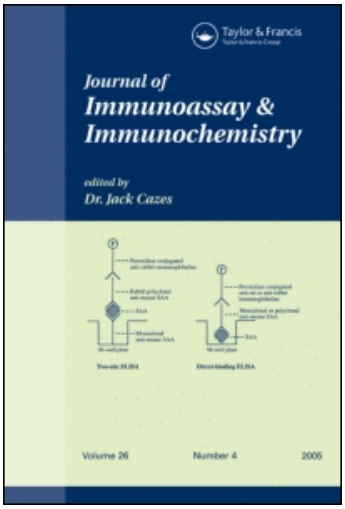

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Induction and Characterization of Monoclonal Anti-Idiotypic Antibodies to Tick-Borne Encephalitis Virus Neutralizing Antibody

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INDUCTION AND CHARACTERIZATION OF MONOCLONAL ANTI-IDIOTYPIC ANTIBODIES TO TICK-BORNE ENCEPHALITIS VIRUS NEUTRALIZING ANTIBODY

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ABSTRACT

Seven monoclonal anti-idiotypic antibodies (anti-ID MAbs, Ab2) were generated against virus-neutralizing and hemagglutination-inhibiting monoclonal antibody (Ab1) specific for tick-borne encephalitis (TBE) virus. Six of these anti-ID MAbs inhibited the binding of Ab1 to the virus antigen, thus classifying these anti-ID antibodies as Ab2β or AB2γ. Inhibition tests with heterologous anti-TBE sera revealed that these anti-ID MAbs were not recognized by anti-TBE antibodies and therefore they do not carry an internal image of TBE virus antigen. Hence, the anti-ID MAbs may be classified as Ab2γ type. None of the anti-1D MAbs induced production of antiviral antibodies nor protective immunity in syngeneic Balb/c mice. Using these anti-ID MAbs four nonoverlapping idiotopes were identified on Ab1 variable region.

(KEY WORDS: anti-idiotypic antibodies, tick-borne encephalitis virus)

INTRODUCTION

Since Jerne proposed his idiotype network theory (1), much attention has been given to anti-idiotypic antibodies as vaccine candidates or tools for study of cell receptors. Since induction of specific immune response to several viruses has been reported after administration of anti-ID antibodies (for review see 2, 3), anti-idiotypic antibodies have been successfully used for identification of virus receptors (for review see 4).

Idiotypes are associated with the variable region of antibody molecules (5, 6). They are composed from multiple epitopes called idiotopes, some of them being associated with the paratope and others with framework determinants of the variable region domain (7). Anti-ID antibodies are divided into four classes depending on the type of the idiotope-anti-idiotope interaction (8). Ab2α anti-idiotype antibodies recognize non-antigen inhibitable idiotopes, binding to idiotopes in the framework region of immunoglobulin . The binding of both Ab2β and Ab2γ anti-idiotypes to Ab1 is inhibitable by antigen. While Ab2γ recognize paratope-related idiotopes but are not an internal image of an antigen epitope, Ab2β

mimic an antigenic determinant and bind to Ab1. Ab2β should recognize idiotypes from several species induced by immunization with the same antigen. Furthermore, Ab2β must be able to induce an antibody response of the same specificity as the epitope which it mimics in different animal species. Anti-idiotype antibodies Ab2β with their unique internal image, are the most important for the development of vaccines or for the study of cell receptors.

Ab2ε anti-idiotypic antibodies are multispecific antibodies recognizing an idiotope on immunoglobulins as well as the epitope for which the paratope of such immunoglobulins has specificity.

The total number of idiotopes belonging to an antibody molecule is not known. Idiotope measurement is limited by the availability of different monoclonal anti-ID probes. Idiotopes are analysed mainly by the competitive binding assay between different anti-ID MAbs (9).

In this paper we described the generation and characterization of seven mouse monoclonal anti-idiotype antibodies to anti-tick-borne encephalitis virus monoclonal antibody with virus-neutralizing and hemagglutination inhibiting activity. One of these antibodies was classified as

Ab2α , the other six anti-ID MAbs were classified as Ab2γ , reactive with private idiotopes of Ab1.Idiotope mapping of Ab1 was carried out using these anti-ID MAbs and with them induced Ab3 sera using reciprocal competitive binding assay. The effect of anti-ID MAbs on different functional activities of Ab1 was discussed.

