santibanez, S., Schlotterbeck, J., Suchard, M.A., Ulrich, M., Widulin, N., Mankertz, A., Leendertz, F.H., Harper, K., Schnalke, T., Lemey, P., Calvignac-Spencer, S., 2020. Measles virus and rinderpest virus divergence dated to the sixth century BCE. Science 368 (6497), 1367–1370. https://doi.org/10.1126/science.aba9411. PMID: 32554594; PMCID: PMC7713999.

Finsterbusch, T., Wolbert, A., Deitemeier, I., Meyer, K., Mosquera, M.M., Mankertz, A., Santibanez, S., 2009. Measles viruses of genotype H1 evade recognition by vaccine-induced neutralizing antibodies targeting the linear haemagglutinin noose epitope. J. Gen. Virol. 90, 2739–2745. https://doi.org/10.1099/vir.0.013524-0.

Griffin, D.E., 2018. Measles vaccine. Viral Immunol. 31 (2), 86–95. https://doi.org/10.1089/vim.2017.0143.

Hashiguchi, T., Kajikawa, M., Maita, N., Takeda, M., Kuroki, K., Sasaki, K., Kohda, D., Yanagi, Y., Maenaka, K., 2007. Crystal structure of measles virus hemagglutinin provides insight into effective vaccines, 19535–1954 Proc. Natl. Acad. Sci. USA 104 (49). https://doi.org/10.1073/pnas.0707830104.

Hashiguchi, T., Ose, T., Kubota, M., Maita, N., Kamishikiryo, J., Maenaka, K., Yanagi, Y., 2011. Structure of the measles virus hemagglutinin bound to its cellular receptor SLAM. Nat. Struct. Mol. Biol. 18, 135–141. https://doi.org/10.1038/nsmb.1969.

Haueis, L., Stech, M., Schneider, E., Lanz, T., Hebel, N., Zemella, A., Kubick, S., 2023. Rapid one-step capturing of native, cell-free synthesized and membrane-embedded GLP-1R. Int. J. Mol. Sci. 24 (3).

Henrich, E., Hein, C., Dötsch, V., Bernhard, F., 2015. Membrane protein production in Escherichia coli cell-free lysates. FEBS Lett. 589 (15), 1713–1722.

Hunt, A.C., Vögeli, B., Hassan, A.O., Guerrero, L., Kightlinger, W., Yoesep, D.J., Krüger, A., DeWinter, M., Diamond, M.S., Karim, A.S., Jewett, M.C., 2023. A rapid cell-free expression and screening platform for antibody discovery. Nat. Commun. 14 (1), 3897.

Katz, S.L., Enders, J.F., Holloway, A., 1962. The development and evaluation of an attenuated measles virus vaccine. Am. J. Public Health Nations Health 52 (Suppl), 5–10.

Kubickova, B., Schenk, J.A., Ramm, F., Markuškienė, K., Reetz, J., Dremsek, P., Tamosiunas, P.L., Cepulyte, L., Trinh, H.A., Scholz, J., Memczak, H., Hovestädt, M., Ryll, R., Petraityte-Burneikiene, R., Corman, V.M., Andersson, A., Becher, D., Groschup, M.H., Kubick, S., Sellrie, F., Johne, R., Ulrich, R.G., 2021. A broadly cross-reactive monoclonal antibody against hepatitis E virus capsid antigen. Appl. Microbiol. Biotechnol. 105 (12), 4957–4973.

Lin, W.W., Moran, E., Adams, R.J., Sievers, R.E., Hauer, D., Godin, S., Griffin, D.E., 2020. A durable protective immune response to wild-type measles virus infection of macaques is due to viral replication and spread in lymphoid tissues. eaax7799 Sci. Transl. Med. 12 (537). https://doi.org/10.1126/scitranslmed.aax7799. PMID: 32238577; PMCID: PMC7895323.

Merk, H., Rues, R.-B., Gless, C., Beyer, K., Dong, F., Dötsch, V., Gerrits, M., Bernhard, F., 2015. Biosynthesis of membrane dependent proteins in insect cell lysates: identification of limiting parameters for folding and processing. Biol. Chem. 396 (9-10), 1097–1107.

Mikami, S., Kobayashi, T., Yokoyama, S., Imataka, H., 2006. A hybridoma-based in vitro translation system that efficiently synthesizes glycoproteins. J. Biotechnol. 127 (1), 65–78.

Minta, A.A., Ferrari, M., Antoni, S., Portnoy, A., Sbarra, A., Lambert, B., Hatcher, C., Hsu, C.H., Ho, L.L., Steulet, C., Gacic-Dobo, M., Rota, P.A., Mulders, M.N., Bose, A.S., Caro, W.P., O'Connor, P., Crowcroft, N.S., 2023. Progress toward measles elimination — worldwide, 2000–2022. MMWR Morb. Mortal. Wkly Rep. 72, 1262–1268. https://doi.org/10.15585/mmwr.mm7246a3.

Mühlebach, M.D., Mateo, M., Sinn, P.L., Prüfer, S., Uhlig, K.M., Leonard, V.H., Navaratnarajah, C.K., Frenzke, M., Wong, X.X., Sawatsky, B., Ramachandran, S., McCray Jr, P.B., Cichutek, K., von Messling, V., Lopez, M., Cattaneo, R., 2011. Adherens junction protein nectin-4 is the epithelial receptor for measles virus. Nature 2011 480 (7378), 530–533. https://doi.org/10.1038/nature10639. PMID: 22048310; PMCID: PMC3245798.

