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Antiidiotypic antibody related to the 84 kD human sperm membrane protein

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ABSTRACT

Wistar rats were inoculated with purified YWK-I antibody. The anti-idiotypic antibodies were isolated from rat sera by successive passage over affinity chromatography columns of YWK-I mAb and normal mouse Igs. Specificity of anti-Id antibody was established by ELISA. The 84 kD protein inhibited the binding of anti-Id to YWK-I mAb, but failed to repress antibody against normal mouse Ig binding to YWK-I mAb. In competitive inhibition assay, 84kD protein had shown the ability to compete with anti-Id binding to YWK-I mkb in a dose-dependent manner. Crude sperm extract showed a lower competitive ability. No effect was found with the irrelevant 36 kD sperm protein. The antisera from the Balb/c mice immunized with Aid contained Ab3 that reacted with 84 kD sperm protein. The binding of anti-Id to YWK-I mAb was inhibited by Ab3 in a dose-dependent fashion and Ab3 was shown to be able to induce human sperm agglutination. These results indicate that anti-Id which may mimic an epitope of the 84 kD protein could be exploited as an antigen to raise antibodies against sperm protein.

Key words: *YWK−I mAb, anti−(anti−Id), internal image*

INTRODUCTION

At the turn of the century, Landsteiner and Metehnikoff first demonstrated that antibodies to spermatozoa could be induced experimentally. Furthermore, high titers of anti-sperm antibodies were demonstrated in the sera and reproductive tract of some infertile men and women. Therefore the occurrence of anti-sperm antibodies appsars to be causally related to infertility. According to these observations, it seems feasible to develop contracep five vaccines composed of specific sperm components ([1−3] Jones, 1986; Anderson *et al*., 1983; Talwar, 1987). Whether for precise analysis of the unique sperm antigen struc-

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ture and function or for application purpose of utilizing sperm component as contraceptive vaccine, in view of substantial practical consideration it seems impossible to get enough quantities of sperm antigens from native sperm. More recent attention has been paid to the potential application of the network concept for producing contraceptive vaccine ([4-7]Kuo et al., 1988; Carron et al., 1988; Taussig et al., 1986; Wang et al., 1988).

In our previous studies, a monoclonal anti-human spermantibody, termed YWK-I has been isolated. The YWK-I mAb can elicit sperm agglutination and interact with 84 kD sperm membrane protein ([8−107) Yan et al., 1983, 1984, 1986a) This antibody significantly in hibited human sperm to penetrate zona-free bamster eggs ([11]Wang et al., 1987) and invitro fertilization. The present report describes the generation and characterization of rat anti-Id angainst YWK-I mA.b. The rat anti-Id was further puritied by immunoaffinitychromatography and used for preparing anti-(anti-Id) antibody, Ab3.

MATERIALS AND METHODS

Animals and materials

Balb/c mice and Wistar rats were supplied by animal facilities of Shanghai Institute of Cell Biology Academia Sinica. CNBr-activated Sepharose 4B and DEAE-Sephacel were purchased from Pharmcia Fine Chemical. Peroxidase-conjugated goat anti-rat Igs and peroxidase conjugated sheep anti-mouse Igs were obtained from Cappel Laboratory. DMEM medium and adjuvant were the products of GIBCO Laboratory. Hepama I mAb against hepatoma antigen was kindly presented by Dr. Xie Hong and G5 mAb against non-histone protein by Dr. Jinde YWK-I, 3F3, 58, 73 were mouse monoclonal anti-sperm antibodies.

Prepar ation and purification of YWK-I mAb

The immunoglobulins were precipitated from culture medium of YWK-I cells or ascitic fluids with ammonium sulfate at 40% of saturated concentration, and further purified by ion-exchange chromatography on a DEAE-Sephacel column. The active material was eluted with gradient sodium chloride.

Preparation of anti-idiotypic antibody

Purified YWK-ImAb was emulsified with complete Freund's adjuvant, and administered by multiple subcutaneous and paw injections to Wistar male rats at 700 μ g per rat. An emulsified YWK-I mAb with incomplete Freund's adjuvant was administered intraperitoneally 2 weeks later. After a lapse of a month and 8 days prior to bleeding, a final booster injection without adjuvant was administered.

