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Original Article

Differences of antigenic profiles on immunoblotting of wild type measles virus and vaccine virus in Indonesia

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Abstract

Background Measles virus has a negative, single strand RNA genome which codes for six important structural proteins. The genes of the wild type measles virus have many variances hence the nucleotide sequences of each wild type virus and vaccine virus are different. This differences lead to the antigenic differences between wild type and vaccine virus.

Objective The purpose of this research is to investigate the differences in the antigenic profiles on immunoblotting between wild type and vaccine virus.

Results The analysis results are 1) the antigen of CAM-70 vaccine virus was less able in cross reacting with the antibodies from G2, G3, D9, CAM-70 and Schwarz; 2) The antibody against CAM-70 was only able to cross react with antigens of N protein and a few of antigens of F proteins; 3) The wild type virus were very immunogenic, hence the antibody titers were very high; 4) The CAM-70 and MMR vaccine virus were less immunogenic, hence their antibody were very low; 5) The antibody responses that always occurred from all immunized mice serum were antibody for N and F proteins. However, the antibody against CAM-70 vaccine virus was still able to react with wild type virus (G2, G3 and D9).

Conclusion All antigen-antibody reaction on immunoblotting resulted in different profiles especially between wild type virus and CAM-70 vaccine virus. Although CAM-70 vaccine virus showed clear differences compared to G2, G3 and D9 genotypes, antibodies against CAM-70 were still able to cross react with antigens from other genotypes (G2, G3 and D9).**[Paediatr Indones. 2008;48:364-73]**.

Keywords: *measles virus, wild type virus, CAM-70 vaccine virus*

Measles is a disease that primarily infects babies and children with symptom of cough, cold, fever, conjunctivitis and occurrence of maculopapular rash on the skin starting from the back of ears spreading through the whole body. Currently this disease is still a major health problem in the world, especially in developing countries. Around 45 million children and babies suffer and one million die every year because of this disease, even though the immunization program has been carried out since the start of 19 century in the world by WHO.[1,2] In Indonesia, measles immunization has been carried since 1980. To improve the success rate of the immunization program, UCI (Universal Child Immunization) has been carried out. In addition, the mass immunization had also been carried out for under-five children in high risk regions. With this program, the infection

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cases had decreased from 92,065 cases in 1990 to 19,238 in 2000.[3] However, cases and outbreaks were still frequently occurred in Indonesia.[4]

Measles is caused by measles virus which belongs to morbillivirus genus, paramyxoviridae family. The virus has an envelope with negative, single strand RNA genome which code for six structural proteins, N, F, P/V/C, M, H and L.[1,5,6] The N, P/V/C and L genes were important in translation and replication of viral RNA, the H and F genes hold major roles in the inclusion of the virus while infecting the host cell, while the M gene functions in the virion maturation.[7]

Measles virus is predicted to be monotype; however there were several genetic variances and different antigenicity between wild type and vaccine virus. Based on the nucleotide sequences of N and H genes, the wild type virus could be classified into eight clades and around 21 genotypes.[8] Hence there were many derivatives of the wild type virus found. Current wild type measles virus had underwent genetic drift against vaccine virus and wild type virus isolated in 1950. This would lead to the differences in the antigenicity between wild type virus and vaccine virus. Neutralization study using vaccine virus and strain of wild type virus showed that serum from current patients neutralized current strain of wild type virus four times better than vaccine virus.[1]

The majority of measles vaccine currently being distributed in Indonesia was CAM-70 and Schwarz vaccine virus. CAM-70 was derived from Tanabe virus which originated from Japan, while Schwarz was derived from Edmonston-wt.[1] The analysis of nucleotide sequences from vaccine virus genome derived from Edmonston-wt did not show any significant differences. However, significant difference was found between CAM-70 vaccine virus and Edmonston-wt derived vaccine virus.[9,10]

The purpose of this research was to observe the differences of the antigenic profiles on immunoblotting between wild type measles virus and vaccine virus in Indonesia.

