

An interspecies idiotope associated with the anti-cholera toxin response detected by a monoclonal auto-anti-idiotypic antibody

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SUMMARY

We have produced an auto-anti-idiotypic (auto-anti-id) monoclonal antibody (mAb) which reacted with syngenic mouse polyclonal anti-cholera toxin (anti-CT) IgG antibody (Ab) and six/eight different anti-CT IgG mAb, but not with normal mouse BALB/c IgG. The binding of this auto-anti-id mAb on one anti-CT mAb was significantly inhibited by polyclonal anti-CT sera of rats, rabbits and mice. Thus the idiotope on anti-CT Ab recognized by this auto-anti-id mAb was public and expressed in different species. Because of the absence of competition between CT and this auto-anti-id mAb for the binding to the six anti-CT mAb, the anti-id mAb was classified as an Ab2 α . The efficiency of this auto-anti-id mAb to induce anti-CT Ab3 was tested with success in rabbits and rats. Auto-anti-id mAb-immunized rats were significantly protected against an intraintestinal CT challenge.

INTRODUCTION

According to Jerne,¹ since lymphocytes are able to recognize virtually every possible molecular shape, they also recognize hypervariable regions (idiotopes) on antibody molecules (Ab) and on receptors of the lymphocytes. Thus, interactions between idiotypes and anti-idiotypes from different lymphocytes form a network: the idiotypic network. Its existence has been demonstrated by the production of auto-anti-idiotypic (anti-id) Ab in normal responses against foreign antigens.^{2,3} The function of this network may be the regulation of the immune system. Jerne thought that all lymphocytes were in a steady state due to the id-anti-id interactions. Introduction of an antigen would disturb that equilibrium: a particular id interaction would then dominate and hence an immune response would be induced. Some idiotopes, called shared id, are expressed on Ab with different specificities. These shared idiotopes were studied with polyclonal anti-id antisera^{4,5} or with monoclonal anti-id Ab^{6,7} specific for these particular idiotopes. Such shared idiotopes were supposed to regulate the immune network. A better understanding of the regulation of immune responses is of great importance for comprehension and treatment of autoimmune diseases.⁸

Another postulate of Jerne's network theory was that, as antibodies could bind by their antigen-binding site to both

foreign antigens and anti-id Ab, this particular anti-id Ab would mimic the antigen. This type of anti-id Ab, called Ab2 β or internal image anti-id Ab, has been extensively used as vaccine as discussed in refs 9 and 10. However, non-internal-image anti-id Ab, called Ab2 α , have also been used successfully to immunize animals.^{11–13} In these cases, animals will develop a response against the antigen if the Ig receptor on their B lymphocytes specific for this antigen carries the idiotope recognized by the anti-id Ab.

We have generated an auto-anti-id monoclonal antibody (mAb) in cholera toxin (CT)-immunized BALB/c mice, that recognized an idiotope shared by six/eight BALB/c anti-CT IgG mAb tested. The aim of this work was to characterize this shared idiotope. In particular, we wished to determine if this idiotope was expressed on polyclonal anti-CT Ab from different species. We also examined the efficiency of this mouse auto-anti-id mAb as an anti-CT vaccine by testing the protection it could induce, in rats, against an intraintestinal CT challenge.

MATERIALS AND METHODS

Production and purification of auto-anti-id mAb

BALB/c mice immunized with CT were used to produce several anti-CT idiotypic mAb (id).¹⁴ During the screening of their fused spleen cell clones, the presence of clones producing anti-(anti-CT) mAb, or auto-anti-id mAb was also examined by particle counting immunoassays.¹⁵ Several well supernatants were able to agglutinate latex particles (Lx) coated with mouse polyclonal anti-CT Ab, but not Lx coated with mouse polyclonal normal

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IgG.¹⁵ Cells of one of these wells were subcloned by limiting dilution, multiplied and injected into pristane-treated BALB/c mice to induce hybridoma ascites. The IgM mAb, called auto-anti-id mAb, was partially purified from ascites by gel filtration on Ultrogel AcA 22.

Polyclonal anti-CT Ab were affinity purified on a CT immunoabsorbent¹⁶ (CT-Iads) from polyclonal anti-CT ascites obtained with Freund's adjuvant, as described earlier.¹⁷ Normal mouse polyclonal IgG was purified from 'non-specific' Freund's adjuvant ascites¹⁷ by chromatography on Protein A-Sepharose CL-4B (Pharmacia, Uppsala, Sweden).