MATERIALS AND METHODS

Animals

Balb/c mice (females, 6-10 weeks old) were purchased from VELAZ Prague.

Cells

Porcine kidney cell line PS was cultured in L-15 medium containing 3% newborn bovine serum. Non-secreting mouse myeloma cells Sp2/O Ag 14 were grown in medium RPMI 1640 supplemented with 10% fetal bovine serum (FBS).

Virus

Prototype TBE virus strain Hypr was used as 10% brain suspension in phosphate buffered saline (PBS) with 10% newborn bovine serum.

Monoclonal Anti-TBE Virus Antibody (Ab 1)

A Balb/c mouse was immunized intraperitoneally three times with live attenuated TBE virus strain 274 (10) without adjuvant at 4-weeks intervals. Spleen cells were fused with SP2/0 myeloma cells using PEG and the hybrids were cloned by limited dilution as previously described (11). The anti-TBE MAb 274-B1B2 was expanded in ascitic fluid and purified by affinity chromatography using a Protein A Sepharose column (Bio-Rad). Purified anti-TBE MAb 274-B1B2 exhibited a titer of 256,000 by TBE-specific ELISA, a titer of 160 by plague reduction neutralization, and a titer of 640 by hemagglutination. This MAb was in turn used for the generation of anti-ID MAbs described below.

Coupling of MAbs to Keyhole Limpet Hemocyanin (KLH)

Purified MAb 274-B1B2 (0.8 mg) in 0.1 ml of PBS was mixed with 1 mg of KLH in 0.2 ml PBS and then coupled by the addition of 60 µl 2.5% glutaraldehyde. After 5 min-incubation at room temperature the reaction was stopped with 60 µl 2.2 M glycine. After addition of 0.58 ml distilled water, the conjugate was dialysed overnight against distilled water.

Production of Monoclonal Anti-idiotype Antibodies

Balb/c mice were immunized intramuscularly with 80 μg Ab1-KLH conjugate mixed with complete Freund s adjuvant (CFS) on day 0 followed by two intradermal/subcutaneous injections of the same dose of antibody in incomplete Freund s adjuvant (IFA) on days 14 and 30. Mice were boosted on day 40, four days before fusion, with 0.5 ml of undiluted ascitic fluid containing MAb 274-B1B2 (Ab1) intraperitoneally. Fusion, clonning and MAbs purification were carried out as described for monoclonal anti-TBE virus antibody. Selection of hybrids was carried by idiotope cross-linking ELISA as described below. Isotyping of MAbs was done by Ouchterlony analysis using goat anti-mouse isotype sera (Sigma).

Production of Ab3 Sera

Seven groups of three Balb/c mice each were immunized with Ab2-KLH conjugate by two intradermal/subcutaneous injections of 80 μg of antibody with CFA (first dose) or IFA (second dose) 14 days apart, the third dose of Ab2-KLH conjugate was given without adjuvant intraperitoneally. Blood was taken 10 days after the last injection and sera were stored frozen until used.

Idiotope Cross-linking ELISA for the Detection of Anti-ID Antibodies (Ab2) or Anti-anti-ID Antibodies (Ab3)

Wells of 96 well microtitre plates (Dynatech Immulon) were coated overnight with 0.2 µg purified MAb 274-B1B2 or control mouse MAb of the same isotype in 50 µl carbonate buffer (pH 9.6) per well.After blocking with 100 µl PBS containing 0.5% gelatine prewarmed to 37°C (incubation 30 min. at 37°C), 50 µl samples of undiluted hybridoma supernatant, ascitic fluid or serum diluted in PBS containing 2% FCS were added to the wells and incubated for 1 hr at 37°C. Next the wells were washed and incubated with 50 µl biotinylated MAb 274-B1B2 diluted 1/500 for 1 hr at 37°C. After washing, streptavidin-peroxidase complex (Sigma) diluted 1/500 was added and plates were incubated for 15 min. at 37°C. Plates were developed with 100 µl per well of substrate solution (0.1 M NaAc buffer pH 5.5, 0.03% H_2O_2, 2.5 mM o-phenylenediamine). The reaction was stopped after 20-30 min. with 100 µl H_2SO_4. The absorbance was read in Titertek Multiskan automatic plate reader (Flow Laboratories) at 492 nm. After each incubation, the plates were washed with PBS containing 0.05% Tween 20.

