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Characterization of an anti-idiotypic MoAb bearing an internal image of the receptor-binding epitope of cholera toxin

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SUMMARY

A mouse anti-cholera toxin (CT) MoAb, mAb1, specific for the GM1-binding epitope of CT, was used to raise a syngenic anti-idiotypic MoAb, mAb2. Purified mAb2 was specific for mAb1 as shown by latex particle counting immunoassay and ELISA. Several experiments of competition between mAb2 and CT for binding to mAb1 demonstrated that mAb2 bore an internal image of the GM1-binding epitope of CT. Binding of mAb2 to GM1 unambiguously corroborated the mAb1-paratopic specificity of mAb2. Furthermore, mAb2 acted as a CT-surrogate antigen: rabbits injected with mAb2 produced some anti-CT antibodies, Ab3, which resembled mAb1 in specificity as expected. The potential use of this mAb2 as vaccine or as prophylactic agent to prevent CT from binding to its cellular receptor is discussed.

Keywords cholera toxin anti-idiotype GM1-ganglioside receptor mimickry

INTRODUCTION

The acute diarrhoeal disease, cholera, is mainly due to the effects of cholera toxin (CT) on the small intestinal epithelium [1]. Other major agents of diarrhoea in man and animals are the heat labile enterotoxins (LT) produced by enterotoxigenic *Escherichia coli* strains. LT are structurally and functionally related to CT [2,3]. The GM1 ganglioside is the major glycolipid membrane receptor for both CT and LT [1,4–6]. Although LT can bind to other cell receptors in humans [7] and rabbits [8,9], prevention of CT- or LT-binding to their GM1-receptor could be extremely useful as this is a necessary step in the disease process.

Anti-idiotypic antibodies are used to study structure–function relationships between ligands and receptors [10–12], such as hormone-receptor binding [13,14], or to purify a receptor [15], or to identify the sequence of the receptor-binding epitope of a ligand. In the case of pathogenic ligands, anti-idiotypic antibodies may be used as vaccine or prophylactic agent, by competition with the ligand for binding to its receptor. Such uses have been explored in the reovirus type 3 anti-idiotype system [16,17]. Anti-idiotypic antibodies are also useful to search for a pathogenic role of anti-receptor antibodies in autoimmune diseases [18,19].

Anti-idiotype vaccination has provided some protection and/or immune responses in various viral [16,17,20,21], parasitic [22] and bacterial [23,24] infections. We partly succeeded [25,26] in protecting rats against an intestinal CT-challenge by anti-idiotype vaccination. Here, we characterize a new mouse anti-idiotypic MoAb, mAb2, that mimics a receptor-binding epitope of CT and elicits anti-CT antibodies, Ab3, after injections into rabbits. The anti-CT specificity of Ab3 was very similar to that of mAb1, as predicted by the idiotype-network.

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MATERIALS AND METHODS

Production and purification of anti-idiotypic mAb2

The anti-CT mAb1 [27] used to produce anti-idiotypic mAb2, as described [26], was an IgG1 (1G4), specific for the B subunit of CT (CT-B). This mAb1 competed with CT for binding to GM1, as demonstrated both by ELISA and latex (Lx) agglutination immunoassay [27]. Another anti-CT IgG1 mAb1 (3B3) had the same apparent specificity as 1G4, but differed in its electrophoretic mobility [27]. Hence, when not specified, mAb1 may be 1G4 or 3B3. Only one specific anti-mAb1 IgM-secreting clone was detected by Lx-particle counting immunoassay (see below). This IgM (1G2.3H7), called mAb2, was purified on a mAb1 (1G4)-Sepharose immunoadsorbent (Iads), as described below. A control IgM anti-idiotypic MoAb, called control mAb2 (Co-mAb2), was raised against another anti-CT mAb1 (4C5) [26]. mAb2 and Co-mAb2 each mimicked a different CT-B epitope. An irrelevant anti-lipopolysaccharide (LPS) MoAb [28], as well as normal polyclonal mouse IgG and IgM [26], and polyclonal mouse anti-CT antibodies [29], were used as controls and/or Iads, as appropriate.

