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Detection of Antigen and Antibody using IgG Fc Linked Hemoagglutination (IFLHA)

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Abstract After refrigeration-fusion of macrophage in mouse ascites, IgG Fc receptor (FcR) on the surface of macrophage is linked to glutaraldehydized sheep red blood cell.
Antigen or antibody is detected by reaction between FcR linked to glutaraldehydized sheep red blood cell and antigen-antibody complexes. This method has high specificity and sensitivity.
Key words antigen, antibody, detection, IgG FC linked hemoagglutination

Introduction

The great leader Comrade **Kim Il Sung** said as follows.

"Our scientists must study subjects which relate to the situation in our country and endeavour to create things which are needed by our people."("**KIM IL SUNG** WORKS" Vol. 19 P. 338)

There are many diagnosis methods for animal infectious diseases such as ELISA, fluorescent antibody test, neutralization test, precipitation test, hemoagglutination[4－6]. These methods are based to prove specific reaction between antigen and immunogloblin Fab.

Immuno-adherence hemoagglutination (IAHA) is based on the principle that antigen-antibody complexes are linked to C_3b receptor on the surface of animal's white blood cell or primates's erythrocyte. It needs human's blood cell and guinea pig's serum[1, 2, 7].

Until now there are no studies on antigen-antibody reaction using linkage between Fc receptor on the surface of macrophage or B cell and antigen-antibody complexes[3].

We studied new method to detect antigen or antibody by reaction between Fc receptor on the surface of refrigeration-fusion macrophage and antigen-antibody complexes.(Fc receptor is linked to glutaraldehydized sheep red blood cell)

1. Materials and Methods

1.1. Materials

Sheep red blood cell, macrophage in white mouse ascites, antigen(RHDV, CSFV, IBDV), specific antibody corresponding to certain antigen, 12～15g mouse are used.

1.2. Methods

1.2.1. Aldehydization of sheep red blood cell

Sheep red blood cell (SRBC) was fixed and aldehydized by glutaraldehyde[1, 2].

1.2.2. Refrigeration-fusion of mouse ascites macrophage

3% starch solution was injected into mouse abdomen by 0.5mL per a head. After 24 hours, ascites was obtained. It was centrifuged and washed with physiological salt water and diluted as 10^7～10^8 cells per a milliliter. This solution was refrigerated and fused 30 times in liquid-nitrogen and its centrifugal upper-liquid was used to linkage.

1.2.3. Linkage of macrophage FcR to aldehydized SRBC

1mL of aldehydized 10% SRBC (pH 8.0) is mixed with 1mL of refrigeration-fusion macrophage and reacted at 18～20℃ or 37～38℃ for 1～2h. Then the mixed solution was centrifuged with physiological salt water 3～4 times. After that, precipitation is diluted with PBS solution as 1% and used to detection of antigen or antibody.

1.2.4. Reaction method

For detection of antigen, antigen is diluted in series in micro plate and the same amount as antigen of antibody is dropped in each hole of it. After reaction for 20 or 30min at 18～20℃ or 37℃ incubator, 1% linked FcR–SRBC(the amount is same as antigen) was dropped in each hole. After 1～2 hours at 18～20℃ or 37℃, the result is estimated.

The index is formation of agglutination.

For detection of antibody, antibody is diluted in series in micro plate and same amount of antigen is dropped.

The remained procedure is same as antigen detection.

2. Results and Discussion

2.1. Optimal condition for linkage SRBC to FcR

2.1.1. The rational rate for linkage SRBC to refrigeration-fusion macrophage

After different amount of SRBC was mixed with refrigeration-fusion macrophage of different protein concentration equally, the sensitivity of reaction was investigated (Table 1).