Two other anti-(anti-CT) anti-id mAb were also utilized: the 3F10 anti-id mAb¹⁵ and the 1G2.3H7 anti-id mAb.¹⁸

Different anti-CT mAb and antisera utilized

Eight anti-CT mAb were used: six [four IgG1 (1G4, 3B3, 4C5, 5B5) and two IgG2a (2B7, 2G9) were described earlier],¹⁴ whereas two more IgG1 (8E11, 9F9) are new ones (G. P. Lucas, C. L. Cambiaso and J. P. Vaerman, unpublished results).

Polyclonal anti-CT antisera from rats, rabbits and mice were utilized to investigate the presence of the cross-reactive idiotope on polyclonal anti-CT Ab from different species (see above). Rats were given CT (50 µg) four times [nine rats by the intraperitoneal route (i.p.), eight rats by the intragastric route (i.g.)] and three times [three rats by the intrajeunal route (i.j.)]. The rabbits ($n=3$) were injected subcutaneously (s.c.) eight to 17 times with 50–300 µg of CT. The BALB/c mice ($n=8$) were given CT (10 µg) three times i.p.

Biotinylation of auto-anti-id mAb

After dialysis against 0.1 M NaHCO₃, pH 8.0, 1.0 ml of auto-anti-id mAb at 1.0 mg/ml, was mixed with 20 µl of *N*-hydroxysuccinimido-biotin (Sigma, St Louis, MO) at 10 mg/ml in dimethylsulphoxide for 2 hr at room temperature, then extensively dialysed against phosphate-buffered saline (PBS), yielding biotinylated auto-anti-id (bio-auto-anti-id) mAb.

Coating of microtitre plates for ELISA

Microtitre plates (96 wells; Greiner, Nürtingen, Germany) were first coated with CT (4 µg/ml) or mAb (5 µg/ml) and then saturated as described in ref. 15. Washings (five times) were performed with PBS containing 0.1% Tween 20 (PBS-Tw). Samples were diluted in PBS-Tw containing 1% bovine serum albumin (BSA) (PBS-BSA-Tw). Background optical density (OD) was the mean OD of wells where only the sample dilutions were omitted.

Binding of bio-auto-anti-id mAb to id-coated plates

Increasing concentrations of bio-auto-anti-id mAb were incubated for 2 hr at 37° in plates coated with anti-CT mAb, or normal polyclonal mouse IgG as control. Binding of the bio-auto-anti-id mAb was revealed by horseradish peroxidase-coupled streptavidin (HRP-Strav) (Amersham, Brussels, Belgium) diluted at 1/1000 in PBS-BSA-Tw for 30 min at 37°. Colour development was as described previously.¹⁵

Inhibition of binding of bio-auto-anti-id mAb on id-coated plates

Increasing concentrations of normal serum or of anti-CT antiserum from different species were preincubated with 1 µg/ml of bio-auto-anti-id mAb for 2 hr at 37°, before incubation for 1 hr on anti-CT mAb id(3B3)-coated plates. Similarly, increasing

concentrations of CT were preincubated in different id-coated plates before washings and addition of 1 µg/ml of bio-auto-anti-id mAb. After incubation, both assays were developed as above. Results were expressed as per cent of inhibition by using the following formula:

% of inhibition =

$$\left[1 - \frac{(\text{mean OD with inhibitor} - \text{background})}{(\text{mean OD without inhibitor} - \text{background})} \right] \times 100.$$

Binding, and inhibition of binding, of rabbit Ab3 on CT-coated plates

Triplicate serial double dilutions of CT-Iads affinity-purified Ab3 (see below) [= rabbit anti-(auto-anti-id)] were incubated for 2 hr at 37° on CT-coated plates. Ab3 was revealed by anti-rabbit-Ig goat bio-IgG (Sigma) at 1/5000 in PBS-BSA-Tw for 1 hr, followed by HRP-Strav. For inhibition, rabbit Ab3 dilutions were preincubated with a fixed amount of soluble CT (100 µg/ml) for 2 hr at 37° before addition to CT-coated plates. ELISA revelation was as above.