Coating of Lx-particles
Lx-particles (50 μl of 1% (w/v) Lx) were coated as detailed [27] with the following amounts: 100 μg of mAb1 (=mAb1-Lx), 100 μg of anti-LPS MoAb (=aLPS-MoAb), 100 μg of mAb2 (=mAb2-Lx), 50 μg of Co-mAb2 previously reduced with dithiothreitol [26] (=Co-mAb2-Lx) and 50 μg of GM1 (=GM1-Lx).

Immunoassays by Lx-agglutination
A mixture of 25 μl of glycine-buffered saline-bovine serum albumin (GBS-BSA) buffer (0·1 M glycine, 0·17 M NaCl, 10 mg/ml BSA, 6 mM NaN_3, adjusted to pH 9·2 with NaOH), 25 μl of coated Lx diluted 1:200 in GBS-BSA, and 25 μl of agglutinating agent was incubated for 30 min at 37°C with continuous vortexing. The reaction was stopped by dilution with 2·0 ml of GBS-buffer. Agglutination was assessed by particle counting, the diluted mixture being aspirated by a peristaltic pump and passed through the flow cell of an optical counter using forward light scattering. Only free, unagglutinated Lx-particles were registered as peak heights or as per cent of free Lx-particles [30,31]. The stronger the agglutination, the less free particles and the smaller the peak height, and conversely. Results are presented as per cent of free particles. Agglutination of GM1-Lx by mAb2 required a second enhancing antibody; this was anti-mouse-immunoglobulin antibody purified from rabbit anti-Co-mAb2 antiserum (see below) on a normal mouse immunoglobulin Iads (see below). Agglutination of mAb2-Lx and Co-mAb2-Lx by Ab3 and Co-Ab3, respectively, also required a second enhancing antibody, a mouse rheumatoid factor [32].

Inhibition of Lx-agglutination
GBS-BSA (25 μl) was replaced by 25 μl of inhibitor, which was preincubated either with the coated Lx or with the agglutinating agent for 15–30 min at 37°C, before performing the final reaction for an additional 15 min at 37°C. Results are expressed as per cent of inhibition calculated as follows:

$$\% \text{ inhibition} = 1 - \frac{\% \text{ of free particles with inhibitor}}{\% \text{ of free particles without inhibitor}} \times 100$$

Coated Lx used as Iads
GM1-Lx (100 μl 1:20 in GBS-BSA) was centrifuged for 10 min at 9000 ***g*** in a Biofuge. The pellet was vortexed with a 100 μl of mAb2 at 10 μg/ml and incubated for 2 h at 37°C. After centrifugation, mAb2 left in the supernatant was titrated by agglutination of mAb1-Lx. This was compared to agglutination by mAb2 not previously incubated with GM1-Lx. The same test was performed with Co-mAb2 to show that GM1-Lx did not bind any IgM MoAb.

Biotinylation of mAb2
After dialysis against 0·1 M $NaHCO_3$, pH 8·0, 1·0 ml of mAb2 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 h at room temperature, then extensively dialysed against PBS, yielding biotinylated mAb2 (bio-mAb2).

Coating of microtitre plates for ELISA
Microtitre plates (96 wells; Greiner, Nurtingen, Germany) were coated overnight at room temperature with CT (4·0 μg/ml) or mAb1 (5·0 μg/ml) in 0·2 M carbonate/bicarbonate buffer, pH 9·5. Residual binding sites were blocked with PBS containing 1% BSA and 0·1% Tween 20 (PBS-BSA-Tw). Washings (five times) were with PBS-Tw. Triplicate serial double dilutions of samples were applied (50 μl) in PBS-BSA-Tw.