Ramm, F., Dondapati, S.K., Thoring, L., Zemella, A., Wüstenhagen, D.A., Frentzel, H., Stech, M., Kubick, S., 2020. Mammalian cell-free protein expression promotes the functional characterization of the tripartite non-hemolytic enterotoxin from Bacillus cereus. Sci. Rep. 10 (1), 2887.

Ramm, F., Dondapati, S.K., Trinh, H.A., Wenzel, D., Walter, R.M., Zemella, A., Kubick, S., 2022. The potential of eukaryotic cell-free systems as a rapid response to novel zoonotic pathogens: analysis of SARS-CoV-2 viral proteins. Front. Bioeng. Biotechnol. 10, 896751.

Santibanez, S., Niewiesk, S., Heider, A., Schneider-Schaulies, J., Berbers, G.A.M., Zimmermann, A., Halenius, A., Wolbert, A., Deitemeier, I., Tischer, A., Hengel, H., 2005. Probing neutralizing-antibody responses against emerging measles viruses (MVs): immune selection of MV by H protein-specific antibodies? J. Gen. Virol. 86, 365–374. https://doi.org/10.1099/vir.0.80467-0.

Seki, F.Yuta, Yamamoto, Y., Fukuhara, H., Ohishi, K., Maruyama, T., Maenaka, K., Tokiwa, H., Takeda, M., 2020. Measles virus hemagglutinin protein establishes a specific interaction with the extreme N-terminal region of human signaling

lymphocytic activation molecule to enhance infection (Article). Front. Microbiol 11, 1830. https://doi.org/10.3389/fmicb.2020.01830.
Spearman, P., Ratner, L., 1996. Human immunodeficiency virus type 1 capsid formation in reticulocyte lysates. J. Virol. 70 (11), 8187–8194.
Tahara, M., Ohno, S., Sakai, K., Ito, Y., Fukuhara, H., Komase, K., Brindley, M.A., Rota, P. A., Plemper, R.K., Maenaka, K., Takeda, M., 2013. The receptor-binding site of the measles virus hemagglutinin protein itself constitutes a conserved neutralizing epitope. J. Virol. 87 (6), 3583–3586. https://doi.org/10.1128/JVI.03029-12. Epub 2013 Jan 2. PMID: 23283964; PMCID: PMC3592127.
Tahara, M., Bürckert, J.P., Kanou, K., Maenaka, K., Muller, C.P., Takeda, M., 2016. Measles virus hemagglutinin protein epitopes: the basis of antigenic stability. Viruses 8 (8), 216. https://doi.org/10.3390/v8080216. Erratum in: Viruses, 2016; 8(11): null. PMID: 27490564; PMCID: PMC4997578.
Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. 38 (7), 3022–3027. https://doi.org/10.1093/molbev/msab120.
Tatsuo, H., Ono, N., Tanaka, K., Yanagi, Y., 2000. SLAM (CDw150) is a cellular receptor for measles virus. Nature 406 (6798), 893–897. https://doi.org/10.1038/35022579. PMID: 10972291.
Thoring, L., Dondapati, S.K., Stech, M., Wüstenhagen, D.A., Kubick, S., 2017. High-yield production of "difficult-to-express" proteins in a continuous exchange cell-free system based on CHO cell lysates. Sci. Rep. 7 (1), 11710.
Thoring, L., Wüstenhagen, D.A., Borowiak, M., Stech, M., Sonnabend, A., Kubick, S., 2016. Cell-free systems based on CHO cell lysates: optimization strategies, synthesis of "difficult-to-express" proteins and future perspectives. PloS One 11 (9), e0163670.
Thoring, L., Zemella, A., Wüstenhagen, D., Kubick, S., 2019. Accelerating the production of druggable targets: eukaryotic cell-free systems come into focus. Methods Protoc. 2 (2).
Wang, X., Liu, J., Zheng, Y., Li, J., Wang, H., Zhou, Y., Qi, M., Yu, H., Tang, W., Zhao, W. M., 2008. An optimized yeast cell-free system: sufficient for translation of human papillomavirus 58 L1 mRNA and assembly of virus-like particles. J. Biosci. Bioeng. 106 (1), 8–15.
Williams, D., Penedos, A., Bankamp, B., Anderson, R., Hübschen, J., Ben Mamou, M., Beck, A., Brown, D., Rey, G., Evans, R., Ghoniem, A., Sangal, L., Byabamazima, C., Dosseh, A., Kfutwah, A., Mulders, M., Brown, K., Rota, P.A., 2022. Update: circulation of active genotypes of measles virus and recommendations for use of sequence analysis to monitor viral transmission. In: Weekly Epidemiological Record, 39, pp. 485–492.
Zhang, X., Lu, G., Qi, J., Li, Y., He, Y., Xu, X., Shi, J., Zhang, C., Yan, J., Gao, G.F., 2013. Structure of measles virus hemagglutinin bound to its epithelial receptor nectin-4. Nat. Struct. Mol. Biol. 20 (1), 67–72. https://doi.org/10.1038/nsmb.2432.