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Original Article

Critical site differences of fusion protein between wildtype and vaccine measles virus strains in Indonesia

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Abstract

Background Measles virus can cause high morbidity and mortality in infants and children. Fusion glycoprotein (F protein) found in the viral envelope is important for the host cell infection mechanism. F protein is immunogenic and may cause specific immune responses in the host. High variability is found in the F protein gene of vaccine viral strains compared to wild type strains. This amino acid sequence variability may result in a less specific immune response against other strains, possibly rendering the vaccine to be less effective.

Objective To determine the amino acid sequence differences in critical sites of F protein in wild type and vaccine measles virus strains in Indonesia.

Methods We compared amino acid sequences of three genotypes of wild type measles virus (G2, G3 and D9) to those of the vaccine strains, CAM-70, Schwarz, and Edmonston-wt type measles virus.

Results Analysis showed that there were differences at F1-F2 cleavage site, B cell epitopes, and H protein binding site between the CAM-70 vaccine viral strains and wild type strains. Schwarz vaccine strain differed from the wild type strains at the H protein binding site. A G3 wild type strain potential glycosylation site was also different from all other strains studied.

Conclusion There were differences in the critical sites of F protein between wild type strains and the CAM-70 and Schwarz vaccine strains. **[Paediatr Indones. 2011;51:123-7]**.

Keywords: *wild type measles virus, measles vaccine, F protein, critical sites*

The etiologic agent of measles is a virus belonging to the morbillivirus genus, and paramyxovirus family. It is highly infectious, especially in infants and children.[1] Measles virus has an envelope and a single negative-strand RNA genome which codes for 6 protein types.[2] Fusion (F protein) and hemagglutinin (H) protein are glycoproteins found on the envelope of the virus. These proteins function to recognize host surfaces, perform membrane fusion, and allow the virus to enter host cells.[3,4] F protein is highly antigenic, and has several epitopes that may be recognized by specific antibodies from infected individuals.[5] Several important sites on the F protein include glycosylation sites, F1-F2 cleavage sites, B-cell and T-cell binding sites. If the amino acid sequences of these sites are changed, the infection process of the virus will not be complete.

In Indonesia, there are 3 known genotypes of measles virus, G2, G3 and D9. Vaccines used to prevent measles in Indonesia are manufactured from the CAM-70 and Schwarz strains. In general,

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the F protein gene is stable. However, the vaccine F protein gene varies greatly compared to the wild type gene.[6] This variability may be caused by the vaccine production process. The CAM-70 F gene has the most substituted amino acids, hence the greatest difference in amino acid sequence from wild type.[7] This research aimed to determine the differences between important sites of F protein amino acid sequences in wild type and vaccine measles virus strains in Indonesia.

Methods

This was a cross-sectional study conducted at Jakarta. Sequencing of the F gene and protein were performed on three wild type virus genotypes (G2, G3 and D9), as well as CAM-70, Schwarz, and Edmonston-wt measles virus strains. Wild type measles strains were obtained from the National Institute of Health Research and Development, Indonesia. Some of the isolated measles viruses were sent to CDC Atlanta, USA for genotyping. The CAM-70 vaccine (PT Bio Farma) was obtained from communicable diseases control, Indonesian Ministry of Health. Schwarz type (MMR) was obtained from PT. Eurindo, while Edmonston-wt nucleotide sequences were provided by NCBI. The genotypes of the Indonesian wild type measles virus were determined in Atlanta and verified in Indonesia.

Extraction of viral RNA isolate derived from a patient was performed using QIAmp Viral RNA Mini Kit from QIAGEN (cat. no. 5204). Amplification of RNA was performed using PCR and primers were designed using sequence analysis based on the Edmonston-wt, substrain AIK-C (AB-46218) from NCBI[8] using Primer-3 software. Reverse transcription was performed using SuperScript III Reverse Transcriptase (Invitrogen). The $_{C}$DNA obtained was amplified by PCR with Platinum Taq DNA Polymerase (Invitrogen). Primers used are shown in **Table 1**. The PCR product was analyzed by gel electrophoresis to determine its molecular weight or length of nucleic acid chain. We used a 2% agarose gel mixed with 10 ul (1 ug/ml) ethidium bromide electrophoresed at 100 volts for 40 minutes.[9] The DNA product was purified from the low melting agarose gel using a QIAQuick Gel Extraction Purification kit (QIAGEN cat no 28704), according to the manufacturer's protocol.