Essentially the same technique was used for the demonstration of Ab3 using plate-bound and biotinylated purified Ab2.

Biotinylation of Monoclonal Antibodies

1 mg MAb in 200 µl PBS was mixed with 0.1 mg biotinyl-ε-caproic acid-N-hydroxy-succinimide ester in 10 µl dimethylsulfoxide and incubated for 4 hr at room temperature. The mixture was then dialysed against PBS at 4°C overnight.

Inhibition ELISA for the Detection of Paratope-related Idiotopes

Samples of hybridoma supernatants or diluted ascitic fluids were incubated with equal volume of such dilution of MAb 274-B1B2 giving 50% of maximal level of binding to TBE virus antigen-coated wells for 90 min. at 37°C. Wells of a 96-well ELISA plate (Dynatech, Immulon) were coated overnight at 4°C with 0.2 µg TBE antigen (12). After coating, blocking was performed by incubation with 200 µl PBS containing 0.05% Tween 20 and 5% FCS for 30 min. at 37°C. After washing, 50 µl samples of hybridoma supernatants previously incubated with MAb 274-B1B2 were added and

incubated for 1 hr at 37°C. The remaining binding of Ab1 to TBE antigen was detected by the addition of peroxidase-conjugated swine anti-mouse gammaglobulin (Sevac, Prague, Czechoslovakia). The plates were washed after each incubation with PBS containing 0.05% Tween 20. The plates were developed with OPD substrate as described above. The percentage of inhibition was calculated according to the formula:

$$\text{Inhibition} = \frac{A_{492}\ \text{uninhibited} - A_{492}\ \text{inhibited}}{A_{492}\ \text{uninhibited}} \times 100$$

Supernatants from non-producing hybridomas or non-immune ascitic fluids preincubated with MAb-274 B1B2 served as uninhibited control.

Inhibition ELISA for Idiotope Mapping

Ab3 sera diluted 1:100 were mixed with equal volume of such dilution of biotinylated Ab2 giving 50% of maximal level of binding to Ab1 coated wells and incubated for 90 min at 37°C. 50 µl aliquots were transfered to the wells coated with Ab1 and incubated for 1 hr at 37°C. The remaining binding of Ab2 was detected by the addition of streptavidin-peroxidase complex (Sigma). The plates were washed, developed and the percentage of inhibition was calculated as above.

Inhibition ELISA for the Detection of Crossreactive Idiotopes

The ability of goat, rabbit, human and mouse polyclonal anti-TBE sera to react with Ab2 was tested. The anti-TBE titres (ELISA) of each sera were 1.600, 6.400, 16.000 and 8.000 respectively. Inhibition of the binding of Ab2 to Ab1 was considered to be indicative for the recognition of interspecies cross-reactive idiotopes. Anti-TBE sera diluted 1:100 and Ab1 (MAb 274-B1B2) ascitic fluid diluted 1:100 (as positive control) were incubated with biotinylated Ab2 and the remaining anti-Ab1 activty was assayed as described above for the inhibition ELISA for idiotype mapping.

Inhibition of Virus-neutralizing Capacity of Ab1

MAb 274-B1B2 diluted 1:20 was mixed with equal volume of serially diluted Ab2 and incubated 90 min. at 37^{O}C. 0.2 ml of this mixture was then incubated with 0.2 ml of TBE virus and the plaque-reduction neutralization test was performed as described earlier (11).

Inhibition of Hemagglutination-inhibiting Capacity of Ab1

25 µl of serially diluted Ab2 (ascites) were mixed

with equal volume of Ab1 in the dilution giving 50% of its HIT titre and incubated 1 hr at 4°C. Two hemagglutination units of TBE antigen in 25 µl borate saline containing 0.4% bovine serum albumin was then added and the mixture was incubated overnight at 4°C. Hemagglutination-inhibition test with goose erythrocytes was then performed according to the method of Clarke and Casals (13).

RESULTS

Preparation and Characterization of Anti-idiotypic MAbs

Initial screening of more than 600 hybridomas by idiotype cross-linking ELISA revealed 7 clones, which were further tested by the inhibition ELISA for the detection of paratope-related idiotopes. Except for one clone, all clones gave positive reaction in both tests (results not shown).

