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ELECTROTRANSFER (Western blot) OF FOOT-AND-MOUTH DISEASE VIRUS C3 RESENDE SUBTYPE CAPSID PROTEINS AND THEIR REACTIVITY WITH MONOCLONAL ANTIBODIES

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Capsid proteins (VP1, VP2, VP3 and VP4) of the foot-and-month disease virus C3 Resende subtype were separated by gel electrophoresis and were electro transferred (Western blot) and immobilized on the membrane. A set of 26 monoclonal antibodies was used for examination of the reactivity with the above mentioned capsid proteins, whereafter reactivity with 12 monoclonal antibodies with VP1 was recorded. No reactivity was observed with any other viral capsid protein. Differences between sequentional and conformational epitopes on the surface of the virus antigen are discussed.

Key words: Foot-and-mouth disease virus, Western blot, monoclonal antibodies.

INTRODUCTION

A blotting procedure can, in principle, be delineated as a method where affinity interactions between ligands and acceptors take place on the spot and where molecular species have been rendered accessible and maintained in a pattern depending on their physichochemical properties. The material (virus) can be separated before the transfer. The main advantages of the blotting principle are due to: (1) concentration of antigen, (2) antigen immobilization, (3) time needed for reaction, (4) economics and (5) flexibility i.e. the blots may be stored and reused. This procedure together with polyacrylamide gel electrophoresis (PAGE) and followed with monoclonal antibody reaction with PAGE separated protein (virus proteins) substructures poses a powerful tool in research on various antigens (Heegard and Bjerrum, 1988).

The somewhat semigeographical terminology used in naming DNA blotting Southern blotting after its inventor (Southern, 1975) and followed by calling RNA blotting Northern blotting and the electrotransfer of protein Western blotting, although widely used, is not recommended. For precise descriptions it is preferable to define the blotting method by stating (1) the class of molecules transferred, (2) the method of transfer and (3) the type of immobilizing matrix (membrane).

Foot-and-mouth disease virus (FMDV), an apthovirus of the picorna virus family, contains the equivalent of 60 copies each of four capsid proteins VP1, VP2, VP3 and VP4, encapsidating a single stranded RNA molecule of positive polarity (Bachrach, 1977). Seven distinct serotypes (O, A and C - so called European, SAT-1, SAT-2 and SAT-3 in South Africa and Asia-1) and more than 60 subtypes have been identified (Cheung et al., 1983). Cloven-hoofed animals are susceptible and, although the disease is not lethal in most cases (except for young animals), productivity is altered and countries where FMD is endemic suffer severe economic losses because of export restrictions.

FMD virus RNA codes for a translatory product which enzymatically splits into several large molecular weight precursors. One of them (p88) is further processed and as a results VP1, VP2, VP3 and VP4 occur. They are of utmost importance since they are the only virus proteins which are surface exposed. Of even more importance is that those proteins are the only ones capable of eliciting neutralizing immunity against whole FMDV (Meloen et al., 1979). It has been shown that animals immunized only with VP1 or VP1 fragments elicit neutralizing immunity (Bachrach, 1982). It was Strohmaier and his co-workers (1982) who analyzed fragments of VP1 obtained from chemical and enzymatic cleavages and who located immunogenic sites within amino acid regions 138-154 and 200-213. Now we know that the critical region, the so called major antigenic site for virus neutralization includes aminoacids 141-160 of VP1 and this part of the virus protein has been subject to strong pressure from neutralizing antibodies (Brown et al., 1989). Another part of VP1 at the carboxy terminus (amino acids 200-213) is also able to generate neutralizing antibodies albeit at a reduced titer (Luo et al., 1988).

With monoclonal antibodies (MAb), Köhler and Milstein (1975) gave us a tool to investigate one single epitope on the antigen surface. Since then MAbs have been in routine use in research and diagnostic work not only concerning viruses but almost all substances which can be defined as an antigen.

The aim of this work was to isolate FMDV capsid proteins on to membrane and to show their reactivity with a set of presinthesized MAbs.

MATERIALS AND METHODS

V i r u s: FMDV C3 Resende subtype was grown in Baby Hamster Kidney cells-21 (BHK-21), partly purified using a polyethylene glycol procedure (Wagner et al., 1970) and finally purified in CsCl gradient (Bachrach et al., 1964).