Binding of bio-mAb2 to mAb1-coated plates
Increasing concentrations of bio-mAb2 were incubated for 2 h at 37°C on mAb1-, non-mAb1 anti-CT MoAb-, polyclonal mouse anti-CT antibody-, and normal polyclonal mouse immunoglobulin-coated plates for 1 h at 37°C. Bio-mAb2 binding 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°C. As positive control, sheep bio-IgG anti-mouse immunoglobulin (Amersham) diluted at 1:5000 in PBS-BSA-Tw was added to all plates and similarly revealed with HRP-Strav. After 3 min reaction with 0·04% (w/v) *o*-phenylene-diamine (Sigma) and 0·001% (v/v) H_2O_2 in 0·1 M citrate-0·2 M phosphate buffer (pH 5·0), development was stopped with 2 M H_2SO_4. OD was read at 620–405 nm in a Titertek Twinreader (Flow Laboratories, Brussels, Belgium). To compare differently coated plates, means of triplicate OD values of the anti-immunoglobulin positive control on a mAb1 (1G4)-coated plate were used as reference. ODs on other plates were multiplied by the mean OD of positive control on mAb1 (1G4)-coated plate divided by mean OD of the positive control on plates coated with other mouse immunoglobulins. Results were expressed as corrected mean OD ± s.e.m.

Inhibition of binding of bio-mAb2 on mAb1-coated plates
Increasing concentrations of mAb1, anti-CT MoAb, polyclonal normal mouse immunoglobulin or rabbit Ab3 (see below) were preincubated with bio-mAb2 at 1·0 μg/ml for 2 h at 37°C, before addition to mAb1-coated plates. Similarly, increasing concentrations of CT were preincubated on mAb1-coated plates before washings and addition of 1·0 μg/ml of bio-mAb2. After incubation, both assays were developed as above. Results were expressed as per cent of OD-inhibition by using the following formula:

$$\% \text{ of inhibition} = 1 - \frac{\text{mean OD with inhibitor}}{\text{mean OD without inhibitor}} \times 100$$

Binding, and inhibition of binding, of Ab3 on CT-coated plates
Triplicate serial double dilutions of CT-Iads affinity-purified Ab3 (see below) (=rabbit anti-mAb2) were incubated for 2 h at 37°C on CT-coated plates. Ab3 was revealed by goat bio-IgG anti-rabbit immunoglobulin (Sigma) at 1:5000 in PBS-BSA-Tw for 1 h, followed by HRP-Strav. Results were expressed as mean OD ± s.e.m. For inhibition, rabbit Ab3 dilutions were incubated with a fixed amount of CT (100 μg/ml) for 2 h at 37°C before addition to CT-coated plates. ELISA-revelation and expression of results were as above.

Sepharose-Iads
mAb1-Iads, as well as CT- and normal mouse immunoglobulin-Iads, were prepared as described [26,29].

Production, purification and absorption of rabbit Ab3-sera
Two rabbits were subcutaneously injected (five times, every fortnight) with 50 μg of mAb2, first in Freund's complete adjuvant (FCA) then in Freund's incomplete adjuvant (FIA),

and finally in PBS with 0·02% gelatin. Two other rabbits were similarly immunized with Co-mAb2 to obtain control Ab3 (Co-Ab3). Serum was obtained before the first and 7 days after each injection. Rabbit anti-CT Ab3 and Co-Ab3 were each affinity-purified on CT-Iads and then passed on normal mouse immunoglobulin-Iads to remove anti-mouse-immunoglobulin rheumatoid factor-like antibody.

RESULTS

mAb2 is a mAb1-specific anti-idiotype

mAb2 was selected because of its unique anti-idiotypic mAb1-specificity, as shown by several methods (Fig. 1). (i) mAb2 dose-dependently agglutinated mAb1-Lx, but not four other anti-CT MoAb-Lx nor the isotype-matched anti-LPS MoAb-Lx (Fig. 1a). (ii) In parallel, mab2-Lx was agglutinated only by mAb1, but not by four other anti-CT MoAbs nor by anti-LPS MoAb (Fig. 1b). Specificity of these experiments was verified by inhibition tests. Only mAb1, but not the four other anti-CT MoAbs nor anti-LPS MoAb, inhibited binding of mAb2 to mAb1 (not shown), confirming true anti-idiotypic specificity for mAb2. (iii) ELISA binding and binding-inhibition tests yielded similar results. Bio-mAb2 bound only to mAb1-coated plates, but not to plates coated with other anti-CT MoAbs, polyclonal mouse anti-CT antibodies or polyclonal normal mouse immunoglobulin (Fig. 1c). (iv) This binding was only inhibited by mAb1, but not by any other anti-CT MoAb, nor by polyclonal normal mouse immunoglobulin (Fig. 1d).