After recloning, anti-ID MAbs were prepared in the form of ascitic fluids and further characterized. In the idiotope cross-linking ELISA with MAb 274-B1B2, titres of anti-ID MAbs ranged from 1:20 to 1:2.560 (Table 1). No binding was demonstrated with isotype-matched control MAb (titres less than 1:20).

TABLE 1

Characteristic of Anti-idiotypic Antibodies

Anti-ID MAb (Ab2)	Isotype[a]	Idiotype[b] cross-linking ELISA	Inhibition[c] ELISA	Inhibition[d] of HI capacity of Ab1
1H9	IgM	640	100	40
2B1	IgM	2.560	<100	<40
2F6	IgG1	80	102.400	160
4H4	$IgG2_a$	160	102.400	40
8C1	IgG1	20	25.600	160
8C3	IgG1	160	409.600	640
9A6	IgG1	20	1.600	80

[a] Isotype was determined by Ochterlony immunodiffusion.

[b] Titres of ascitic fluid were expressed as reciprocals of the highest dilutions which gave A_{492} of twice the control.

[c] Titres were expressed as reciprocals of the highest dilutions giving 50% inhibition of Ab1 binding to viral antigen.

[d] Titres were expressed as reciprocals of the highest dilutions giving the inhibition of HI capacity of Ab1 in 50% of wells.

Individual anti-ID MAbs differed in the slope of the titration curves (Fig. 1).

Another type of ELISA, based on the inhibition of the binding of Ab1 to TBE virus antigen by anti-idiotype, was chosen for further classification

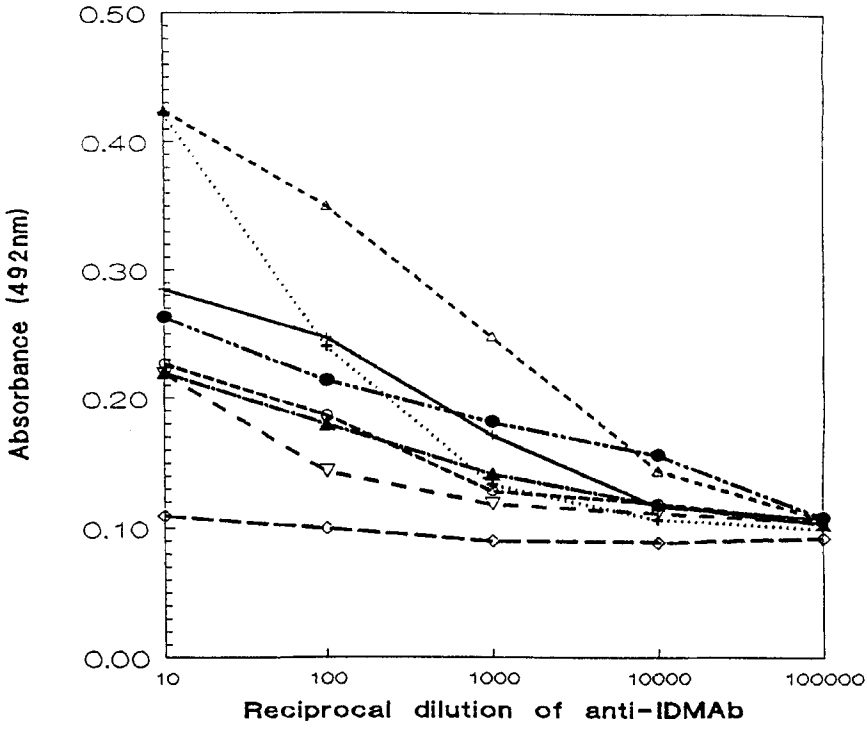

FIGURE 1. Direct binding of anti-ID MAbs 1H9 (+), 2B1 (△), 2F6 (○), 4H4 (+), 8C1 (▲), 8C3 (●), 9A6 (▽) and non-immune ascitic fluid (◇) to MAb 274-B1B2 (Ab1) as determined by idiotope cross-linking ELISA.

of anti-ID MAbs as either Ab2α or Ab2β + Ab2γ. As shown in Fig.2, 5 of 7 anti-ID MAbs exhibited high titres in this ELISA, MAb 1H9 showed low titre and MAb 2B1 had no inhibitory activity. According to these results anti-ID MAb 2B1 could be classified as Ab2α, while remaining 6 MAbs were considered to be type Ab2β or Ab2γ.