Assay of polyclonal anti-CT Ab in sera of CT-immunized rats, rabbits and mice

A CT-sandwich ELISA was set up to measure the levels of polyclonal anti-CT Ab in anti-CT sera of different species. Four serial 10-fold dilutions of each serum, in duplicate, were incubated for 2 hr on CT-coated plates in PBS-BSA-Tw buffer at 37°. The anti-CT Ab were revealed by an additional 2 hr incubation with HRP-CT, prepared as described in ref. 19, at 0.15 µg/ml. Development was as above. The results were expressed as the log₁₀ of the anti-CT serum dilution giving an OD > 0.1. Preimmune or normal sera of all examined species never yielded an OD greater than 0.05 at any concentration, up to the 1/10 dilution.

Levels of serum anti-CT IgG and bile anti-CT IgA in auto-anti-id mAb- and CT-immunized rats

The levels of serum anti-CT IgG and bile anti-CT IgA in auto-anti-id mAb-immunized rats, expressed in arbitrary units, were estimated as in ref. 15 and compared to the levels obtained in the serum and bile in i.p. CT-immunized rats. Serial double dilutions of serum or bile of immunized rats were incubated on CT-coated plates and revealed by HRP-labelled affinity-purified polyclonal rabbit IgG Ab specific for rat α - or γ -chains.¹⁵ Standard IgG and IgA anti-CT curves were included in each plate, as well as control curves derived with serum and bile from unimmunized rats. The serum of a rat immunized four times i.p. with CT served as standard serum anti-CT IgG. A pool of bile from rats immunized twice i.j. with CT was utilized as standard anti-CT IgA. A 1/1000 dilution of the standard anti-CT serum and a 1/200 dilution of the standard anti-CT bile were defined as containing 1000 arbitrary units of anti-CT IgG and IgA, respectively.¹⁵ The quantity of anti-CT IgG and IgA in individual rat serum and bile was expressed in arbitrary units, calculated from the optical densities of at least three serial double dilutions of serum or bile falling in the sensitive range of the corresponding standard curve.

Production and purification of rabbit anti-CT Ab3 and absorption of Ab3-sera

Two rabbits were s.c. injected (five times, every fortnight) with 50 µg of the mouse auto-anti-id mAb, first in complete Freund's adjuvant (CFA), twice in incomplete Freund's adjuvant (IFA), and then in PBS with 0.02% gelatin. Serum was obtained before the first injection and 7 days after each injection. Anti-CT Ab3 in the rabbit sera were affinity purified on CT-Iads and then passed on normal mouse IgG-Iads to remove anti-mouse-Ig rheumatoid factor-like Ab, yielding affinity-purified Ab3. In other cases, the rabbit sera were only adsorbed on normal mouse IgG-Iads in order to eliminate rabbit anti-mouse-Ig Ab, yielding mouse-adsorbed Ab3 (mouse-abs-Ab3).

Production of anti-CT Ab3 in rats

Two groups of outbred Sprague Dawley rats (250–300 g; IFFA Credo, Brussels, Belgium) were injected with our mouse auto-anti-id mAb.²⁰ Briefly, group A (*n* = 6) received five and group B (*n* = 9) four i.p. doses, every 15 days, of 50 µg of auto-anti-id mAb in CFA, as above. A third group (C) of eight rats received four i.g. doses, 2 weeks apart, of 50 µg auto-anti-id mAb, in 1.0 ml containing 3% NaHCO₃ with a small catheter during a light ether anaesthesia. Sera were collected before the first injection and 7 days after each injection.

Rat intrainestinal CT challenge

Briefly, under ether anaesthesia, two 5-cm long intestinal loops were prepared by transversal jejunal ligatures in 24-hr fasted rats, in both immunized (imm) and control (unimm) rats 5 days after the last immunization. The loops were injected with 0.5 ml of buffer (PBS with 0.2% of gelatin) or CT (1 µg in PBS-gelatin) and gently replaced thereafter in the abdominal cavity which was then closed. The rats were placed in restraining cages for bile collection during this CT challenge. The bile duct was cannulated as described previously.²¹ After 4 hr, the rats were killed by ether overdose and bled out. The two ligated loops were carefully dissected, excised and weighed. The fluid accumulation [weight/length ratio (mg/cm) of CT-injected loop minus that of PBS-injected loop] was calculated for each animal.¹⁶ For comparison, our results are expressed as percentage of protection (P%), calculated as follows:

$$P\% = \left[1 - \frac{(\text{mg/cm of CT loop}_{\text{imm}} - \text{mg/cm PBS loop}_{\text{imm}})}{(\text{mg/cm of CT loop}_{\text{unimm}} - \text{mg/cm PBS loop}_{\text{unimm}})} \right] \times 100.$$

The mean fluid accumulation of PBS-injected loops was taken arbitrarily to be 100% protection, and the mean fluid accumulation of CT-injected loops in unimmunized control rats was arbitrarily taken to be 0% protection.

