Specificity of the Monoclonal Antibody and its Application to Forensic Medicine

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INTRODUCTION

Studies on the molecular level of antibody are one of the most advanced in the fields of medicine and biology. Since antibody has a high-specific binding activity, it has been used in various fields as a qualitative and quantitative reagent. However, antiserum is a mixture of antibodies which react to different antigen determinant groups. This indicates that antiserum can be chemically impure. Many immunologists have attempted to obtain the antibody which recognizes a specific antigen determinant group. Köhler and Milstein (1975) succeeded in obtaining monoclonal antibody with cell fusion between myeloma cells and spleen cells of immunized mice.

We examined the serological specificity for blood group substances by using monoclonal antibodies (anti-A: SMA, anti-B: SMB) which were prepared by us, commercialized monoclonal antibodies (anti-A: CMA, anti-B: CMB) and commercialized polyclonal antibodies (antiserum).

MATERIALS AND METHODS

1. IMMUNIZATION AND CELL FUSION

We immunized BALB/c mice with human erythrocytes (5×10^8 erythrocytes per injection every 5 day over a period of 4 weeks), and checked the titer of antibody of the mice every week. In the 5th week, all the mice were sacrificed and the spleen cells were obtained. Spleen cells and myeloma cells were fused by the method of Schreier (1980) with some modifications. The procedures for the preparation of monoclonal antibodies were previously described (Ikeda and Nagai 1984).

2. SPECIFICITY OF THE MONOCLONAL ANTIBODIES AND POLYCLONAL ANTIBODIES

We examined the specificity among CMA, CMB, SMA, SMB and antisera using human erythrocytes (A_1, A_2, B, O, A_1B and A_2B) by agglutination reaction. Moreover, 2 % erythrocyte suspension of monkey

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(Rhesus and Cynomolgus), dog (Beagle), horse, pig, sheep, cat, rabbit, guinea-pig, mouse and hen was tested with these monoclonal antibodies.

3. APPLICATION TO BLOODSTAIN

Whole blood of human and animals mentioned above was droped on the filter paper and dried up at room temperature. Bloodstains were tested by absorption-elution method.

4. ABSORPTION TEST BY HUMAN SALIVA

CMA, CMB, SMA and SMB (1:8) were absorbed with Se and se saliva which were boiled for 30 minutes before use. After the mixture had been left at room temprature for 2 hrs. and at 4°C overnight, 2 % erythrocyte suspension was added to the mixture, and the activity of the monoclonal antibodies was examined.

5. AGGLUTINATION INHIBITION TEST BY MONOSACCHARIDE

10 mM N-acetyl-D-galactosamine was added to CMA and SMA (1:8), but 10 mM D-galactose was added to CMB and SMB for absorption. After the mixture had been left at room temperature for 2 hours and then at 4°C overnight, the activity of monoclonal antibodies was measured by using 2 % erythrocyte suspension.

6. 2-MERCAPTO-ETHANOL TREATMENT

CMA, CMB, SMA and SMB were treated with 2-mercapto-ethanol in the same method as described by Grubb (1958).

7. ANALYSIS OF THE MONOCLONAL ANTIBODY BY GEL-DIFFUSION METHOD

The immunogloblin type of CMA, CMB, SMA and SMB obtained in our study was determined by micro-Ouchterlony method (1958).

8. EXAMINATION OF THE LOCALIZATION OF BLOOD GROUP SUBSTANCE IN THE TISSUES

The localization of blood group antigen in human tissues was investigated by mixed-cell-agglutination reaction (MCAR) method using all monoclonal antibodies and antisera.

RESULTS AND DISCUSSION

The specificity of the CMA, CMB, SMA and SMB which are biologically homogenous was investigated. Commercialized anti-A serum (polyclonal antibody) agglutinated erythrocytes of human A_1, A_2, A_1B, A_2B as well as pig, sheep, rabbit, cat, monkey (Rhesus and

Table 1. Comparison of agglutination reaction of antiserum (polyclonal antibody) and monoclonal antibody against erythrocytes of human and various animals

Erythrocyte	Number of Examples	Anti-A Serum[1)]	Monoclonal Anti-A Antibody[2)]	Anti-B Serum[1)]	Monoclonal Anti-B Antibody[2)]
Human A_1	100	+++	+++	-	-
A_2	8	+	+	-	-
B	100	-	-	+++	+++
O	100	-	-	-	-
A_1B	100	+++	+++	+++	+++
A_2B	6	+	+	+++	+++
Monkey:					
Rhesus	5	+	-	+	-
Cynomolgus	5	+	-	+	-
Dog:					
Beagle	5	++	-	+++	-
Horse	5	++	-	-	-
Pig	10	+++	+~++	+++	-
Sheep	5	++	-	-	-
Cat	5	++	-	++	-
Rabbit	20	+++	-	+++	+++
Guinea-pig	10	-	-	++	-
Mouse	20	-	-	++	-
Hen	3	-	-	-	-

1: Ortho Diagnostics Inc.