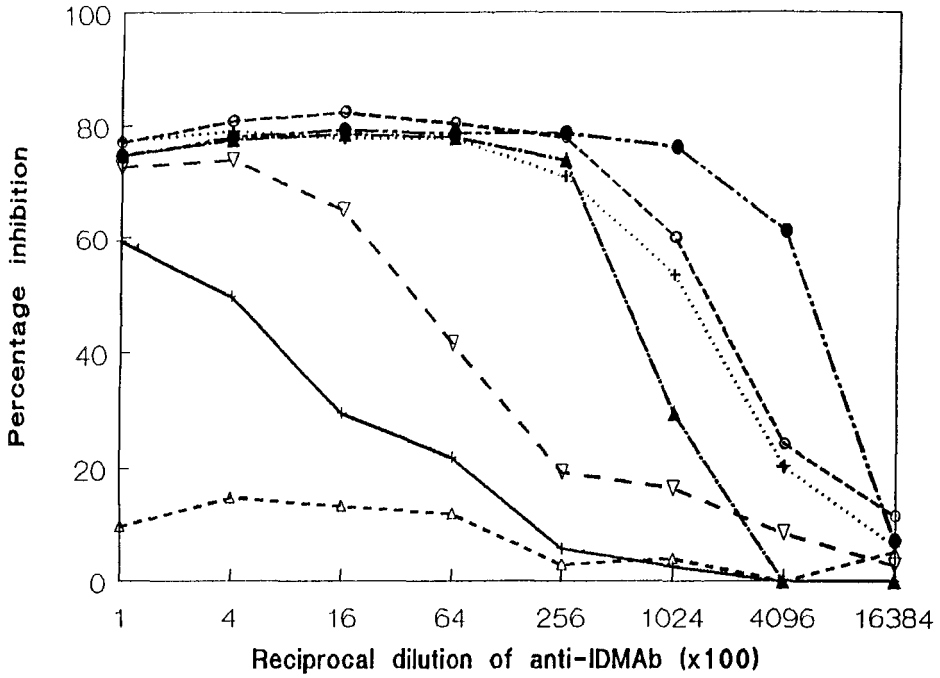

FIGURE 2. Inhibition of binding of MAb 274-B1B2 (Ab1) to TBE virus antigen by anti-ID MAbs 1H9 (+), 2B1 (△), 2F6 (○), 4H4 (+), 8C1 (▲), 8C3 (●) and 9A6 (▽) in inhibition ELISA. Dilutions of ascitic fluids were incubated with a dilution of Ab1 giving 50% of maximal binding to TBE virus antigen for 90 min. at 37°C. The remaining binding of Ab1 to TBE antigen was then determined by ELISA in wells coated with 0.2 μg of TBE antigen. Percentage of inhibition was calculated as described i materials and methods.

Inhibitory effect of anti-ID MAbs on the binding of Ab1 to TBE virus antigen was studied in two other functional assays, virus-neutralization and inhibition of hemagglutination. For the inhibition of TBE virus neutralization we chose anti-ID MAb 8C3 with highest

activity in the inhibition ELISA. As shown in Fig.3 this anti-ID MAb could inhibit the neutralizing capacity of Ab1 up to 10^{-4} dilution.

Results similar to the inhibitory ELISA were obtained in the hemagglutination inhibition test (Table 1). While Ab2 2B1 failed to inhibit the hemagglutination-inhibiting capacity of Ab1, titres of remaining anti-ID MAbs varied from 40 to 640.

Determination of Interspecies Cross-reactivity of Anti-ID MAbs and Preparation of Ab3 Sera

In order to determine if some of our anti-ID MAbs had the characteristics of internal image Ab2β antibody, we examined the ability of goat, human, mouse and rabbit anti-TBE sera to inhibit the binding of 6 paratope-related Ab2 to Ab1 by inhibition ELISA. As shown in Table 2, while homologous Ab1 used for the generation of anti-ID MAbs efficiently inhibited this binding (55-75%), none of the anti-TBE sera tested exhibited any inhibitory activity in this assay (inhibition less than 10%).