Isolation of anti-idiotypic antibldy

Affinity columns (0.8×4.5cm) of Sepharose 4B conjugated with YWK-I antibody or normal mouse Igs (nMIg) were prepared according to the manufacture's instruction. Aliquot of 10 ml of rat antisera against YWK-I antibody was applied to the affinity column. The column was washed with PBS and eluted subsequently with 0.2M Glycine, pH 2.8. Fractions of 1 ml per tube were collected at a flow rate of 12ml per hour. The

fractions eluted with Glycine were pooled and neutralized with 0.2 *M* Tris-HCl, pH 8.0. The pooled fraction was dialyzed against PBS and concentrated by ultrafiltration method. The concentrated fraction was then applied to an affinity column of nMIg-conjugated Sepharose 4B. Void fractions and those eluted with Glycine were collected separately.

Preparation of anti-(anti-Id) antibody

Anti-(anti-Id) antibody to anti-Id was generated by similar procedure of immunization. Balb/c mice were immunized by subcutaneous injections of purified anti-Id emulsified with complete Freund's adjuvant. Anti−Id emulsified with incomplete Freund's adjuvant was administered subcutaneously one week later. Subsequently, anti-Id was administered intraperitoneally weekly for 5 weeks without adjuvant. Antisera were isolated and stored at −20℃ until use. For controls, antisera were isolated from Balb/c mice immunized with nMIg or partially purified normal rat sera.

Microplate enzyme-linked immunoassay

ELISA was performed according to the procedure described previously ([12) Yuan et al., 1986b). Wells of microtiter plates were coated with 50 μl of antigen dissolved in Na_2CO_3 $NaHCO_3$ buffer (50m*M*), pH 0.6) by overnight incubation. A fter washing with PBS/Tween 20, free binding sites were blocked with PBS/BSA at 37℃ for one hour, and the plates were washed with PBS/Tween. Samples of antibody were added and incubated at 37℃ then washed again. Peroxidase-conjugated second antibody was added to each well. After washing, the calorimetric enzyme reaction was initiated by adding 0.04% OPD in 50 m*M* Citric-phosphate, and 0.003% H_2O_2. The plates were incubated at 37℃ for 45 minutes and the reaction was stopped with the addition of 1 *M* H_2SO_4. Absorbance of each well was measured at 492 nm.

ELISA inhibition assay

Serial dilutions of the 84 kD human sperm membrane protein (HSMP) were incubated with 0.1 μg of YWK-I mAb absorbed on wells of microplate at 37 for 2 hours. Following three washings with PBS/Tween, 50 μl of 2 μg/ml anti-Id, nMIg or non-absorbed proteins from first affinity column were added and incubated for half an hour. After three washings, peroxidase-conjugated goat anti-rat Igs antibody were added and then the colorimetric enzyme reaction was developed.

Another inhibition assay was done as following: 50 μl of 2 μg/ml of anti-Id was added to each well coated with YWK-I mAb. Following incubation and washings with PBS/Tween, serial dilutions of the 84 kD protein, crude human sperm extract, or an irrelevant 36kD sperm protei were added and incubated for 2 hours. Peroxidase-conjugated goat anti-rat Igs antibody was then added, and the colorimetric enzyme reaction was developed.

In additional experiments, mixture of A b3 and anti-Id were incubated at 37 for half an hour. Then the mixture was added to the wells coated with YWK-I mAb. The subsequent procedure was the same as above.

Sperm agglutination assay

Human sperm agglutination assay was described previously ([8] Yan et al., 1983).

RESULTS

Purification of YWK-I mAb

YWK-I mAb was purified from ascitic fluid by preoipifation with 40% of saturafed ammonium sulfate followed by ion-exchange chromatography on DEAE-Sephacel column. A major peak of immunoglobulin was eluted with a gradient of 0−300 m*M* sodium chloride.

Purified YWK-I mAb was analyzed by SDS-PAGE under reducing and non−reducing conditions. The mAb migrated under nonreducing condition as a single band with an apparent MW of 150kD, and under reducing condition, two bands, corresponding to heavy and light chains of IgG, were found (data not shown).

Generation and isolation of Anti-Id against YWK-I mAb

After successive immunizations, antisera against YWK-I mAb were raised in Wistar rats. The tilers of specific antibodies against YWK-I mAb were determined by ELISA. Using YWK-I Sepharose 4B affinity chromatography column, the rat immunoglobulins reacting with YWK-I mAb were isolated following elution with Glycine-HG1, pH 2.8. The first peak eluted with PBS contained irrelevant proteins. The purified immunoglobulin fractions were then applied to the column of Sepharose 4B-conjugated with normal mouse Igs. Fractions ehted with PBS contained anti-Id antibody, and the second peak eluted with Glycine, pH 2.8, contained Igs recognizing not only YWK-I mAb but also nMIg, suggesting that anti-nMIg antibodies reacted with determinants of constant region of immunoglobulin common to YWK-I mAb and nMIg.