2: Seraclone®, Biotest-Serum-Institut GmbH and prepared by us

Agglutination grade: +++, Strongly positive; ++, Moderately positive; +, Weakly positive; -, Negative

Cynomolgus) and dog (Beagle) erythrocytes whereas the CMA and SMA agglutinated only human and pig erythrocytes. Similarly commercialized human anti-B serum agglutinated erythrocytes of human B, A_1B, A_2B and pig, rabbit, dog, guinea-pig, mouse and monkey erythrocytes. CMA and SMA agglutinated only human and pig erythrocytes, and CMB and SMB agglutinated human and rabbit erythrocytes, showing that the monoclonal anti-A and anti-B antibodies have extremely high specificity, compared with the commercialized polyclonal antibodies (Table 1).

The carbohydrate chain of pig erythrocytes and human blood group A antigen is quite similar. That of rabbit and human blood group B is also very similar.

We examined the agglutinin activity of CMA by using different phenotypes, A_1 and A_2 human erythrocytes. It was obserbed that group A_1 was higher than group A_2 in the agglutination reaction. Group A_1 was also higher than group A_1B. A similar result was obtained when commercialized anti-A serum and SMA were used. These results suggest that high agglutinin titer or low agglutinin titer is related to the number of the receptor of blood group substance which is localized on the surface of erythrocytes.

The titer of CMA was lost after absorption with human secretory group A saliva, and the same result was observed for CMB and human secretory group B saliva. The activity of CMA and SMA was lost after absorption with N-acetyl-D-galactosamine, and that of CMB and SMB were also lost with D-galactose.

CMA, CMB, SMA and SMB produced a single precipitation band with anti-mouse-IgM by the micro-Ouchterlony method. The results in this study also demonstrated that the monoclonal antibodies are IgM molecules.

On the examination of bloodstain, CMA and SMA were strongly reacted with human bloodstain of A and AB, but SMA was weakly reacted with pig bloodstain. These results suggest that if we use monoclonal antibodies for examination of bloodstain, we must check the specificity of monoclonal antibodies and the origin (human or animals) of bloodstain for successful wark of forensic medicine.

In this study, CMA, CMB, SMA and SMB were used for examination of the localization of blood group antigens in esophagus mucous membranes by using MCAR method. It was clearly confirmed that the blood group antigens are localized in squamous cells of esophagus mucous membranes (Fig. 1a and 2a). The distribution of blood group substance in serous gland was examined by using CMA, CMB, SMA SMB and antisera. When the titer of monoclonal antibodies and antisera was more than 1:128, MCAR in serous gland was positive. MCAR in serous gland was negative on the use of antisera (the titer 1:64), but that was positive on the use of monoclonal antibodies (the titer; 1:64)(Fig. 2a and 2b).

The difference of specificities of monoclonal antibodies and antisera can be useful for the study on localization of blood group antigens in various tissues and monoclonal antibodies will be more useful in forensic serology in the future.

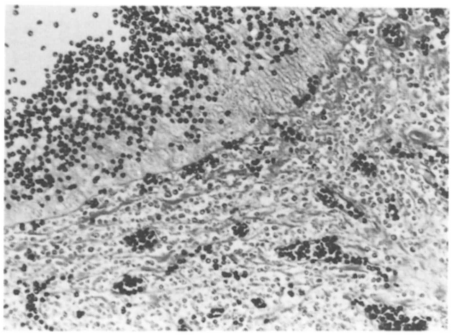

Fig. 1a

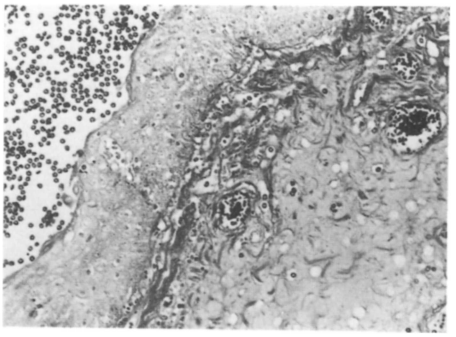

Fig. 1b

Fig. 1 Monoclonal anti-A (CMA) was used for examination of the localisation of A-group substance in esophagus mucous membranes by using MCAR method; magnification, 1x40. 1a: positive reaction. 1b: negative reaction.

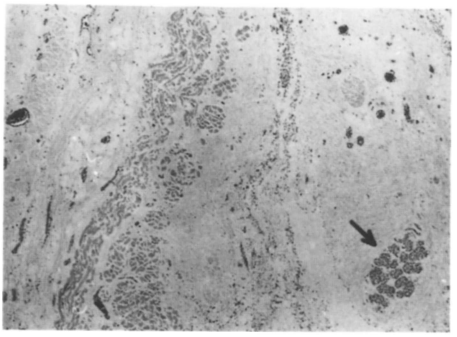

Fig. 2a

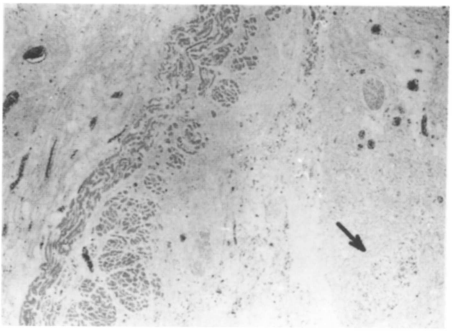

Fig. 2b

Fig. 2. Localisation of B-blood group substance in serous gland by using MCAR method; magnification, 1x40. 2a: MCAR in serous gland was positive reaction on the use of monoclonal anti-B (CMB. titer: 1:64). 2b: negative reaction on the use of anti-B serum (1:64).

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