The other criterion for classifying anti-ID MAbs as Ab2β antibodies is the induction of Ab3 sera containing antibodies which can react with the original antigen. Three Balb/c mice were immunized

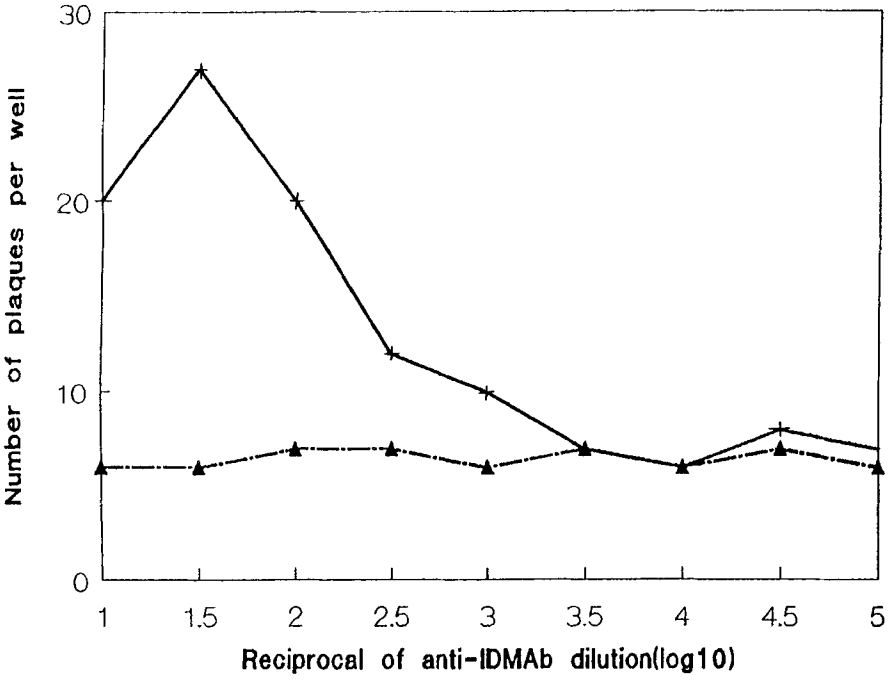

FIGURE 3. Inhibition of neutralization of TBE virus by MAb 274-B1B2. Serial dilutions of anti-ID MAb 8C3 (+) or non-immune ascitic fluid (▲) were mixed with MAb 274-B1B2 diluted 1:20 and capacity of anti-virus MAb was then tested by plaque-reduction neutralization test. Each value represents the mean of triplicate determinations.

with each anti-ID MAb previously coupled to KLH. Although all the mice developed high anti-Ab2 antibody titres as measured by the idiotope cross-linking ELISA (titres 10^2-10^3), no TBE virus antigen-reactive antibodies were detected by ELISA or by indirect immunofluorescence assay. Thus all anti-ID MAbs

TABLE 2

Detection of Interspecies Cross-reactive Idiotopes Recognized by Selected Anti-ID MAbs

Inhibitor	Biotinylated anti-ID MAbs					
	1H9	2F6	4H4	8C1	8C3	9A6
MAb 274-B1B2 (Ab1)	66[a]	75	65	55	56	60
Goat anti-TBE serum	1	0	3	0	4	0
Mouse anti-TBE serum	3	3	7	0	0	5
Human anti-TBE serum	1	0	9	1	1	3
Rabbit anti-TBE serum	0	0	0	0	0	0

[a] Percentage inhibition of binding of anti-ID MAbs to Ab1 by TBE-immune sera from different species and by Ab1. The values represent the mean of triplicate determinations.

reactive in the inhibition ELISA were classified as Ab2γ.

Idiotope Mapping Using Ab3 Sera

Our panel of anti-ID MAbs was further applied to topological mapping of idiotopes on monoclonal antibody 274-B1B2. We used Ab3 sera prepared by the immunization of Balb/c mice with different anti-ID

TABLE 3

Inhibition of Anti-ID MAbs Binding to Ab1 by Ab3 Sera

Inhibitor Antiserum to anti-ID	Biotinylated anti-ID MAbs						
	1H9	2B1	2F6	4H4	8C1	8C3	9A6
1H9	77[a]	13	**91**	14	**92**	**88**	33
2B1	9	**56**	37	2	22	0	40
2F6	**75**	12	**90**	3	**92**	**88**	23
4H4	1	12	14	**84**	0	0	1
8C1	**72**	15	**89**	0	**92**	**85**	9
8C3	**71**	17	**91**	6	**92**	**85**	5
9A6	7	7	22	13	4	0	**93**

[a] Percentage inhibition of binding of anti-ID MAbs to Ab1 by syngeneic antisera to particular anti-ID MAbs. The values represent the mean of determinations with three Ab3 sera. Values over 50% inhibition are boldfaced.