Methods

To observe the difference of antigenic profiles between several wild type measles virus and vaccine virus in Indonesia, experimental works were performed by injecting each strain of measles virus to mice. The next step was to perform immunoblotting on each of the formed antibody serum. The strains of measles virus to be analyzed were G2, G3, D9, CAM-70 and Schwarz. Half of the sample of cultured wild type measles virus had been sent to ICDC Atlanta to determine their genotypes, which then reconfirmed back in Indonesia.[11] The wild type measles virus were provided by Litbangkes-RI, the CAM-70 (Bio Farma) vaccine virus was provided by Subdit Imunisasi and Schwarz (MMR) vaccine was obtained from PT Eurindo. The wild type viruses which were isolated to be analyzed are presented in **Table 1.**

Table 1. Wild type viruses which were isolated to be analyzed

No.	Code	Location/ Source	IgM	Age (yr)	Sex	Geno -type
1	MVi/INA/06.02/161Yo	Subang-West Java	+	4,5	L	D9
2	MVi/INA/05.02/Ba	Gresik-East Java	+	4	P	G3
3	Mvi/INA/03.04/362 Sep	Pekalongan	+	3,8	P	G2
4	CAM-70 vaccine virus	PT. BioFarma				
5	Schwarz vaccine virus	PT. Eurindo				

Measles virus stock preparation

The stock preparation of the virus followed the instruction given by WHO.[12] First, the passage of B95a or Vero cell was performed as follows:

The materials needed were 500 ml DMEM (Dulbecco's MEM) supplemented by penicillin 100 U/ml, streptomycin 100 ug/ml and foetal bovine serum (FBS) 10% and culture bottles.

The medium inside T-25 bottle was discarded. PBS (5 ml) was added for washing, and then discarded. Trypsin (1-2 ml) was added, and then left for a while (no need to be inside incubator), then discarded. Add 5 ml DMEM. Move to other smaller qtube to mix it evenly. Cell from one bottle can be divided into 1:2, 1:3 or 1:4 (no more than 1:4). Then the cell was added to the prepared culture bottle filled with DMEM-PBS. Cell was ready to be infected after two days.

When the monolayer cell in the bottle had grown successfully (around 75-80%), the liquid virus stock was inoculated into the culture bottles as much as 1.5-2 ml for T-25 bottle, incubated at 37 degree for one hour, and then observed under inverted microscope to check for cell changes (such as floating cell or shape

changes) as a result of intoxication. If this happens, the medium had to be replaced immediately. If all was good, DMEM (10 ml) containing 2% FBS and the antibiotics was added to the culture bottle. The culture bottles were then incubated at 37 degree. The cytopatic effect was examined every day. When the effect reached 75-80%, the virus was harvested, by scratching the wall of the bottle, centrifuged with 1500x g and filtered with Millipore 0.45 μl. The supernatant was stored as virus stock for further treatment.

Animal experiment

Animal used for the trials was BALB/c mouse, because it gave good antibody response when injected with measles virus, and it was inbreed hence genetically identical. Thus the variances within and between groups can be reduced.[13] Each group consists of 22 mice weighted around 25-28 grams and mixed between males and females.

The trials consisted of six groups: group I was immunized with placebo as control, II with G2 genotype, III with G3 genotype, IV with D9, V with CAM-70 vaccine and VI with Schwarz (MMR) vaccine (**Table 2**).

Blood (100 μl) was taken one week prior to immunization to make sure that mice had not been infected with measles by using neutralization test, and another 1 ml was taken two week after the third immunization for laboratory analysis. The laboratory analysis performed was antibody examination by ELISA method and cross neutralization test to find out the differences in antigenic property of each immunogenic protein.

Sample size

The animal trials consisted of six groups as had been discussed above. Because a group would be tested with four different types of antigens, each group could be assumed as two paired groups. Based on the data obtained from references, the mean and expected variance from the discrepancy of the results of neutralization tests reacted with wild type virus were different with vaccine virus. Hence, the calculation for the variance from the neutralization tests of wild type virus was split from the vaccine virus. This data was one of the important components in determining the sample size.