Statistical analysis

Significance was determined by using the Student's *t*-test for unpaired data.

RESULTS

Detection of the cross-reactive idiotope by the auto-anti-id mAb

The bio-auto-anti-id mAb was able to bind to plates coated with several anti-CT IgG1 mAb (5B5, 1G4, 9F9, 3B3, 4C5, 8E11) with different efficiencies according to the anti-CT mAb, but did

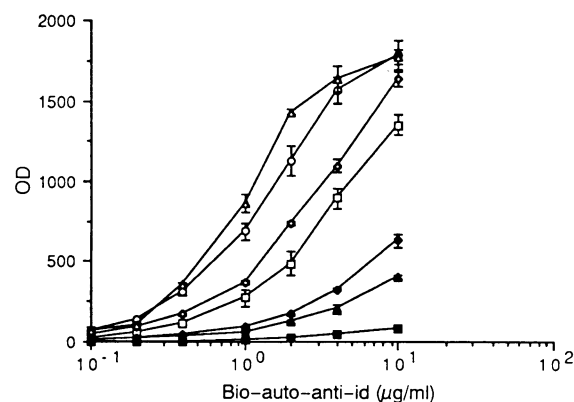


Figure 1. Binding of bio-auto-anti-id mAb (1 µg/ml) on ELISA plates coated with six different IgG1 anti-CT mAb [1G4 (○); 3B3 (◇); 5B5 (□); 9F9 (Δ); 4C5 (◆); 8E11 (▲)], two different IgG2a anti-CT mAb and normal polyclonal mouse Ig [the latter three represented by a single curve (■)] as revealed by HRP-Strav. Absorbance values represent OD means of triplicate wells minus background.

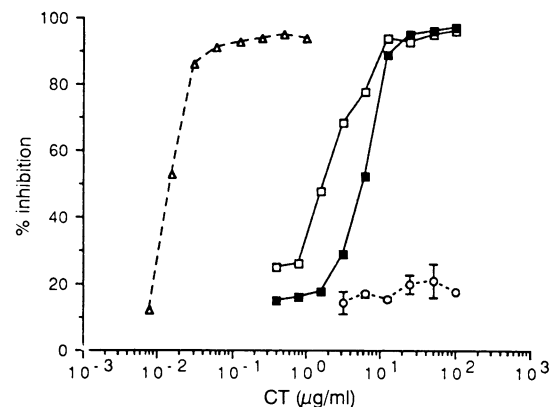


Figure 2. Inhibition of binding of three different bio-anti-id mAb (1.0 µg/ml) on ELISA plates coated with id mAb by preincubation of the coated plates with increasing concentrations of CT. Mean inhibition ± SEM of bio-auto-anti-id mAb on 1G4 and 3B3 anti-CT mAb-coated plates (○); inhibition of 1G2.3H7 anti-id mAb on 1G4 (■) and 3B3 (□) anti-CT mAb-coated plates and inhibition of 3F10 anti-id mAb on 4C5 anti-CT mAb-coated plate (Δ).

not bind to mouse normal polyclonal IgG-coated plates, nor to two IgG2a (2B7, 2G9) anti-CT mAb-coated plates (Fig. 1). The mouse normal polyclonal IgG contained at least 50% of IgG1 (pH 6.0 eluate from protein A-Sepharose). All these anti-CT mAb had different specificities for CT, as determined by their lack of competition in the Lx particle counting immunoassay (data not shown).