The purity of anti-Id and anti-nMIg antibodies were analyzed by SDS-PAGE. Both showed single band under non-reducing condition by coomassie blue staining (data not shown), From 1ml of rat antisera, about 0.12 mg of anti-Id and 0.25 mg of anti-nMIg antibodies could be obtained.

Characterization of anti-ld antibody

The binding specificity of anti-Id antibody was established by measuring the reactivity of anti-Id with YWK-I mAb, irrelevant mAbs (Hepama I, 58, 73, 3F3 and G5), and normal mouse Igs. The anti-Id eluted with PBS from Sepharose 4B-conjugated with nMIg reacted with YWK-I mAb only, whereas rat antisera against YWK-I mAb, the fractions eluted by Glycine from Sepharose 4B-conjugated with YWK-I mAb, and anti-nMIg antibody fractions reacted with all of these mAbs and nMIg. The irrelevant fractions failed to bind these coated antibodies (Table 1).

Inhibition of binding of anti-ld to YWK-I mAb by the 84 kD HSMP

Anti-Id antibody binding to YWK-I mAb was inhibited by the 84 kD protein in a dose-dependent fashion, whereas the 84 kD protein failed to inhibit anti-nMIg antibody binding to YWK-I mAb. The irrelevant proteins did not react with YWK-I mAb (Fig. 1).

Table 1. Binding specificity of anti-idiotypic antibody

Coated Antigens	YWK-I Antisera	YWK-I Conjugated Sepharose 4B Eluted with PSB (pH 7.4)	YWK-I Conjugated Sepharose 4B Eluted with GLYCINE (pH 2.8)	nMIg Conjugated Sepharose 4B Eluted with PBS (pH 7.4)	nMIg Conjugated Sepharose 4B Eluted with GLYCINE (pH 2.8)
YWK-I, IgG1	2.09±0.05	0.09±0.01	1.81±0.02	1.41±0.05	1.77±0.05
Hepama I, IgG1	0.74±0.03	0.05±0.01	0.43±0.01	0.03±0.02	0.66±0.03
58, IgG	0.60±0.03	0.04±0.02	0.36±0.05	0.01±0.01	0.42±0.02
73, IgM	0.81±0.03	0.06±0.02	0.32±0.02	0.04±0.02	0.59±0.08
3F3, IgM	0.59±0.03	0.04±0.01	0.30±0.05	0.04±0.01	0.57±0.04
G5, IgE	0.60±0.02	0.05±0.01	0.40±0.01	0.01±0.01	0.36±0.03
nMIg	1.63±0.08	0.65±0.06	0.96±0.05	0.02±0.01	1.82±0.05

Binoing of anti-Id to YWK-I mAb, a panel of other monoclonal anti-sperm antibodies (3F3, 58, 73), monoclonal antibodies against other proteins (G5, Hepama I), and normal mouse immunoglobulins, was determined by ELISA. The values represent the mean of quadruplicate determinations.