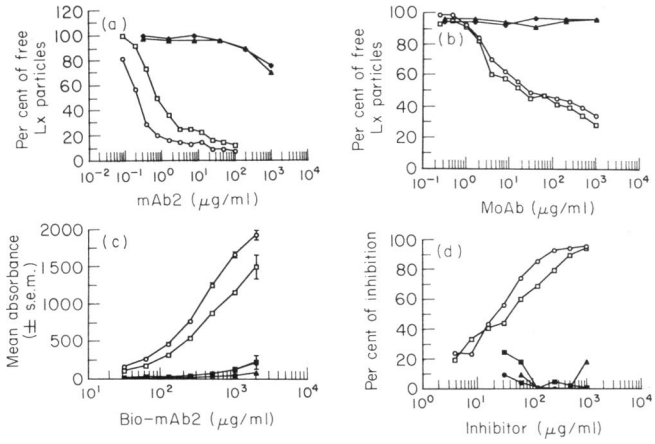

Fig. 1. Specificity of the mAb1-mAb2 reaction by Lx-agglutination. (a) Agglutination curves of mAb1-Lx (□, 1G4; ○, 3B3), non-mAb1 anti-CT MoAb-Lx (▲; only one of four different anti-CT MoAb-Lx is shown), and isotype-matched anti-lipopolysaccharide (LPS) MoAb-Lx (◆) induced by increasing concentrations of mAb2. (b) Agglutination curves of mAb2-Lx by increasing levels of mAb1 (□, 1G4; ○, 3B3), non-mAb1 anti-CT MoAb (▲; only one of four different anti-CT MoAb-Lx is shown), and isotype-matched anti-LPS MoAb (◆). (c) Binding of bio-mAb2 (1 μg/ml) on ELISA-plates coated with mAb1 (□, 1G4; ○, 3B3), non-mAb1 anti-CT MoAb (■; mean of four different non-mAb1 anti-CT MoAb), normal polyclonal mouse immunoglobulin (▲), and with polyclonal mouse anti-CT immunoglobulin (●), as revealed by horseradish peroxidase-coupled streptavidin (HRP-Strav). Absorbance values represent corrected means of triplicate wells. (d) Inhibition of (c) by increasing concentrations of mAb1 (□, 1G4; ○, 3B3), non-mAb1 anti-CT MoAb (■; mean of four different anti-CT MoAb), normal polyclonal mouse immunoglobulin (▲), and polyclonal mouse anti-CT immunoglobulin (●), all preincubated with 1·0 μg of bio-mAb2.

The mAb1-mAb2 system is thus a true idiotype-anti-idiotype interaction. We next assayed if mAb2 bore an internal image of CT, by inhibition of mAb1-binding to mAb2 with CT.

mAb2 bears an internal image of CT

Various CT-competition tests were attempted: (i) saturation of mAb1-Lx by CT entirely inhibited its agglutination by mAb2 (Fig. 2a); (ii) similarly, mAb1 preincubated with CT no longer agglutinated mAb2-Lx (Fig. 2b); (iii) by ELISA also, bio-mAb2 bound less to mAb1-coated plates preincubated with increasing levels of CT (Fig. 2c).

Thus, reacting the mAb1-paratope with CT prevented binding of mAb2 to the mAb1-CT complex. Two explanations were possible: (i) mAb2 binds to a mAb1-idiotope which is hidden when CT is bound to its paratope (= steric hindrance); or (ii) mAb2 binds as well as CT in the mAb1-paratope, meaning that mAb2 mimics CT. To confirm the paratopic specificity of mAb2, binding of mAb2 to the GM1-binding site of CT should be demonstrated because mAb1 had the same CT-reactivity as GM1 [27]. A direct mAb2-GM1 interaction would corroborate this hypothesis.

mAb2 binds to GM1

Figure 3 illustrates mAb2-binding to GM1; (i) mAb2, but not Co-mAb2, bound to, but did not agglutinate, GM1-Lx; to achieve this, anti-mouse-immunoglobulin antibody had to be added. However, to avoid interferences due to reaction between anti-mouse-immunoglobulin antibodies and non-Lx-bound mAb2 or Co-mAb2, fixed amounts of mAb2 and Co-mAb2, both preincubated with GM1-Lx, received increasing doses of