The auto-anti-id binds to a cross-reactive non-paratopic idiotope of anti-CT mAb

The idiotope reacting with our auto-anti-id mAb was shown to be located outside the paratope of six/eight anti-CT mAb, as shown by the absence of competition, between CT and the auto-anti-id mAb, for binding to six different anti-CT id mAb-coated

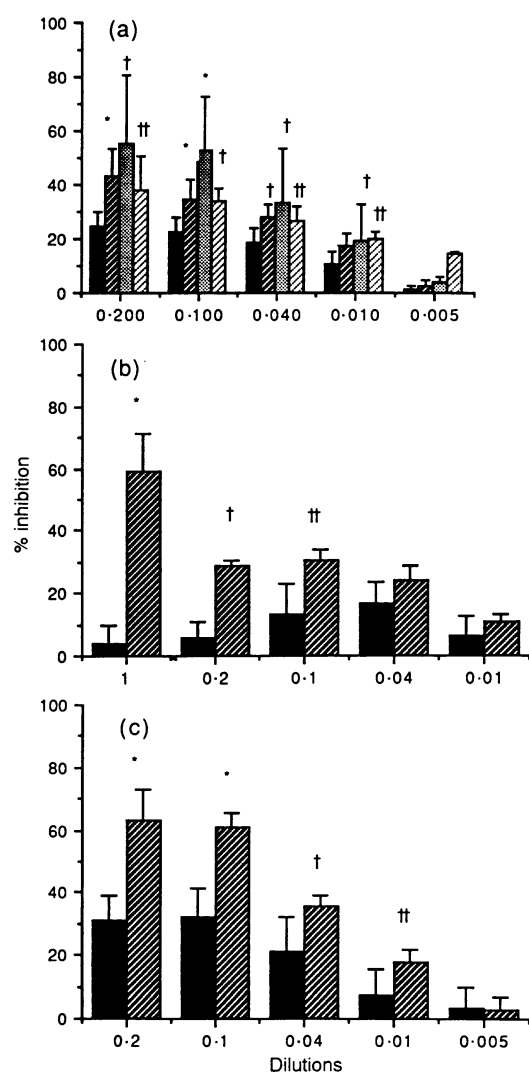


Figure 3. Inhibition of binding of bio-auto-anti-id mAb (1 µg/ml) on mAb1 (3B3)-coated plates by (a) increasing concentrations of sera from normal rat ($n=9$, ■), anti-CT rat i.p. ($n=7$, ▨), anti-CT rat i.g. ($n=8$, ▩), anti-CT rat i.j. ($n=3$, ▪); (b) increasing concentrations of sera from normal rabbit ($n=4$, ■), anti-CT rabbit ($n=3$, ▪); (c) increasing concentrations of sera from normal BALB/c mice ($n=8$, ■) and anti-CT BALB/c mice ($n=8$, ▪). Data represent mean per cent inhibition $\pm$ SEM. Significance inhibition as compared to normal sera: * $P \leq 0.001$; † $P \leq 0.01$; †† $P \leq 0.05$.

plates, by ELISA (Fig. 2). In this figure a mean curve is shown for only two anti-CT mAb (1G4 and 3B3) id-coated plates; the curves for the four other anti-CT mAb id-coated plates were similar by their lack of inhibition. Thus, increasing blockade by CT of the paratope of the coated anti-CT mAb did not modify its further binding of the bio-auto-anti-id mAb. In contrast, binding of two other anti-id mAb to their specific anti-CT id mAb1 was almost completely inhibited, either with the same concentration of CT for anti-id 1G2.3H7, or with 100-fold less CT for 3F10 (Fig. 2). These two anti-id mAb had been previously characterized as Ab2 β .^{15,18} All these results indicate that our auto-anti-id mAb is specific for a 'public' cross-reactive

Table 1. Titres of anti-CT Ab in individual anti-CT sera of rats, rabbits and mice and per cent of inhibition of the id-auto-anti-id mAb interaction for these anti-CT sera