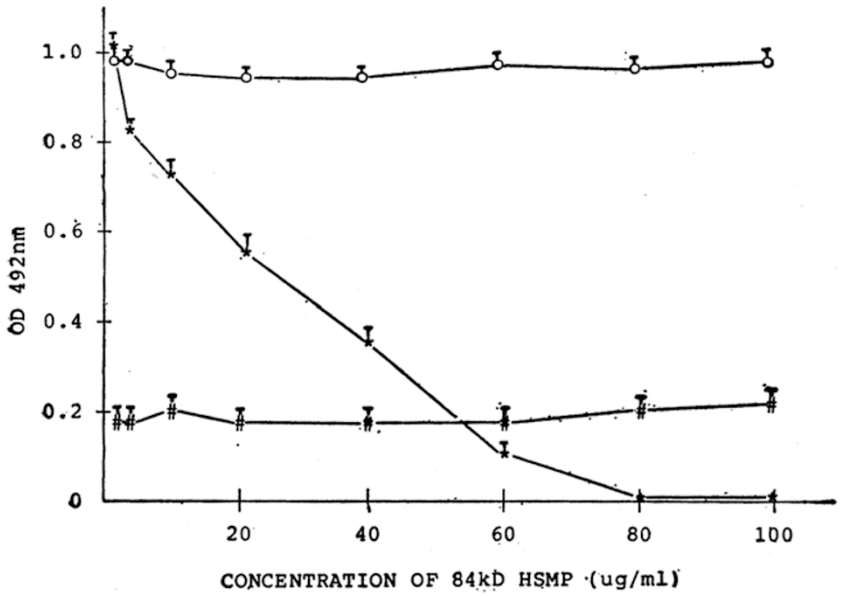

Fig. 1 Inhibition of the binding of the anti-Id to YWK-I mAb by the 84 kD human sperm membrane protein was determined by ELISA. The 84 kD HSMP inhibited the binding of anti-idiotypic antibody (*), but not anti-nMIg antibody (○), to YWK-I mAb. The proteins from first peak of YWK-I affinity column did not react with YWK-I mAb (#). Each point represents the mean of quadruplicate determinations (+SD).

Competitive inhibition of binding of anti-Id to YWK-I mAb by the 84 kD HSMP

The binding of anti-Id to YW K - I mAb was markedly inhibited by the 84 kD protein in a dose-dependent manner, with a maximal inhibition of 80%. The crude extract of human sperm membrane showed a lower inhibitory activity. No effect was found with irrelevant 36 kD sperm protein (Fig. 9).

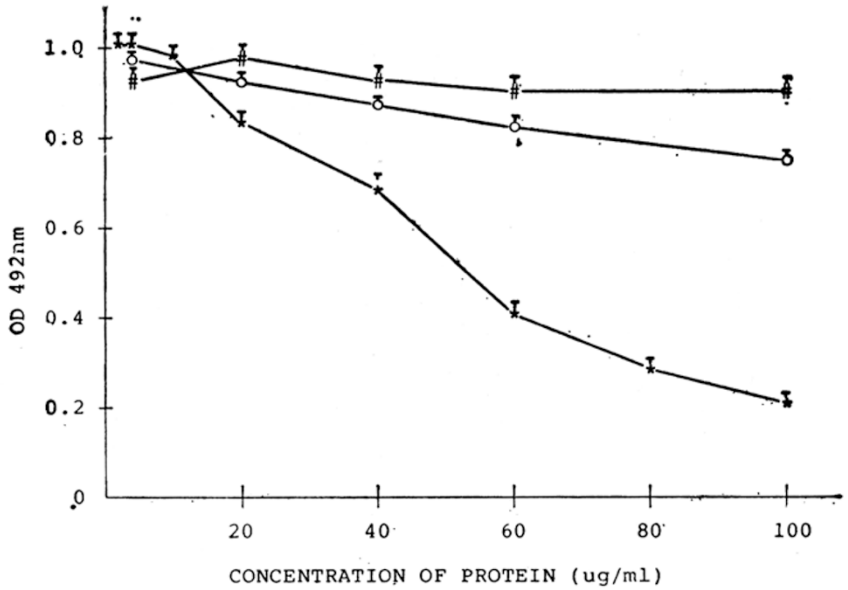

Fig. 2 Competitive inhibition of the binding of anti-Id to YWK-I mAb by the 84 kD human sperm membrane protein was determined by ELISA. The 84 kD HSMP (∗), but not irrelevant 36 kD sperm protein (#), inhibited the anti-ld binding to XWK-I mAb. The total human sperm extract (◦)shewed a lower inhibition. Each point represents the mean of quadruplicate determinations (+SD).

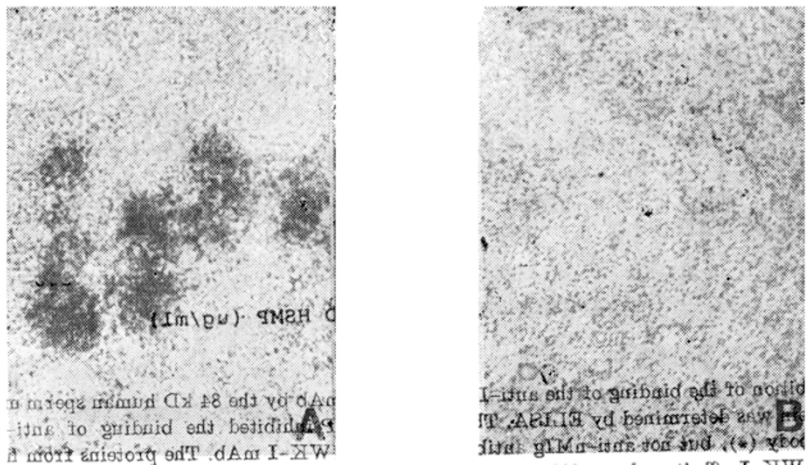

Fig. 3 Sperm agglutination induced by Ab3 (A) Negative control (B). ×100

Generation and characterization of anti-(anti-Id) antibody, Ab3

17 male and female Balb/c mice were divided into three groups immunized with different doses of anti-Id (10, 25 and 50 μg). The immunoreactivity of Ab3 to the 84 kD protein was determined by ELISA. Antisera from 11 mice positively reacted with the 84 kD protein (OD 492nm >0.2). Preimmune sera of the mice were used as control(Table 2).