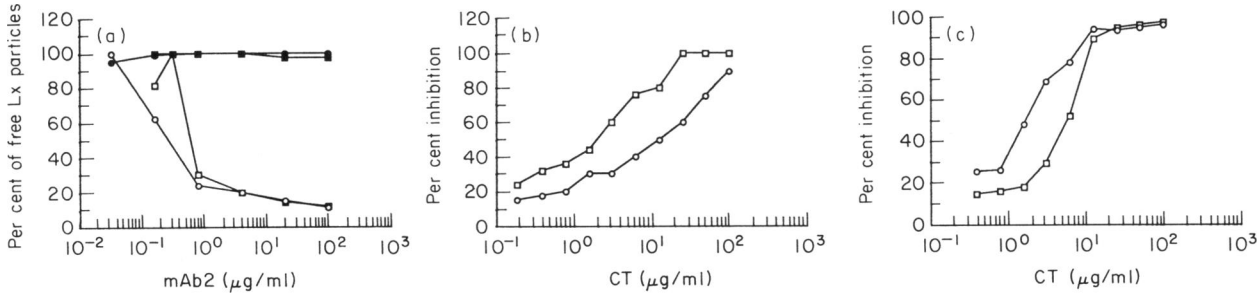

Fig. 2. Competition between mAb2 and CT for binding to mAb1. (a) Agglutination of mAb1-Lx (□, 1G4-Lx; ○, 3B3-Lx), preincubated in presence (closed symbols) or in absence (open symbols) of 1 mg/ml CT, by increasing levels of mAb2. (b) Inhibition, by preincubation with increasing concentrations of CT, of agglutination of mAb2-Lx by mAb1 (□, 1G4 50 μg/ml; ○, 3B3 500 μg/ml). (c) Inhibition of binding of bio-mAb2 (1 μg/ml) to mAb1 (□, 1G4; ○, 3B3)-coated ELISA plates, by increasing concentrations of CT, as revealed by horseradish peroxidase-coupled streptavidin (HRP-Strav).

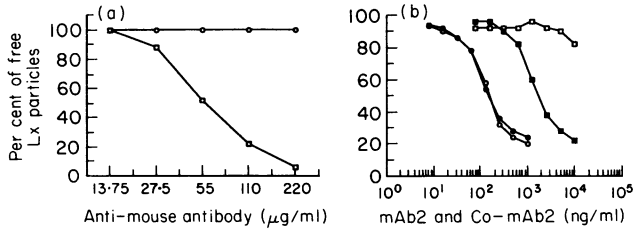

Fig. 3. Binding of mAb2 to GM1. (a) Double antibody agglutination of GM1-Lx by a constant amount of mAb2 (25 μg/ml, □), or of Co-mAb2 (25 μg/ml, ○) and increasing concentrations of rabbit anti-mouse-immunoglobulin antibody. (b) Immunoabsorption of mAb2, but not of Co-mAb2, by GM1-Lx. After incubation (open symbols) or not (closed symbols) with pelleted GM1-Lx, residual mAb2 (□) or Co-mAb2 (○) was titrated by its agglutination of mAb1 (1G4)-Lx or of non-mAb1, anti-CT MoAb (4C5)-Lx.

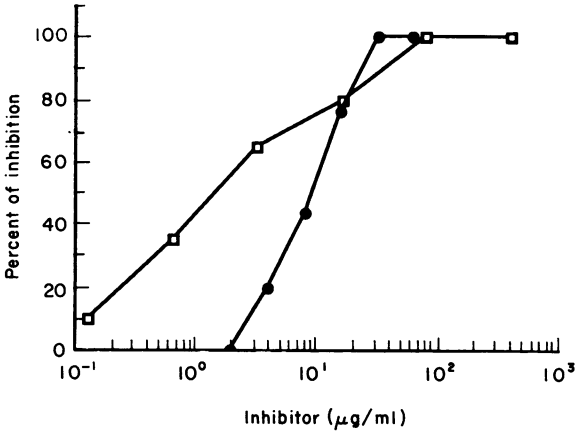

Fig. 4. Preincubation of GM1-Lx with increasing concentrations of CT (□) and heat labile enterotoxin (LT) (●) inhibit the double antibody agglutination of GM1-Lx by mAb2 (10 μg/ml) followed by anti-mouse-immunoglobulin antibody.