Table 1. Agglutination sensitivity according to content of aldehydized SRBC and protein concentration of refrigeration-fusion macrophage

Content of SRBC/%	Protein concentration of refrigeration-fusion macrophage/%				
	0.01	0.02	0.04	0.08	0.16
5	256	512	1 024	2 048	2 048
10	256	1 024	4 096	4 096	4 096
15	128	256	256	1 024	−
20	32	256	−	−	−

− self-agglutination, RHDV detection reaction

Table 1 shows that reaction sensitivity is the highest when the content of SRBC is 10% and protein concentration of refrigeration-fusion macrophage is 0.04% for linkage.

2.1.2. The suitable pH for linkage SRBC to refrigeration-fusion macrophage

The sensitivity of specific agglutination was investigated under different pH condition, after 10% aldehydized SRBC were mixed with 0.04% of protein concentration refrigeration-fusion macrophage (Table 2).

Table 2. Agglutination sensitivity according to pH for linkage

pH	7.0	7.5	8.0	8.5	9.0	9.5
Sensitivity/times	512	1 024	4 096	4 096	2 048	128

The result shows that the suitable pH for linage is 8.0～8.5.

2.1.3. The rational temperature and time for linkage SRBC to refrigeration-fusion macrophage

Under the condition of different temperature and time, the sensitivity of specific reaction was investigated after linkage SRBC to refrigeration-fusion macrophage FcR (Table 3).

Table 3 shows that linkage temperature and time are 2h at 18～22℃ and 1h at 28～30℃ or 37～38℃ respectively.

Table 3. Sensitivity of specific reaction according to linkage temperature and time

Temperature	Time/h				
/℃	0.5	1	1.5	2.0	2.5
4～10	128	256	256	1 024	1 024
18～22	512	512	2 048	4 096	4 096
28～30	1 024	4 096	4 096	4 096	4 096
37～38	1 024	4 096	4 096	4 096	4 096

2.2. Specificity and sensitivity of IgG Fc receptor Linked Hemoagglutination (IFLHA)

2.2.1. Specificity

After specific or cross-reaction of different antigen and antibody, the specificity was investigated.(Table 4)

Table 4. Specificity of IFLHA

Antigen	Antibody		
	RHDV	CSFV	IBDV
RHDV	+	−	−
CSFV	−	+	−
IBDV	−	−	+

Table 4 shows that IFLHA has very specificity because antigen only reacts on the certain antibody specially, not cross-reaction.

2.2.2. Sensitivity

Sensitivity of IFLHA was compared with several serological tests, the result is as following (Table 5).

Table 5. Sensitivity of antigen-antibody detection according to tests (times)

Tests	Groups					
	RHD		CSF		IBD	
	Antigen	Antibody	1	2	1	2
IFLHA	4 096	1 024	2 048	512	1 024	1 024
HA	1 024					
HAI		512				
ELISA			2 048	1 024		
PT					8	4

Table 5 shows that the sensitivity of IFLHA is same or a little lower than ELISA, but is far higher than other tests. That is, it shows that IFLHA has high sensitivity.

2.3. Discussion

There are many serological tests to detect antigen-antibody and diagnose infectious diseases. According to methods, sensitivity, specificity, rapidity and simplify, equipments, instruments and reagents of test are different.

There is no study on serological test by using the principle that Fc of antigen-antibody complexes link to FcR on the surface of macrophage.

We studied on new serological test to detect antigen or antibody using linkage SRBC to FcR on the surface of refrigeration-fusion mouse macrophage.

The SRBC for FcR linkage can detect all kinds of antigen or antibody specially. And it has high reappearance and accuracy.

Because it doesn't need special equipments and instruments in test and it is rapid, this test can be used in practice possibly.

Conclusion

1) The optimal condition for linkage SRBC to FcR is as follows.

Content of SRBC is 10%, protein concentration of refrigeration-fusion of mouse macrophage is 0.04%, linkage pH is 8.0～8.5, linkage temperature and time are 2h at 18～22℃, 1h at 28～30℃ or 37～38℃ respectively.

2) IFLHA is very specific and sensitive.

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