Serum source	Inhibition (%)*	Anti-CT†
Rat i.p.		
animal no. 1	52	4.00
animal no. 2	76	4.22
animal no. 3	54	4.10
animal no. 4	65	4.07
animal no. 5	68	4.52
animal no. 6	49	4.60
animal no. 7	47	3.89
animal no. 8	3	4.40
animal no. 9	47	4.35
Rat i.g.		
animal no. 1	31	3.67
animal no. 2	27	3.85
animal no. 3	27	3.33
animal no. 4	41	4.39
animal no. 5	37	2.47
animal no. 6	43	3.32
animal no. 7	29	3.32
animal no. 8	45	4.39
Rat i.j.		
animal no. 1	40	4.66
animal no. 2	33	4.66
animal no. 3	28	4.35
Mouse		
animal no. 1	62	4.04
animal no. 2	65	3.72
animal no. 3	58	3.76
animal no. 4	63	3.97
animal no. 5	60	3.80
animal no. 6	49	3.78
animal no. 7	61	3.76
animal no. 8	68	4.14
Rabbit		
animal no. 1	25	5.72
animal no. 2	32	6.22
animal no. 3	34	6.15

* Per cent of inhibition of the id-auto-anti-id mAb interaction for these individual anti-CT sera for the 1/10 dilution.

† Titres expressed as log₁₀ of the end dilution (see Materials and Methods).

idiotope located outside of the paratope of six different anti-CT IgG1 mAb. It may thus be classified in the Ab2 α group of anti-id.

The occurrence of this public idiotope, as recognized by our auto-anti-id mAb, was also investigated on polyclonal anti-CT Ab from mice and other species. This was studied both by (1) specific inhibition of the id-auto-anti-id mAb interaction with polyclonal anti-CT Ab from different species and (2) the ability of the auto-anti-id mAb to induce anti-CT Ab-responses (Ab3) in animals from different species.

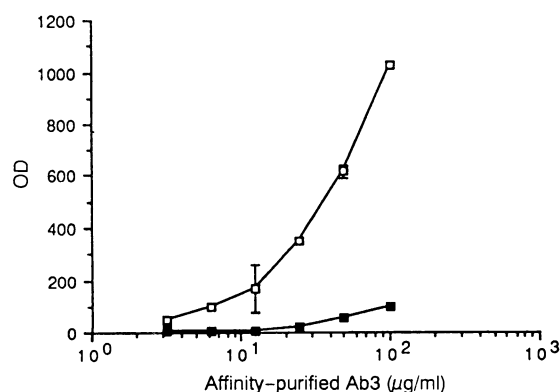


Figure 4. Binding of affinity-purified Ab3, in the presence (■) or absence (□) of a constant amount (100 μg/ml) of CT, on CT-coated plates, revealed by ELISA. Mean OD ± SEM of triplicates.

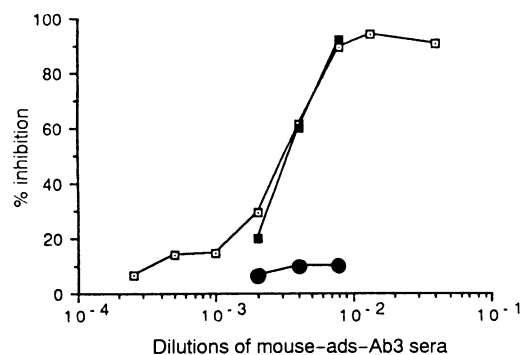


Figure 5. Inhibition of binding of bio-auto-anti-id mAb (1 μg/ml) to mAb1(3B3)-coated plates by increasing concentrations of mouse-ads-Ab3 preincubated with buffer (□), with auto-anti-id mAb-Lx (●) or with another isotype-matched anti-id-Lx (■).

Detection of a cross-reactive idiotope on polyclonal anti-CT Ab responses in rats, rabbits and mice

The expression of the cross-reactive idiotope on polyclonal anti-CT Ab, and not on polyclonal normal Ig, from different species was investigated by the efficiency of normal and CT-immunized individual animal sera to inhibit the id-auto-anti-id mAb interaction. The results are expressed as inhibition per cent, as described in Materials and Methods. We tested anti-CT antisera of 20 rats (nine immunized i.p., eight i.g. and three i.j.) and, as controls, sera of nine normal rats. The sera of three CT-immunized rabbits and of three normal ones were also tested. Anti-CT antisera of eight BALB/c mice, and their preimmune sera as controls were further used for comparison (Fig. 3). All anti-CT sera tested, excluding one from an i.p.-immunized rat, inhibited the binding of the bio-auto-anti-id mAb on anti-CT mAb-coated plates. These inhibitions were significantly larger than those obtained with normal or preimmune sera at the same dilution.