MAbs in an inhibition ELISA. In this ELISA the binding of Ab2 to Ab1 was inhibited by preincubation of Ab2 with individual Ab3 sera (Table 3). All Ab3s generated to anti-ID MAbs 1H9, 2F6, 8C1 and 8C3 were able to inhibit all of these anti-ID MAbs similarly. As the inhibition pattern of Ab3 sera were the same either for the corresponding immunizing anti-ID MAb or the other three anti-ID MAbs (data not shown), we concluded that these anti-ID MAbs recognize the same idiotope on Ab1 molecule. It is of interest to note

that while anti-ID MAbs 2F6, 8C1 and 8C3 exhibited comparable reactivity in both idiotope cross-linking and inhibition ELISA as well as in HIT, anti-ID MAb 1H9 revealed the highest titre in the cross-linking ELISA, but very low reactivity in both the inhibition ELISA and HIT.

Conversely, the Ab3 sera to anti-ID MAbs 2B1, 4H4 and 9A6 reacted only with the corresponding immunizing MAb. The results suggest that these MAbs recognize three nonoverlapping idiotopes on the Ab1 molecule, two of them (4H4, 9A6-specific) being closely related to the antigen-combining site of Ab1 and third (2B1-recognized) localized outside the Ab1 paratope.

Taken together, we have been able to identify at least four distinct nonoverlapping idiotopes in the 274-B1B2 idiotype, three of them being closely related to the antibody paratope.

DISCUSSION

In this report we describe 7 anti-ID MAbs generated to the MAb 274-B1B2 in turn specific for the *glycoprotein* E of TBE virus. This was chosen because of its functional characteristics, namely virus-neutralizing and hemagglutination inhibiting

activities. Initially we aimed to produce some anti-ID MAbs, which could mimic "protective" epitopes of the virus (14) and/or epitopes reacting with virus receptors on susceptible cells (4).

According to the classification proposed by Bona and Kohler (15), 6 of our 7 anti-ID MAbs belong to the $Ab2_{\gamma}$ subclass, because they can inhibit the interaction between idiotype and antigen, but they do not react with anti-TBE antibodies from heterologous sera and are not able to induce TBE antigen-reacting antibodies in Ab3 sera. From these data we concluded that they do not carry an internal image of TBE antigen and therefore they can not be considered as Ab2β. Anti-ID MAb 2B1 with the highest activity in the idiotope cross-linking ELISA, but no activity in the inhibition ELISA was considered to be Ab2α type binding to the idiotope outside the paratope of the Ab1.

The differences in anti-ID antibody titres in cross-linking and inhibitory ELISA (Table 1) can be, in addition to the differences in the specific antibody content in an antibody preparation, connected with different affinity of these antibodies or different epitopes recognized by these antibodies. The highest difference in cross-linking and inhibitory

ELISA titres among our γ-type anti-ID MAbs was observed in MAb 1H9, which can be explained by the IgM isotype of this antibody. In the cross-linking ELISA MAb 1H9 exhibited higher avidity perhaps due to the bonus effect of its 10 antigen-binding sites, while in the inhibitory ELISA the MAb 1H9 appeared to have some failure in occupying both antigen-binding sites in Ab1. The latter can be attributed to some form of steric restriction.

Comparisons on the effect of our anti-ID MAbs on hemagglutination inhibitory activity of Ab1 showed that while MAb 2B1 (Ab2α) had no effect, the remaining anti-ID MAbs inhibited this activity to various extents. The lack of correlation between anti-ID antibody titres in the inhibition ELISA and HIT can be due to different idiotopes recognized by these antibodies as discussed below.

Using anti-ID MAb 8C3, which was the most powerful in both the inhibitory ELISA and HIT, we proved its ability to inhibit the neutralizing activity of Ab1. This neutralization-inhibition effect of anti-ID MAbs was exploited for their titration in a modified ELISA initially described by Oosterlaken et al. (16). Lower activity of our anti-ID MAbs in the neutralization-inhibition test (60-70% inhibition) in

comparison to the 100% inhibition achieved by these authors can be explained by much lower neutralizing capacity of our Ab1 and therefore lower sensitivity of this test.