TOPO TA Cloning Kit (Invitrogen cat. no. K4500-01) was used to clone the PCR product. Plasmid extraction was performed with QIAprep Spin Miniprep (QIAGEN cat. no. 27104), according to manufacturer's instructions. Filtrate was labeled and stored for further sequencing. DNA sequencing was performed by Sanger method (dideoxy termination method) with automatic ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit on an automatic sequencer. Primers for sequencing

Table 1. Primers used for PCR and sequencing

No.	Primers	Nucleotide sequences	Position
4	Primer F 1	gAC CAA AAg ATC AAT CCA CCA C	5360-5381
	2	ggC CgA TTA AAT CAC AAg ATA gTT	6032-6055
5	Primer F 3	CTg TTC Agg gTg TCC AAg ACT AC	5978-5600
	4	CTT gAT TAA TgA TCg TTC CTg TTg	6653-6676
6	Primer F 5	gTA CAC TCg TAT CCg ggT CTT TT	6550-6572
	6	gCA CCC TAA gTT TTA ATT AAC TAC Cg	7232-7257

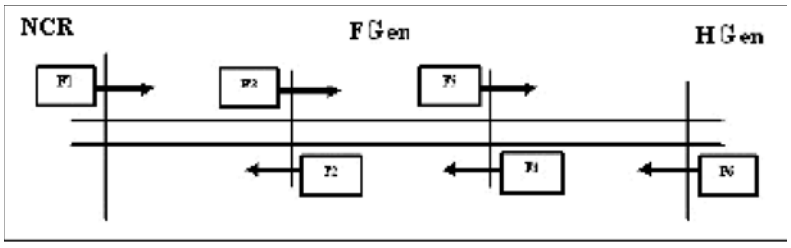

Figure 1. Strategy for sequencing F gen in overlapping regions. This strategy using three pairs of primers (see table 1: F1-F2, F3-F4, F5-F6) which overlapping on F gen region. F gen = Fusion gen; H gen = hemagglutinin gen; NCR = non coding region.

are shown in **Table 1**. The obtained DNA fragments were checked, reedited, then assembled into complete contiguous sequences of the F gene using Genetyx and Bioedit software. The complete F gene sequences from all measles virus isolates were compared. Comparisons of both DNA and protein sequences of the 5 isolates were performed using Genetyx, ClustalW and Bioedit software. **(Figure 1)**

Results

For B-cell epitope analysis of F protein, we used 2 previously discovered epitope locations. The first epitope was residues 388-402 with an abundance of protected cysteines. The map of this epitope showed that 15 amino acids were important for strong affinity to anti-measles antibodies.[10] The second epitope location was residues 397-420,[5,10] another protective B-cell epitope. From the alignment analysis of both epitopes, we found differences in the CAM-70 amino acid residue 401 H (histidine) and residue 420 D (aspartic acid), compared to the Edmonston-wt, Schwarz, G2, G3 and D9. All non-CAM-70 strains had 401 Y (tyrosine) and 420 A (alanine).

The F1 and F2 cleavage site consisted of 5 amino acids (residues 108-112) (Arg-Arg-His-Lys-Arg). Arginine 112 is a critical determinant of cleavage. If a mutation occured in this site, the rate of transport to the cell surface would decrease, causing abnormal Fo cleavage and loss of fusogenic activity.[11] From the alignment analysis of residues 108-112, a difference was found in residue 110. The CAM-70 vaccine virus had a G (glycine) at that site, while Edmonston-wt, Schwarz, G2, G3 and D9 had S (serine) as shown in **Figure 3**.

The fusion process requires F protein to be co-expressed with H protein. The F1 protein site contacts H protein on residues 337-381, which are rich in cysteine.[11] We found two differences in the F1 protein site, residues 365 and 369. Residue 365 was Y (tyrosine) in the Schwarz vaccine, F (phenylalanine) in the CAM-70 vaccine, and S (serine) in the Edmonston-wt, G2, G3 and D9 strains. Residue 369 was S (serine) in CAM-70, but C (cysteine) in the Edmonston-wt, Schwarz, G2, G3 and D9 strains **(Figure 4)**.