To verify if individual inhibitory capacity of sera was related to their anti-CT Ab level, anti-CT Ab levels were measured in all individual sera by the CT-sandwich ELISA. Results are listed in Table 1. No significant correlation was found between anti-CT

Table 2. Protection of rats immunized with auto-anti-id mAb and CT against CT intestinal challenge and mean levels of anti-CT IgG and IgA detected by ELISA in serum and bile of these rats

Protection P%	Anti-CT†	
	Serum IgG	Bile IgA
Autoimmunized Group A i.p. (n=6)		
91	5440	4520
72	7930	160
72	27,360	100
97	18,060	No bile
61	3910	0
100	151,460	220
Mean	82.2	12,974.3
SEM	14.5	8184.3
Group B i.p. (n=9)		
100	3420	2790
100	6150	3490
100	2600	2940
82	4510	1980
98	2510	860
72.5	4980	1320
95	3250	2020
100	1910	4350
100	2290	2820
Mean	94.2	3513.3
SEM	9.4	1336.8
CT immunized i.p.		
16	4,790,000	47,000
16	2,130,000	0
30	871,000	17,740
38	979,000	18,820
57	717,000	34,000
63	4,213,000	0
90	526,000	6600
Mean	44.29	2,708,570
SEM	10.14	752,370

* P% as described in Materials and Methods.

† Arbitrary units ± SEM, defined by standard serum or bile of CT-immunized rats (see Materials and Methods).

content and the ability to inhibit the id-auto-anti-id mAb interaction for those various anti-CT antisera.

Production of anti-CT Ab3 in rabbits

Anti-CT Ab (Ab3) produced by the auto-anti-id mAb immunized rabbits were affinity purified on CT-lads. These Ab3 bound in a dose-dependent way to CT-coated plates, and this binding was inhibited by preincubation of purified Ab3 with a constant amount of CT (100 μg/ml) before adding the mixture to the CT-coated plate (Fig. 4). This result suggests that the public idiotope is well expressed by anti-CT Ab3. The inhibition of bio-auto-anti-id mAb binding on id-coated plates by increasing amounts of mouse-ads-Ab3 confirmed this result (Fig. 5). Moreover, preincubation of mouse-ads-Ab3 with auto-anti-

id mAb-Lx, used as a Iads, completely inhibited the ability of mouse-ads-Ab3 sera to inhibit the binding of bio-auto-anti-id mAb on id-coated plates. In contrast, adsorption of increasing amounts of mouse-ads-Ab3 sera with Lx particles coated with a different isotype-matched anti-id mAb (3F10) had no effect (Fig. 5). This indicates that the inhibition observed was due to presence of the public idiotope on Ab3, and not to that of anti-mouse-Ig Ab. Lx coated with CT also failed to prevent inhibition of id-auto-anti-id mAb interaction by mouse-ads-Ab3 sera (not shown). This suggests that the mouse-ads-Ab3 sera also contained a quantity of Ab ($\text{id}^+ \text{Ag}^-$) recognizing the auto-anti-id mAb, but not CT, thus able to inhibit the id-auto-anti-id mAb interaction. Ab that both recognized auto-anti-id mAb and CT ($\text{id}^+ \text{Ag}^+$) were probably present in a smaller quantity or had a relatively low affinity for CT.

Production of Ab3 in rats and protection of their gut against CT challenge

All rats i.p. immunized with the auto-anti-id mAb (groups A and B) developed an anti-CT response (Table 2). In contrast, rats injected with the auto-anti-id ig (group C) did not develop any anti-CT response. In addition, those i.g.-immunized rats also had no ELISA-detectable serum Ab against mouse Ig epitopes, in contrast to groups A and B (data not shown). We also compared the i.p. auto-anti-id-immunized rats Ab3 anti-CT response to that obtained by immunization with CT via the same route. The anti-CT Ab3 titres of auto-anti-id-immunized rats, although much lower than those of CT-immunized rats, were significantly higher than those of control mAb-immunized rats (anti-CT Ab never exceeded 30 arbitrary units: not shown).

When Ab3-rats were intraintraintestinally challenged with CT to evaluate their protection,²⁰ both groups A and B of i.p.-immunized rats displayed a high P% (mean $\text{P}\% \pm \text{SEM}$ of 82 ± 6.5 and 94 ± 3 for groups A and B, respectively). In comparison, the P% of rats similarly i.p. immunized with another isotype-matched anti-id mAb (3F10, an Ab2 β)¹⁵ was only 29.25 ± 14.31 .