The sample size determination of each group is explained as follow.[13]

A. The animal group immunized with vaccine virus

The formula used to determine the animal trial sample size for neutralization test for each group that received vaccine immunization is described as below. The laboratory tests performed on three groups is the same as on other groups. The data used to calculate the sample size in this group is:

N = sample size
$Z\alpha$ = normal standard deviation for α = 0,0025 ($z\alpha$ two-side = 2,813)
$Z\beta$ = normal standard deviation for k β = 0,005 ($Z\beta$ =2,57)
Sd = predicted standard deviation from average = 277,61
d = significant average difference between 2 group = 326

Using the formula for sample size:

$$N = \left[\frac{(Z\alpha + Z\beta)\ Sd\ 2}{d} \right]$$

then N was obtained as 22.

Table 2. Group of trial animals

Group	Size	Weeks									
		-I	I	II	III	IV	V	VI	VII	VIII	IX
Placebo	22	♠	♥				♣		♣		♦
G2 genotype	22	♠	♥				♣		♣		♦
G3 genotype	22	♠	♥				♣		♣		♦
D9 genotype	22	♠	♥				♣		♣		♦
CAM-70 vaccine	22	♠	♥				♣		♣		♦
Schwarz vaccine	22	♠	♥				♣		♣		♦

Note :
♠ : Blood taken for base data
♥ : Antigen injection
♣ : Booster
♦ : Blood taken from the heart after anastetion
The animal used is mouse from BALB/c strain

B. The animal group immunized with wild type virus

The formula used is the same as above, while the data for calculation is as follow:

N = sample size
Zα = 2,575 (normal standard derivative for α= 0,01)
Zβ = 1,960 (normal standard derivative for β= 0,025).
Sd = 277,61
d = 326
therefore, N= 18.

To avoid the mismatch samples at the end of the experiments, samples were added to all groups to 22 mouse (20% addition).

Control group

One of the groups was immunized with vaccine diluents or media solution as the control group. The ELISA test and cross neutralization test would then be performed on the serum of the group.

The categorization and selection of experiment animals

The selection of trial animals was carried out by proportional random sampling with the same population size of males and females for each group.[24]

Antigen for injection

As had been previously described, antigen injected for group A was media solution, group B was G2 genotype of wild type virus, group C in G3 genotype, group D was D9 genotype, group E was CAM-70 vaccine virus from Bio Farma with batch number 250463, and group F was Schwarz MMR TRIMOVAX vaccine from Aventis with batch number X6104-1.

Dosage of antigen

All groups were given the same titer of virus as follows: first injection was given 10^3 TCID50, while the first and second booster were $5x10^2$ TCID50. The virus was injected to the mouse intrapertoneally.[23]

Injection

Antigen was injected in disguise where the staff who was going to inject did not know the type of the antigen as all antigens were numbered only. In addition, the laboratory staff did not know the antigen of the obtained serum.

Blood sampling

Blood was taken through the tails of mice before immunization, while after immunization blood was taken by plexus choroideus, continued by intracardiac by opening the mouse chest. Before the second blood sampling, the mice was anesthesied with ether.[14]

Antigen proteins isolation

We added cultured virus with 1 liter solution, then centrifuged with the speed of 1500X g for 15 minutes. We filtered the supernatant with 0.45 μm paper filter. The filtered solution was then centrifuged with 26000X g for 3 hours at 4^{o}C to get the viral particle pellets. The pellets was then suspended with Tris NaCl EDTA (TNE), and was then purified further with column chromatography using Sepharose CL-4B beads, a cross-linked agarose with certain pore sizes. The eluent from the column chromatography was suspended in Tris NaCl EDTA (TNE) buffer, and then recentrifuged with 6700X g for 90 minutes. Then the formed pellet was re-suspended in 100-300 ul TNE buffer and was stored in 4 C for overnight. At this step, the viral suspension or virion was ready to be used for next experiment.[15,16] The antigen protein content was then measured using BCA Protein Assay Kit with Bradford method. This method was quite sensitive, quick and stable. The standard solution used bovine serum albumin (BSA) with various concentration, and then standard curve was plotted on 560 nm wavelength. The antigen protein content could be calculated by measuring absorbance and comparing it to the standard curve.