anti-mouse-immunoglobulin antibody. Double antibody agglutination of GM1-Lx was then obtained (Fig. 3a); (ii) GM1-Lx functioned as an Iads for mAb2, but not for Co-mAb2 (Fig. 3b). The lack of adsorption of Co-mAb2 after incubation with GM1-Lx shows that this adsorption was specific. In ELISA also, mAb2 bound to GM1-coated plates (not shown). But this assay was not very significant as the ODs were rather low (maximal OD = 0·7), requiring both high levels of GM1-coating (1·0 mg/ml) and of bio-mAb2 (100 μg/ml).

CT inhibition of binding of mAb2 to GM1 should confirm that mAb2 mimics a receptor-binding epitope of CT.

mAb2 competes with CT and LT for binding to their GM1 receptor

Both CT and LT toxins have cell membrane-bound GM1 as major receptor. Preincubation of GM1-Lx with increasing concentrations of CT totally inhibited its agglutination by mAb2 plus anti-mouse-immunoglobulin antibodies. Also, increasing doses of closely related LT inhibited mAb2–GM1 interaction (Fig. 4).

Collectively, these results confirm that: (i) mAb2 is bearing an internal image of the CT receptor-binding epitope; and (ii) mAb1 has the same CT-reactivity as GM1. Our anti-idiotypic mAb2 could thus represent a surrogate antigen for CT, as tested by immunizing rabbits with mAb2.

Rabbits immunized with mAb2 produce some mAb1-like Ab3

Rabbits immunized with mAb2 were assayed for anti-CT Ab3 by measuring the binding of rabbit antibodies to CT-coated plates in ELISA. Two other rabbits, similarly immunized with Co-mAb2, also produced anti-CT antibodies (Co-Ab3), because Co-mAb2 also bore an internal image of a different epitope of CT [26]. Ab3 and Co-Ab3 were compared: (i) both specifically bound to CT-coated plates. The ODs obtained when increasing amounts of CT-Iads affinity-purified Ab3 and Co-Ab3 were incubated on CT-coated plates, in presence and absence of a constant amount of CT (100 μg/ml), are shown in Fig. 5a. These data (CT affinity-purification and binding on CT-coated plates) demonstrate that both rabbit groups had developed anti-CT antibodies; (ii) after additional purification of Ab3 and Co-Ab3 on normal mouse immunoglobulin-Iads, to remove anti-mouse rheumatoid factor activity coeluted with anti-CT antibodies, we tested anti-CT Ab3 for agglutination of both mAb2-Lx and Co-mAb2-Lx. Purified Ab3 still agglutinated mAb2-Lx, but not Co-mAb2-Lx, an isotype-matched