The glycosylation site contained a consensus sequence (Asn-X-Thr or Asn-X-Ser) which was found in F2 but not in F1. In the gene sequence, these glycosylation sites were located at 667-675 bp, 763-771 bp and 781-789 bp,[11] corresponding to amino acid residues 25-27, 57-59 and 63-65, respectively.[12] The alignment analysis of F protein showed the sequence of T-G-Q (Thr-Gly-Gln) on 25-27, L-V-I (Leu-Val-Ile) on 57-59, and P-N-I (Pro-Asn-Ile) on 63-65 in all strains. However, these three glycosylation sites differed from the consensus sequence, hence other glycosylation

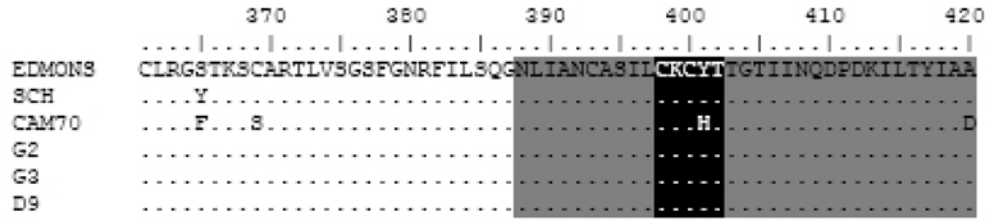

Figure 2. F protein B-cell epitopes (388-402, 397-420) (Black region indicates overlap of epitopes)

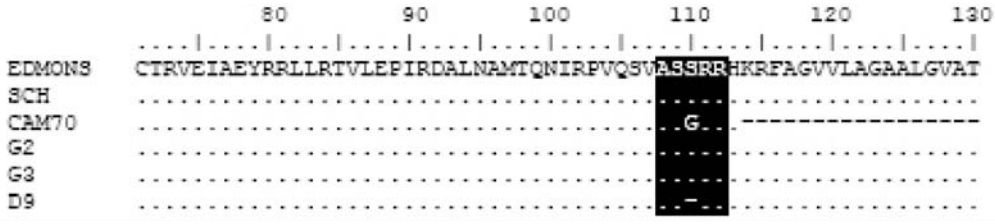

Figure 3. Cleavage site of F1 and F2 protein (black shadow indicates residues 108-112).

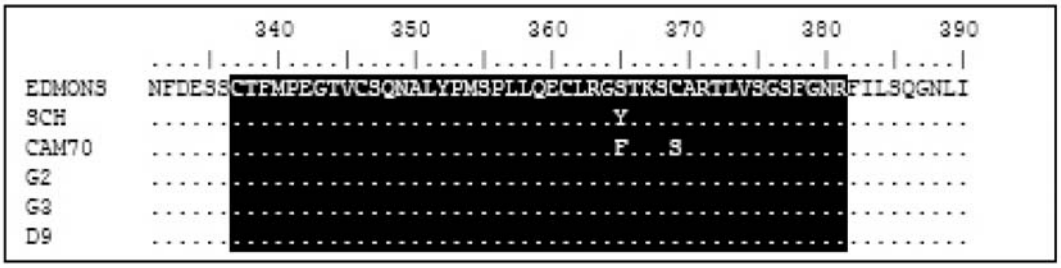

Figure 4. Binding site of H protein on F protein (black shadow indicates residues 337-381)