Immunoblotting experiment

To observe antibody profile from each antigen of measles virus, immunoblotting experiment was performed. Because each antigen proteins were mixed, separation were performed by sodium dodecyl sulfat polyacrilamide gel electrophoresis (SDS-PAGE) using concentration of gel gradient of 7.5 – 17.5% where the differences in the position of the protein were

a function of their molecular weight. Proteins with heavier molecular weight would move slower than proteins with lighter molecular weight. Hence, heavier proteins would form bands behind the lighter proteins. The fractionated proteins are transferred to a thin membrane of nitrocellulose which was placed over the gel. Protein transfer was accomplished by capillary and also by electric current. The membrane, to which the proteins are tightly bound, was incubated with a specific antibody couple to alkaline phosphatase. Bound antibody was detected by the addition of a substrate (Bromo chloro Indolyl phosphate-Nitro blue tetra sodium) that was converted to a luminescent compound after reaction with the enzyme.

Data analysis

The analysis of the results of immunoblotting was done by evaluating the visibility and intensity of the bands in the paper.

Results

Serum from mice immunized with G2 genotype of measles virus showed different immunoblotting bands when reacted with antigens from G2, G3, D9 and CAM-70 vaccine virus. The serum showed very complete, very dark bands (all bands for L, H, P, N, F1 and M were visible) when reacted with antigens from both G2 and G3 genotypes. However, when reacted with antigens from D9 genotype, bands for H, P and M were very light and thin. Likewise, when reacted with CAM-70, only bands for N and F1 proteins appeared thinly (**Figure 1**).

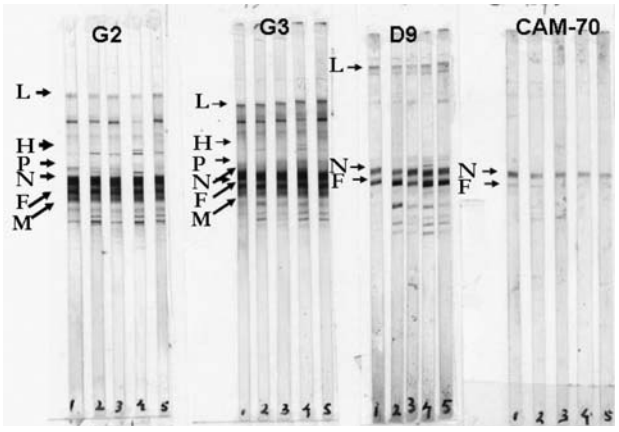

Figure 1. The result of immunoblotting of G2, G3, D9 and CAM-70 antigens reacted with serum from mice immunized with G2 genotype of measles virus

Serum from mice immunized with G3 genotype also showed different bands when reacted with antigens of G2, G3, D9 and CAM-70 genotypes. The differences appeared noticeably when the serum was reacted with CAM-70 antigens which only showed three clear specific bands for H, N, and F1 proteins while dark, thick bands for L, H, P, N, F1 and M proteins were visible when the serum was reacted with G2, G3 and D9 antigens, even though the bands were thinner and lighter for D9 antigen (**Figure 2**).