Immunization of Balb/c mice with our anti-ID MAbs induced an anti-Ab2 antibody response. However these Ab3 antibodies did not recognize TBE virus antigens, nor immunized mice were protected against a lethal dose of TBE virus (data not shown). There are several papers demonstrating induction of nominal antigen-specific antibodies by α or γ anti-ID antibodies (17, 18, 19, 20), which is mostly genetically restricted and does not cross species barier. Therefore such non-internal image anti-ID antibodies have a significant limitation as putative vaccine candidates. Unfortunately, none of our type anti-ID MAbs were able to induce TBE-specific antibody response, nor protection of mice against TBE virus infection. The potential mechanism of non-internal image anti-ID MAbs inducing anti-viral response is the recognition of a regulatory idiotope predominantly associated with one particular paratope specific for a nominal antigen (21). Our results suggest that idiotopes recognized by our anti-ID MAbs are not preferentially associated with TBE-specific Abs and

therefore the anti-viral response have been not detected. Similar results have been demonstrated on the canine parvovirus model (22).

Our anti-ID MAbs were further used for the idiotope mapping on the variable region on the anti-TBE virus MAb 274-B1B2. In cross-blocking studies we used Ab3 sera specific for different anti-ID MAbs rather than simple competition experiments with different anti-ID MAbs because of better differentiating ability of the former arrangment (23). Using this approach we identified at least four non-overlapping idiotopes on the 274-B1B2 antibody. Four of our anti-ID MAbs seem to recognize identical or very closely overlapping determinants, because Ab3 sera prepared to individual anti-ID MAbs inhibited their binding to Ab1 similarly. Nevertheless, the comparison of reactivities of these four anti-ID MAbs in cross-linking and inhibitory ELISA revealed a different pattern for MAb 1H9, which exhibited very low capacity to inhibit the binding of Ab1 to the TBE virus antigen, but the highest binding to Ab1 in cross-linking ELISA. Whether the IgM isotype of 1H9 antibody enabled its binding to Ab1 coated wells with higher affinity due to the bonus effect of its

multivalency, or the different idiotope recognized by this antibody remains to be elucidated.

Determination of the number of idiotopes borne on an antibody molecule is limited by the availability of different anti-ID MAb probes. Streicher et al. (24) identified three clusters of overlapping idiotopes on anti-myoglobin MAb detected by 6 syngeneic anti-idiotypic MAbs. The same number of anti-ID MAbs was used for the determination of 6 discrete overlapping idiotopes on the TEPC 15 immunoglobulin molecule (9).

Using our panel of 7 anti-ID MAbs we determined four discrete idiotopes on the MAb 274-B1B2. While four anti-ID MAbs recognized probably identical paratope-related idiotopes, three anti-ID MAbs bound to three distinct non-overlapping idiotopes. The relatively lower inhibition of binding of anti-ID MAbs 1H9 and especially 2B1 by homologous Ab3 sera seemed to be due to higher affinity of these two anti-ID MAbs belonging to the IgM class. Non-reciprocal inhibition with Ab3 sera can be explained, similarly to non-reciprocal competition between anti-ID MAbs, by different affinities of respective MAbs or by changes in the fine conformation of antibody molecule, which can change the availability of other epitopes (9). The actual number of idiotopes borne by a simple

immunoglobulin molecule is probably much higher than has been determined by means of our anti-ID MAbs. Novotny et al. (25), using a computer model for detecting epitopes on protein antigens, estimated that there are potentially 15-20 epitopes on the variable region of a single immunoglobulin molecule.

In conclusion, we have used an antiviral neutralizing and hemagglutination-inhibiting MAb to produce anti-idiotypic MAbs. These anti-ID MAbs allowed us to study their effect on some of functional activities of Ab1 as well as to map idiotopes on the variable region of the Ab1 antibody molecule. We are now producing further anti-ID MAbs with the aim to use them for TBE virus receptor study. We intend to use two ways to get β anti-ID MAbs: (i) by increasing the efficiency of immunization using intrasplenic immunization (26) and (ii) by positive selection of splenic B lymphocytes reactive with heterologous hyperimmune anti-TBE sera using fluorescent-activated cell sorter (27, 28).

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