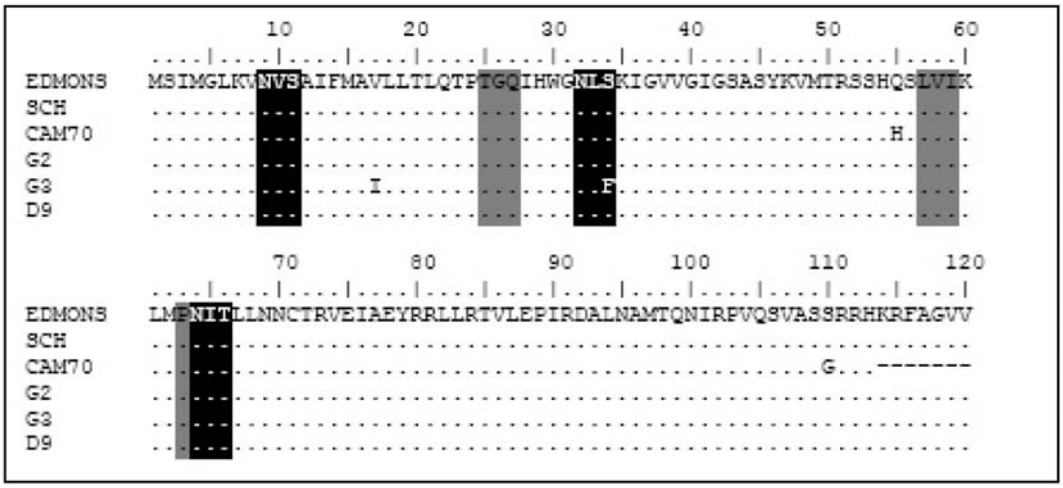

Figure 5. Glycosylation sites on F protein of measles virus (25-27, 57-59, 63-65) (black shadows contain consensus sequences for glycosylation sites, while grey shadows are reported glycoyslation sites11, 12)

sites were searched for and found on residues 9-11 (N-V-S) (Asn-Val-Ser), 32-34 (N-L-S) (Asn-Leu-Ser), and 64-66 (N-I-T) (Asn-Ile-Thr). G3 residue sequence 32-34 was N-L-F (Asn-Leu-Phe), differing from the other strains. Since this sequence differed from the consensus sequence, the glycosylation site may be lost in the G3 strain **(Figure 5)**.

Discussion

Alignment of a B-cell epitope on residues 388-402[10] of F protein showed a difference at residue 401: H (histidine) in CAM-70 and Y (tyrosine) in all other genotypes. At the epitope at residues 397-420,[5] a difference was found in residue 420: D (aspartic acid) in CAM-70 but A (alanine) in the other genotypes. Differences in amino acids may lead to decreased in vitro or in vivo affinity between antibody and antigen.

The cleavage site of F1 and F2 protein consists of 5 amino acids located at residues 108-112 (Arg-Arg-His-Lys-Arg). Arginine 112 is a critical determinant of cleavage. Mutation in this amino acid results in a reduced rate of transport of the protein to the cell surface, an aberrant cleavage process of the Fo protein, and abolition of fusogenic activity.[2]

We found that arginine at residue 112 was conserved in all genotypes of the measles virus. However, amino acid alignment of these sites showed differences at residue 110, G (glycine) in CAM-70 and S (serine) in G2, G3, D9, Schwarz and Edmonston-wt. These amino acids may have different characteristics. Amino acid sequences 337-381 are important for H protein attachment to complete the fusion process between virus and host cell.[11] If the binding site has an anomaly, the infection process may be hindered.[13] Amino acid sequence alignment of binding sites showed two amino acid differences, at residues 365 and 369. At residue 365,

Schwarz vaccine had Y (tyrosine), CAM-70 vaccine had F (phenylalanine), and Edmonston-wt, G2, G3 and D9 had S (serine). Since these amino acids have different characteristics, the binding process may be affected. At residue 369, CAM-70 had S (serine) while Edmonston-wt, Schwarz, G2, G3 and D9 had C (cysteine). Serine and cysteine have similarities, nevertheless, an amino acid change may affect the biological function of F protein in vivo or in vitro, so that the binding affinity to H protein may be decreased.