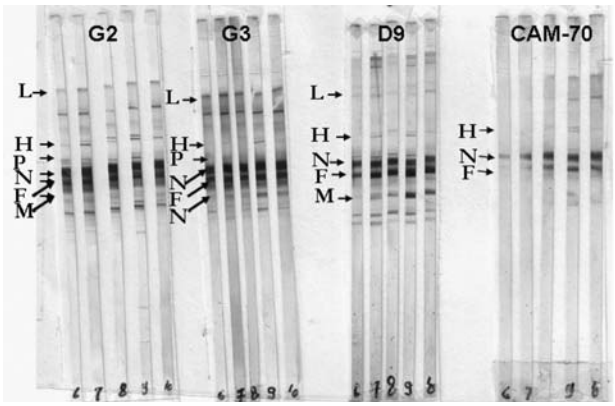

Figure 2. The result of immunoblotting of G2, G3, D9 and CAM-70 antigens reacted with serum from mice immunized with G3 genotype of measles virus

Serum from mice immunized with D9 genotype showed comparable bands with similar intensities when reacted with G2, G3, D9 and CAM-70 genotypes. Only small differences on the bands were appeared when the serum was reacted with CAM-70 where bands for F1 and M proteins were lighter and thinner. Overall, all formed bands of proteins were appeared very clearly. This means that anti-D9 serum cross reacted very well with all four types of antigens (G2, G3, D9 and CAM-70). All bands also were appeared completely, except several proteins were not appeared on Western blotting of CAM-70 vaccine virus.

Serum from mice immunized with CAM-70 vaccine virus showed very few bands when reacted with antigens from G2, G3, D9 and CAM-70 genotypes. Only two bands appeared in the

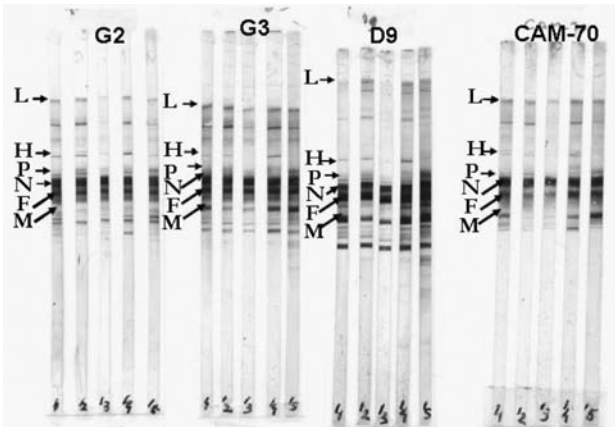

Figure 3. The result of immunoblotting of G2, G3, D9 and CAM-70 antigens reacted with serum from mice immunized with D9 genotype of measles virus

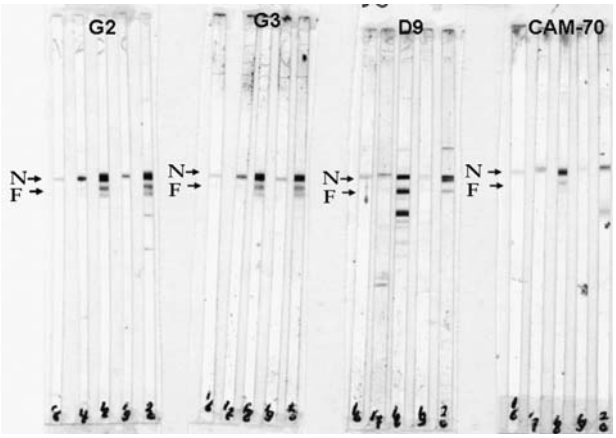

Figure 5. The result of immunoblotting of G2, G3, D9 and CAM-70 antigens reacted with serum from mice immunized with Schwarz (MMR) vaccine virus

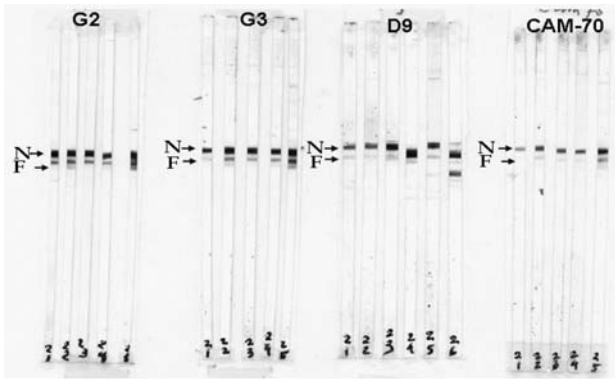

Figure 4. The result of immunoblotting of G2, G3, D9 and CAM-70 antigens reacted with serum from mice immunized with CAM-70 vaccine virus