Antisera from 8 mice were found capable of inducing human sperm agglutination. YWK-I mAb was used as positive control and Baker's buffer, normal mouse sera and preimmune sera were used as negative controls (Fig. 3, Table 2).

Table 2. Characterization of anti-(anti-Id)antibody, Ab3

Balb/c mice		Immunized with 10μg AId		Immunized with 25μg AId		Immunized with 50μg AId	
Sex	Group	Reacting with 84 kD HSMP	Human sperm agglutination	Reacting with 84 kD HSMP	Human sperm agglutination	Reacting with 84 kD HSMP	Human sperm agglutination
	A	-	+	-	-	+	+
Male	B	+	-	-	-	-	-
	C	+	+	-	-	+	+
	D	+	+	+	-	+	-
Femae	E	+	-	+	+	+	+
	F			+	+	-	-

The results were determined by ELISA. 50μl of a 1:500 dilution of anti-idiotypic antisera was tested. The OD 492nm absorbance over 0.2 was considered positive.

Inhibition of the binding of anti-Id to YWK-I mAb by Ab3

Anti-Id antibodies were incubated with serial dilutions of anti-(anti-Id) antisera for half an hour. The mixtures were then added into the wells precoated with YWK-I mAb. It was found that the absorbance values decreased as the concentrations of Ab3 antisera increased. These results indicated that anti-Id binding to YWK-I mAb was inhibited by Ab3 antisera in a dose-dependent manner. Ab3 antisera from the mice immunized with 10μg anti-Id showed the highest inhibitory activity. A complete inhibition was observed at a dilution of 1:16 of antisera from mice immunized with 10μg of anti-Id. Mouse antisera against normal rat Igs did not show any inhibitory effect, A mixture of normal rat Igs and Ab3 did not react with YWK-I mAb (Fig. 4).

DISCUSSION

Jerne ([13,14]Jerne, 1982, 1984) proposed that the immune system as a homeostatiic network which, in essence, is maintained by the interaction of idiotypes and complementary an tiidiotypes. Amplo experimental evidences now exist of the presence of antiidiotypes in