M o n o c l o n a l a n t i b o d i e s: The series 15 and 16 MAbs were isolated from Balb/c mice infected intraperitoneally with subimmunizing doses of virus (100 suckling mice LD_{50}) 7 and 14 days before fusion, respectively. Two days before fusion mice were inoculated with infectious virus (1000 LD_{50} and 7000 LD_{50}) for fusions 15 and 16, respectively. The fusion of cells in order to obtain hybridomas followed an already described procedure (Letchoworth and Appleton, 1984).

SDS - PAGE and electrotransfer of FMDV proteins; The method followed that of Laemmli (1970) with some differences. Briefly, 12,5% (w/v) polyacrylamide-bisacrylamide (30:0,8) gel was used as the separation gel and 5% as the stacking gel. PBS (5% BSA) was used for saturation all remaining protein binding sites on the nitrocellulose paper (Immobilon-p transfer membranes, Millipore Corp, Bedford, MA 02730, USA). The paper was cut into strips which were marked and incubated with 1/5 diluted MAb hybridoma cell supernatants (1 hour, 37^{0}C).

After washing with PBS-T (PBS-Tween 20, 0,05% and 1% BSA) strips were incubated (1 hour, 37^{0}C) with radiactively labelled (^{125}Iodogen procedure, Stave et al. 1986) Protein A (Pharmacia Inc., Piscataway, NJ USA) adjusted to 300.000 cpm per 1 ml. After washing the strips were aligned and exposed to Kodak Blue Brand X-ray film (Eastman Kodak Co.Rochester, NY) for 24 hours at-70^{0}C, using an intensifying screen.

RESULTS

Table 1 shows a list of MAbs used to characterize FMDV C3 Resende and their isotype. The firs number is a fusion number. The next letter is the plate used for hybridoma culturing. The next letter and number shows the position of the particular well from where the clone was picked out.

Table 1. List of MAbs and their isotype used to characterize FMDV.

Monocolonal antibody	Isotype	Western blot rectivity
1. 15 AC-3.1.1	IgM	-
2. 15 AG-1.1.2	IgM	-
3. 15 AH-2.1.2	IgG2b	+
4. 15 BB-12.1.1	IgM	-
5. 15 BC-12.1.1	IgM	-
6. 16 AE-12.1.1	IgG1	-
7. 16 AF-11.1.1	IgG3	+
8. 16 DE-9.1.1	IgG3	-
9. 16 HB-3.1.1	IgG3	-
10. 16 IB-4.1.1	IgG2a	-
11. 16 JB-4.1.1	IgG2a	+
12. 15 BD-3.1.2	IgM	+
13. 16 AC-1.1.1	IgG2a	+
14. 16 BE-12.1.1	IgG2a	+
15. 16 BF-1.1.1	IgG2b	+
16. 16 CB-5.1.1	IgM	+
17. 16 CB-6.1.1	IgG2a	+
18. 16 CB-8.2.1	IgM	+
19. 16 CH-3.1.1	IgG2a	+
20. 16 ID-10	IgM	-
21. 16 IE-9.1.1	IgG2a	-
22. 16 IF-9.1.1	IgG3	-
23. 15 AC-5.1.3	IgG2b	+
24. 15 AF-9.1.1	IgG1	-
25. 15 AE-12.1.1	IgG1	-
26. 16 AG-8.1.1	IgG2b	-

After checking the control gel as far as separation of virus capsid proteins in PAGE was concerned this gel (Coomassie blue stained) was aligned with the X-ray film used in the test. Figure 1 shows the specific reactivity between MAbs and VP1 separated in the gel and transfered on to the membrane.

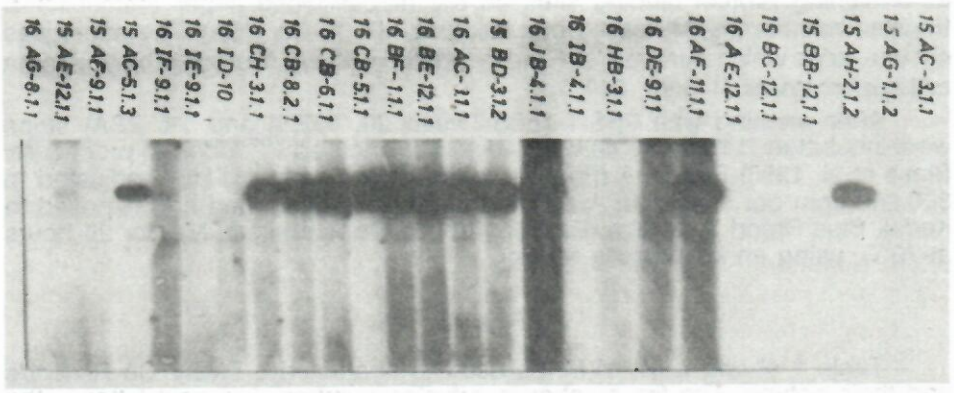

Figure 1. MAbs binding pattern in Western blot.