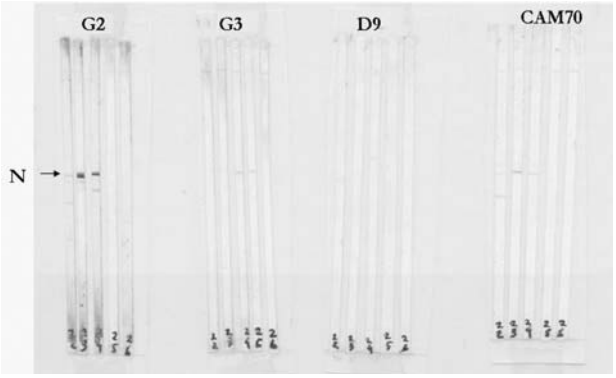

Figure 6. The result of immunoblotting of G2, G3, D9 and CAM-70 antigens reacted with serum from mice immunized with placebo

immunoblotting paper, the quite dark N protein band and very light F1 protein band. Both band showed in all four types of antigens with almost the same intensities. Bands for proteins other than N and F1 were not visible at all.

Serum from mice immunized with Schwarz vaccine virus showed even fewer bands when reacted with antigens from G2, G3, D9 and CAM-70 genotypes. Bands that appeared on the paper were those from N and F1 proteins. The band for N protein always showed up in all mice with different intensities while the band for F1 protein only showed up several times with different intensities. The bands for CAM-70 antigen were relatively thinner and fewer than bands for wild type virus antigens.

To assess the existence and intensity of bands from reaction of each serum with antigens from all genotype on Western blotting, the following criteria was used:

++++	:	complete, very dark and clear bands on all five samples
+++	:	complete, quite visible bands on all samples
++	:	incomplete, quite visible bands on all samples
+	:	incomplete, barely visible bands
-	:	no visible bands

The summary of the assessment of the result from antigen-antibody reactions on western blotting

Tabel 3. Distribution of bands of CAM-70, G2, G3, and D9 viral antigens after being reacted with antibody serum on immunoblotting

Antigen component	Sample serum anti G2				Sample serum anti G3			
	Type of viral antigen				Type of viral antigen			
	G2	G3	D9	CAM-70	G2	G3	D9	CAM-70
Polymerase 210 kd	+++	+++	+++		++	++	++	++
Glycoprotein H 78 kd	+++	++	-		++	+	+	+
Phosphoprotein 72 kd	++++	++++	++		+++	+++	+++	-
Nucleoprotein 60 kd	++++	++++	+++	++	++++	++++	+++	++
Glycoprotein F1 40 kd	++++	++++	+++	+	++++	+++	+++	++
Protein M 38 kd	+++	+++	++		++	++	+	-

Table 4. Distribution of bands of CAM-70, G2, G3, and D9 viral antigens after being reacted with antibody serum on immunoblotting

Antigen component	Sample serum anti D9				Sample serum anti CAM-70			
	Type of viral antigen				Type of viral antigen			
	G2	G3	D9	CAM-70	G2	G3	D9	CAM-70
Polymerase 210 kd	+++	+++	+++	+++	-	-	-	-
Glycoprotein H 78 kd	+++	+++	+++	++	-	-	-	-
Phosphoprotein 72 kd	+++	+++	+++	++	-	-	-	-
Nucleoprotein 60 kd	++++	++++	++++	+++	+++	+++	+++	++
Glycoprotein F1 40 kd	++++	++++	++++	+++	+++	+++	+++	++
Protein M 38 kd	+++	+++	+++	++	-	-	-	-