Glycosylation is necessary for antigenicity of measles virus proteins. Glycosylation is also critical for proteolytic processing and protein transportation to cellular surfaces.[14,15] N-linked glycosylation is more important then O-linked glycosylation.[16] There are 3 glycosylation sites on F protein, located in amino acid sequences 25-27, 57-59, and 63-65.[11,12] From the amino acid alignment of F protein, we found that the location of the glycosylation sites differed from the studies of Richard *et al*.[11] and Tsukiyama *et al*.[12] Using typical glycosylation site sequences (Asn-X-Ser) and (Asn-X-Thr), we found 3 possible sites at residues 9-10-11 (Asparagine-Valine-Serine), 32-33-34 (Asparagine-Isoleucine-Serine), and 64-65-66 (Asparagine-Isoleucine-Threonine). In addition, we found that the amino acid sequence of a glycosylation site in the G3 strain (Asn-X-Phe) did not conform to typical glycosylation site requirements. Hence, glycosylation at this location in G3 genotype may be lost. This loss may influence the biological function of F protein in vitro or in vivo, reducing the antigenicity of F protein for this wild type strain.

In conclusion, there were differences in the F protein B-cell epitopes, F1-F2 cleavage site, and H protein binding site for CAM-70 vaccine virus compared to several wild type strains. Also, the Schwarz strain H protein binding site differed from the wild type strains. A G3 wild type strain potential glycosylation site was also different from all other strains studied.

References

1. World Health Organization. Global measles mortality reduction and regional elimination, 2000-2001. Part 1. Weekly Epidemiological Record. 2002;77:49-56.
2. Griffin DE, Bellini WJ. Measles Virus. In: Fields Virology. 3rd ed. Philadelphia-New York: Lippincott-Raven; 1996. p. 1267-312.
3. Tyrell DIJ, Norrby F. Structural polypeptides of measles virus. J Gen Virol. 1978;39:219-29.
4. Rima, BK. The proteins of morbilli viruses. J Gen Virol. 1983;64:1205-19.
5. Partidos CD, Salani FB, Ripley J, Steward MW. Deconstructing the antigenic profile of a protective epitope from measles virus fusion protein using overlapping peptides. Vaccine. 2000;18:321-4.
6. Rota JS, Wang ZD, Rota PA, Bellini WJ. Comparison of sequences of the H, F, and N coding genes of measles virus vaccine strains. Virus Res. 1994;31:317-0.
7. Rota JS, Hummel KB, Rota PA, Bellini WJ. Genetic variability of the glycoprotein gene of current wild-type measles isolates. Virology. 1992;188:135-42.
8. Komase K, Suzuki N, Nakayama T, Miki K, Kawanishi R, Fukuda K. Genome sequence of measles virus. NCBI no. accession: AB046218 (2001). Availble from: *http://www.ncbi.nlm.nih.gov/nuccore/AB046218*
9. Coligan JE, Kruisbeek AM, Margulies DH, Shevach EM, Strober W. Current protocols in immunology. Vol II. National Institute of Health of USA: Current Protocols Wiley; 1996. p. 841-96.
10. Atabani SF, Obeid EO, Chargelegue D, Aaby P, Whittle H, Steward MW. Identification of an immunodominant neutralizing and protective epitope from measles virus fusion protein by using human sera from acute infection. J Virol. 1997;71:7240-5.
11. Richardson C, Hull D, Greer P, Hasel K, Berkovich A, Englund G, et al. The nucleotide sequence of the mRNA encoding the fusion protein of measles virus (Edmonston Strain): A comparison of fusion protein from several different paramyxoviruses. Virology. 1986;155:508-23.
12. Tsukiyama K, Yoshikawa Y, and Yamanouchi K. Fusion glycoprotein (F) of rinderpest virus: Entire nucleotide sequence of the F RNA, and several features of the F protein. Virology. 1988;164:523-30.
13. Fournier P, Brown NHC, Berbers GAM, Wiesmueller KH, Fleckenstein BT, Schneider F, et al. Antibodies to a new linear site at the topographical or functional interface between the haemagglutinin and fusion proteins protect against measles encephalitis. J Gen Virol. 1997;78:1295-302.
14. Alkhatib G, Shen SH, Briedis D, Richardson C, Roder J. Functional analysis of N-linked glycosylation mutants of the measles virus fusion protein synthesized by recombinant vaccinia virus vectors. J Virol. 1994;68:1522-31.
15. Hu A, Sheshberandaran H, Norbby E, and Kovamees J. Molecular characterization of epitopes on the measles virus hemagglutinin protein. Virology. 1993;192:351-4.