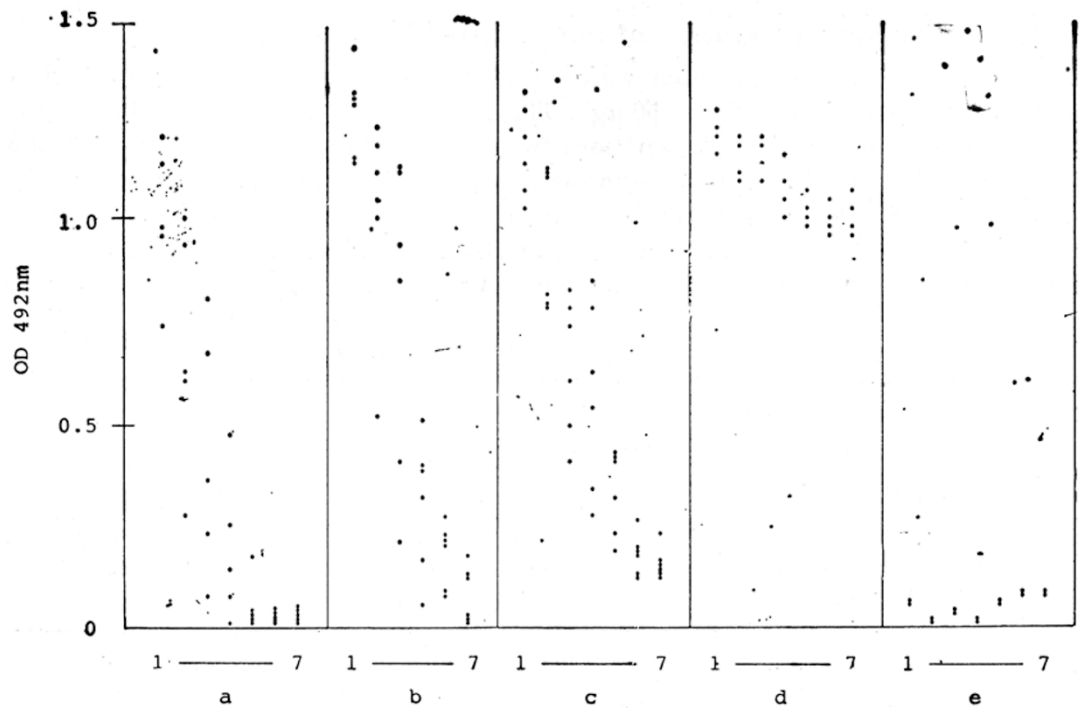

Fig. 4 Inhibition of the binding of anti-Id to YWK-I mAb by Ab3 was determined by ELISA. 1-7 means a serial dilution of Ab3 antisera (1:256, 128, 64, 32, 16, 8, 4,). a, b, c: Anti-Id mixed with antisera of Balb/c mice immunized with 10, 25, 50 fig purified anti-Id, respectively, d: Anti-Id mixed with antisera of mice immunized with 10μg normal rat immunoglobulins, e: Normal rat immunoglobulins mixed with antisera of mice immunized anti-Id. Each point represents the mean of duplicate determinations.

normal responses and of their roles in the regulation of antibody synthesis. According to present elassifieation of subclasses adopted ([15, 16] Kohter, 1985; Bona, 1984), three types of unti-Id Abs eould be distinguished: Ab2α, Ab2β and Ab2γ,. Ab2α binds to a determinant on Ab1 that is antigen binding site unrelated. Ab2T binds to a dete rminant that is binding site related or very close to the paratope. However, it could not mimic an antigenic determinant to fit the paratope. In other words, they are not internal images of external antigen. Ab2β binds also to paratope, but though its idiotype to the binding site of Abl, mimioing an antigen binding to the paratope. They are internal images of external antigen. With respect to network hypothesis, Ab2γ has ability to inhibit partially the binding of YWK-I mAb to the 84 kD protein, however Ab2γ, is different from Ab2β , since Ab2γ does not mimic an antigenic structure which fits the paratope of YWK-I mAb. The present study suggests that anti-Id antibodies could react with determinants located closed to, or within the antigen binding site of the monoclonal anti-sperm antibody, YWE-I, which induces human sperm agglutination and reacts with 84 kD human sperm protein. Polyclonal rat anti-Id raised against YWE-I mAb was isolated from rat sera. The binding specificity studies indicated the anti-Id was specific for YWK-I mAb, and not for other mAbs and nMIg. Furthermore, the purified 84 kD protein was shown to be able to inhibit specific binding of anti-Id to YWE-I mAb, suggesting that the anti-Id might contain Ab2β or

Ab2 γ,. It has been suggested that the Ab3 in response to tmmunization with Ab2 β may contain a type of immunoglobulin which is able to bind antigen although it did not necessarily share idiotypes with Ab1 ([17] UytdeHaag, 1986). In the present study, Balb/c mice were immunized with anti-Id and positive immunoresponses were shown by ELISA. The responses seemed to vary significantly among individual mice. Among antisera from 17 mice, sera from 11 mice (9 female and 2 male) showed positive reaction with 84 kD protein, with sera samples showing negative reaction in 2 females and 4 from males. Eight sera samples from 17 immunized mice were able to elicit human sperm agglutination: The result is in consisetence with the data from immunoreaction with 84 kD protein by ELISA, except one sample serum. The present results show that Ab3 which not only reacted with the 84 kD protein but also induced sperm agglutination as YWK-I mAb is similar or identical to YWK-I mAb in functional ways.

The inhibition of binding of anti-Id to YWK-I mAb by Ab3 indicates that Ab3 sera may contain a certain type of antibody which could inhibit the binding through reacting with the determinant of anti-Id. The inhibition efficiency by Ab3 seemingly varied with immunization dosage of anti-Id. Higher inhibition efficiency was obtained by Ab3 from low dosage immunized mice. The mechanism is unknown. However, Similar results have been reported.

The present results suggest that Ab2 β anti-YWK-I-Id represents a particular antibody which carries the internal image of 84 kD protein. Since it could mimic the epitope of antigen, it is suggested that the Fab portion of anti-YWK-I-Id should have a similar three dimensional structure of 84 kD protein. Therefore it is possible to utilize anti-Id as substitute for sperm antigen to resolve the problem of its shortage of supply and to raise antibodies in quantity against sperm protein.

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