Tabel 5. Distribution of bands of CAM-70, G2, G3, and D9 viral antigens after being reacted with antibody serum on immunoblotting

Antigen component	Sample serum anti Schwarz			
	Type of viral antigen			
	G2	G3	D9	CAM-70
Polymerase 210 kd	-	-	-	-
Glycoprotein H 78 kd	-	-	-	-
Phosphoprotein P 72 kd	-	-	-	-
Nucleoprotein N 60 kd	++	++	++	+
Glycoprotein F1 40 kd	+	+	+	+
Protein M 38 kd	-	-	-	-

was shown on **Table 3, 4, 5**. It was obvious that bands for L, H, P and M always showed up lightly, and sometimes appeared and sometimes not appeared depending on the type of antibody in the sample and the type of antigens to be reacted. Bands for N and F1 proteins were always showed up clearly on all western blotting reaction except on antibody against Schwarz vaccine virus.

Discussion

To find the differences of the antibody profiles from each group of serum against each genotype of wild type virus and CAM-70 vaccine, immunoblotting examination was carried out. In this research, only five samples from each group with highest antibody

determined by ELISA tests were examined, hence the differences in the formed bands were very clear. Evaluation of immunoblotting was based on the existences and the intensities of particular bands as a result of the occurrences of cross reaction from each viral antigen against antibody serum from each group.

Immunoblotting examination of serum from mice immunized with G2 genotype of measles virus reacted with antigens from G2, G3, D9 and CAM-70 on nitrocellulose showed very different bands. The differences on the bands were very obvious for mice serum reacted with antigen of CAM-70 vaccine virus. Only light bands of N and F protein were appeared, while bands from other proteins were not seen. When reacted with antigens from D9 genotype, the same serum showed quite dark bands for L, N and F proteins, while bands for other proteins were pretty light. When reacted with antigens from G2 and G3 genotypes, the serum gave decent reactions with dark bands for L, H, P, N, F and M proteins. This might indicate that antibody against G2 genotype was less reactive to antigens of CAM-70, quite reactive to antigens of D9, and very reactive to antigens of G2 and G3 genotypes. This confirmed the antigenic differences between wild type measles virus and CAM-70 vaccine virus which might be affected by the differences in the antibody epitopes on each pair of proteins.

Serum from mice infected with G3 genotype showed bands very light bands for L, H, P, N, F and M proteins when reacted with antigens from G2, G3 and D9 genotypes. When reacted with antigens from CAM-70 vaccine virus, the same serum showed dark bands for N protein, quite light bands for F proteins, and very light bands for other proteins. This indicated that the serum antibody for G3 genotype cross reacted very well with antigens from G2, G3 and D9 genotypes, and reacted poorly with antigens from CAM-70 vaccine virus. This also indicated that the antigenicity profiles of wild type measles virus were different with CAM-70 vaccine virus.

Serum from mice immunized with CAM-70 vaccine virus showed comparable bands when reacted with antigens from either G2 or G3 genotype, with quite dark bands for N and F proteins while bands for other proteins were not visible. When reacted with antigens from D9 genotype, the same serum showed very dark bands for N protein and light bands for F proteins. When reacted with antigens from CAM-70, only bands for N protein appeared lightly compared to other bands for N proteins from other immunoblotting reaction. Hence it could be concluded that antibody serum against CAM-70 could only react with N and F proteins from antigens of G2 and G3 genotype, and could only react with N protein from antigens of D9 genotype. This clearly showed that antibody responses were only targeted to N and F proteins.

Serum from mice immunized with Schwarz vaccine virus showed comparable bands when reacted with antigens from G2, G3 and D9 genotypes, with uneven bands for N protein (sometimes visible, sometimes invisible) and very light bands for F protein which were not always visible. This serum only showed very few bands when reacted with antigens from CAM-70. Hence, it could be concluded that antibody against Schwarz vaccine virus was only able slightly to cross react with N and F proteins from antigens of G2, G3 and D9 genotypes and was not able to cross react with proteins from antigens of CAM-70 vaccine virus. This might be affected by the low existence of antibody responses which can only response to N and F proteins.