Despite expectations that radioactively labelled Protein A would not react with IgM immunoglobulins such a reaction occurred.

DISCUSSION

A set of 26 MAbs has been used to define their affinity for isolated capsid proteins of FMDV C3 Resende subtype. It should be noted that as a result of the first fusion (15) 50% of the clones produced immunoglobulins of the IgM isotype, an indication of a primary immune reaction. All MAbs had kappa light chains which is in accordance with already published data (Stave et al., 1986).

Electrotransfer of proteins and their immobilization is a method widely used by immunologists in order to characterize antigens. In combination with MAbs this method gives valuable data concerning the reactivity of certain antigen molecules and their importance for animal immunity. Since the method provides virtually pure peptide molecules in the case of FMDV capsid proteins, it is possible to say that significant affinity of MAbs specific for VP1 capsid protein used in the test has been registered.

Inside the structure or on the surface of a certain antigen (virus for example) there are antigenic sites which are made up of epitopes. Epitopes are, as a counterpart of paratopes, the simplest units of antigenicity and could depend exclusivelly on a continuous (primary) amino acid sequentional epitopes). However, such epitopes are in a minority comparing with conformationally dependent epitopes. The structure of such epitopes depends not only on the primary amino acid sequence but mainly on secondary and tertiary structures that influence the conformation of the molecule (conformational epitopes) (Beck et al., 1983). Since the method of separating capsid proteins

included SDS (reduced conditions) which neutralize the tertiary structure of peptid molecules, it could be concluded that all MAbs that reacted with VP1 showed affinity for sequentionally dependent epitopes (parts of the VP1 primary sequence).

It has already been published that VP1 FMDV capsid protein is crucial, since antibodies against its structure can neutralize virus infectivity (Barnett et al., 1989). The reactivity of this capsid protein with certain MAbs shows that neutralizing MAbs had been synthesized as a result of both fusions. Of course, along with sequentionally dependent epitopes one should predict the presence of conformationally dependent epitopes. Such epitopes have been shown to be more important in the phenomenon of neutralization (Grubman et al., 1985). Taking that fact into consideration, there is a possibility that some MAbs, although not reacting with sequentionally dependent epitopes, could be very effective as far as neutralization is concerned. The next step is to characterize the MAbs in order to define their reactivity with conformationally dependent epitopes and the role of VP2 and VP3 in defining the structures of such epitopes. Their amino acid chains might influence the expression and nature of both kind of epitopes on the FMDV surface.

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ELEKTROTRANSFER (Western blot) KAPSIDNIH PROTEINA VIRUSA SLINAVKE I ŠAPA I NJIHOVA REAKTIVNOST SA MONOKLONSKIM ANTITELIMA

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SADRŽAJ

Koristeći poliakrilamid gel elektroforezu izvršena je separacija kapsidnih proteina virusa uzročnika slinavke i šapa (VP1, VP2, VP3 i VP4) C3 Rezende subtipa. Ovi su proteini fiksirani elektrotransfer (Vestern blot) metodom na nitroceluloznu membranu. Ovako fiksirani proteini (na trakama) su upotrebljeni za reakciju sa 26 prethodno sintetisanim monoklonskim antitelima (MAt) pri čemu je ustanovljena reaktivnost 12 MAt samo sa VP1 kapsidnim proteinom. Nije ustanovljena reaktivnost ni jednog MAt sa ostalim kapsidnim proteinima virusa koji su bili separisani upotrebom redukcionog sistema (SDS-PAGE). Diskutovane su razlike između sekvencionalno zavisnih epitopa na površini antigena i epitopa čija struktura prevashodno zavisi od sekundarne i tercijalne strukture molekula proteina (konformaciono zavisni epitopi).