Table 2 lists the P% of individual auto-anti-id and CT i.p.-immunized rats of groups A and B together with their corresponding serum IgG and bile IgA anti-CT titres in arbitrary units.

DISCUSSION

In this report, an auto-anti-id mAb is described specific for a public idiotope present on several anti-CT Ab. Indeed, this auto-anti-id mAb recognized 6/8 anti-CT mAb with different specificities, as well as polyclonal anti-CT Ab from rats, rabbits and syngenic mice. The absence of competition between CT and the auto-anti-id mAb, and its efficiency to bind anti-CT mAb with different specificities, suggest that this auto-anti-id mAb was not a paratopic Ab2 β 'internal image', but rather an Ab2 α -type anti-id mAb. This Ab2 α induced the production of anti-CT Ab3 when injected into rats and rabbits. This was not surprising as anti-CT Ab from these two other species were also recognized by the auto-anti-id mAb. The first report on a vaccination effect in anti-id Ab-immunized animals dealt with protection of mice against African trypanosomiasis, by injections with a pool of three mouse polyclonal anti-id IgG. None, however, of these anti-id Ab competed with the antigen,

suggesting that they were Ab2 α anti-id¹¹ without internal image. Another group obtained a good response against hepatitis B surface antigen in mice, by immunization with rabbit polyclonal anti-id, also of the Ab2 α type.¹² Zhou *et al.*¹³ also immunized mice against human immunodeficiency virus (HIV) glycoprotein (gp41) by injection of a mAb2 α . We obtained an unexpectedly high P% against CT by immunizing rats with our auto-anti-id mAb, in comparison to our results with Ab2 β (3F10)-immunized rats by the same route¹⁵ ($\text{P}\% = 29$, instead of the present 82 and 94% for groups A and B, respectively). One explanation could be that the rat anti-CT Ab3 response induced by this auto-anti-id mouse mAb was more 'polyclonal' than that induced by the mouse internal image anti-id mAb, which could be nearly monoclonal. Thus, it sometimes seems more profitable to induce a 'polyclonal' Ab response for effective protection, especially when dealing with an antigen comprising many different epitopes, several of which could be necessary to stimulate the various aspects of immunity required for protection. This was well illustrated by Sacks *et al.*¹¹ who found that a cocktail of three anti-id Ab was more efficient than one anti-id alone. Our auto-anti-id mAb, by virtue of its specificity for a public idiotope, could replace such a mixture of several anti-id mAb. Huang *et al.*²² have compared the efficiency of one Ab2 β , one Ab2 α and the original antigen: phosphorylcholine, a protecting epitope of *Streptococcus pneumoniae*. They observed that the best way to immunize mice against *S. pneumoniae* was to prime them with Ab2 α before boosting with the antigen. They thought that the idiotope recognized by their Ab2 α was a regulatory idiotope of the anti-phosphorylcholine response. Our auto-anti-id could also be specific of such a regulatory idiotope. This could explain its efficiency as vaccine in comparison to another mAb2 β and to the CT itself.

Anti-id Ab specific for shared idiotopes are also attractive in the treatment of autoimmune diseases. It has been shown that many auto Ab detected in autoimmune disease bear shared idiotopes and that patients who recover from autoimmune disease have produced auto-anti-id Ab against such shared idiotopes.²³⁻²⁷

Rats i.g. immunized with our auto-anti-id (group C) produced no detectable serum anti-CT Ab3. Explanations could be that this immunization route induced the so-called 'oral tolerance' effect,²⁸ an explanation also suggested by their lack of production of anti-mouse Ig Ab (data not shown).

We were unable to explain our high rat P% against intestinal CT challenge, considering the i.p. route of auto-anti-id injections as well as the rather low concentrations of anti-CT IgA and IgG found in bile and serum. Indeed, it is known that secretory IgA Ab play a major protective role in intestinal protection against CT^{6,29,30} and that enteric administration should be the best way to induce such local sIgA Ab.

We have thus demonstrated that a non-internal image anti-id Ab was sufficient to vaccinate and protect against an intraintraintestinal challenge with CT, and that sometimes it could be more profitable to use this type of anti-id Ab rather than internal image anti-id Ab.

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