The differences on the immunoblotting bands might be the consequences of the differences in the amino acid sequences of the epitope as a result of differences in the nucleotide sequences of the genes. Differences in the amino acid sequences on the epitopes could cause the monoclonal antibody to be unable to bind with homolog epitope on the antigens or to decrease the affinity and making the bonds easier to break. Setiawan found several differences in the amino acid sequences of important B-cell epitopes on H and F proteins between wild type measles virus and vaccine virus in Indonesia. Differences of F protein between wild type and vaccine virus were around 29-31 amino acids, while differences of H protein were around 24-29 amino acids. This differences were quite substantial and it was predicted that these differences could change the structure of both proteins. Moreover, amino acid differences were also found on B-cell epitopes that were important in neutralizing the virus.[17,18]

Differences in the amino acid sequences and in the immunobloting profiles would lead to the differences in the cross neutralization test between

wild type virus and vaccine virus. Setiawan also found the differences in the cross neutralization tests between wild type virus (G2, G3 and D9) and CAM-70 and Schwarz vaccine virus.[19]

Birrer found differences in the epitope of H proteins between measles Edmonston-wt vaccine virus and wild type virus, by performing neutralization test using monoclonal antibody.[20] Truong also found the differences in the result of neutralization test between A, B3, D2 and D4 genotypes using monoclonal antibodies.[21] However, Zhou did not find any significant differences of the neutralization test between C1, D3, D5 and H1 against serum with high antibody titer. However, several D3 and H 1 virus could not be neutralized by antibodies with low titer.[22]

The forming of bands of antigen-antibody reaction on nitrocellulose paper (immunoblotting) could be affected by antigens that injected to mice and the product of viral purification from culture. Before injected into mice, measles virus was cultured on B95-8 cell (wild type virus). CAM-70 vaccine virus was injected straight from the bottled package from the factory (Bio Farma). Protein components that might mix in the viral antigen were protein components originated from B95-8 cell (wild type viral antigen) or Vero cell (CAM-70). In addition, other proteins that might be mixed in the viral antigen were proteins originated from serum, as during culturing the virus, calf serum was added to the media for cell growth. Hence, antibody against those proteins also formed inside mice and could affect the bands on nitrocellulose paper. The intensity on the immunoblotting could also be affected by the intensity of the antigen separation from each virus on electrophoresis. This was confirmed as the result of electrophoresis of each component of viral antigens showed different intensity.

The following conclusions can be drawn from the analysis of immunoblotting examination:

1. Antigens of CAM-70 vaccine virus were less able to cross react with antibodies of G2, G3, D9, CAM-70 and Schwarz genotypes.
2. Antibodies against CAM-70 vaccine virus were only able to cross react with antigens of N protein and a small part of F protein.
3. Wild type measles virus (G2, G3 and D9) were very immunogenic as their antibody titter were very high.
4. CAM-70 and Schwarz/MMR vaccine virus were less immunogenic as their antibody titter were very low.
5. Antibody responses that always occurred from all serum obtained from immunized mice were antibodies against N and F proteins. This agreed with the result of Grave which found the high frequency and titter of antibody serum against N protein from newly recovered measles patients, hence the antibody against N protein was being used as an indicator for diagnosing measles disease until now.

All antigen-antibody reaction on immunoblotting resulted in different profiles especially between wild type virus and CAM-70 vaccine virus. Although CAM-70 vaccine virus showed clear differences compared to G2, G3 and D9 genotypes, antibodies against CAM-70 were still able to cross react with antigens from other genotypes (G2, G3 and D9).

As CAM-70 and Schwarz vaccines were less immunogenic, it was suggested to reevaluate giving measles immunization once to children by performing clinical test to immunize twice on separate time based on the clinical and serological epidemiology.

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