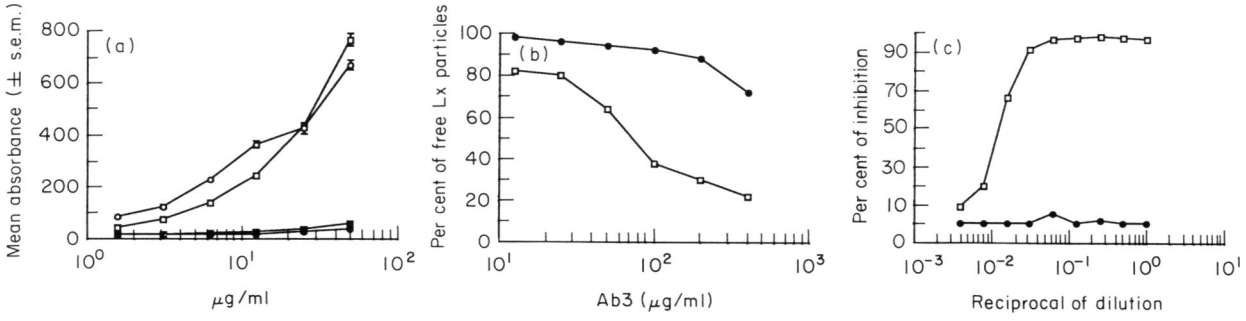

Fig. 5. Reactivities of Ab3. (a) Binding of affinity-purified Ab3 (□) and Co-Ab3 (○) in presence (closed symbols) or absence (open symbols) of a constant amount (100 μg/ml) of CT on CT-coated plates, revealed by ELISA. (b) Agglutination of mAb2-Lx (□), but not of Co-mAb2-Lx (●), by increasing concentrations of affinity-purified Ab3. (c) Inhibition of 1 μg/ml of bio-mAb2-binding on mAb1-coated plates by increasing concentrations of Ab3 (□) and Co-Ab3 (●).

control (Fig. 5b), and conversely (not shown). Both Ab3 and Co-Ab3 were thus adequately absorbed for anti-mouse-immunoglobulin reactivity and specific for their respective immunizing anti-idiotype. This also means that anti-CT Ab3 recognizes both CT and mAb2. Theoretically, polyclonal anti-CT antibodies could recognize mAb2 and Co-mAb2 as these both bear an internal image of CT (except perhaps if the mimicked epitope is genetically restricted). The lack of cross-reaction between Co-mAb2 and Ab3, and between mAb2 and Co-Ab3, shows that these interactions were highly specific and not due to any spontaneous rabbit anti-CT antibodies. Thus, these Ab3 should have the same specificity as mAb1, and this was also tested; (iii) indeed, Ab3 competed well with mAb1 for binding to mAb2. Preincubation of bio-mAb2 with increasing concentrations of Ab3, but not at all of Co-Ab3, inhibited the binding of bio-mAb2 to mAb1-coated plates (Fig. 5c). This result confirmed the mAb2-specificity of Ab3. Such competition between Ab3 and mAb1 for binding to mAb2 was also obtained by Lx-agglutination assay (not shown).

A particular feature of mAb1 was to ignore CT bound to its GM1 receptor [27]. So far, however, no significant difference has been found between bindings of Ab3, or Co-Ab3, onto CT- and GM1-CT-coated plates (not shown). This means that the Ab3 do not recognize perfectly the receptor binding-epitope of CT as they still bind to CT even if CT is previously fixed to GM1.

DISCUSSION

A specific idiotype-anti-idiotype reaction was demonstrated between mAb1 (1G4 or 3B3) and our affinity-purified IgM mAb2 by several methods: (i) mAb2 only agglutinated mAb1-Lx; (ii) mAb1 agglutinated mAb2-Lx, but not isotype-matched Co-mAb2-Lx; (iii) mAb2 only bound to mAb1-coated plates; (iv) only mAb1 inhibited this binding. Furthermore, mAb2 bore an internal image of CT, competing with CT for mAb1-binding: (i) mAb1-Lx agglutination by mAb2 was totally prevented by preincubation with CT; (ii) preincubation of CT with mAb1 completely prevented agglutination of mAb2-Lx by mAb1; (iii) CT inhibited binding of mAb1 to mAb2-coated plates. The anti-CT mAb1 (1G4) [27] used to raise this mAb2 recognized a GM1 receptor-binding epitope of CT, and thus reacted with CT like GM1. Proof of this mimickry relied on mAb2-binding to GM1, and its inhibition by CT and LT (Figs 3 and 4). However, the mAb2–GM1 interaction was rather weak. Being an IgM, mAb2 should strongly agglutinate GM1-Lx; however, a second antibody was required to achieve agglutination. Similarly, mAb2-binding on GM1-coated plates required both high levels of GM1-coating (0·1–1·0 mg/ml instead of 2 μg/ml for CT binding) and high concentrations of mAb2. Furthermore, GM1 did not interfere with the Ab3–CT interaction, whereas it did for mAb1–CT. Several hypotheses might explain this problem.

First, mAb2 had a low affinity for mAb1 (1G4 or 3B3), as compared with that of Co-mAb2 (3F10) for its corresponding mAb1 (4C5), based on concentration of NH_4SCN necessary to inhibit by 50% the agglutination of mAb1-Lx by mAb2 (not shown). This suggests that mAb2 did not fit perfectly into the paratope of mAb1, and thus mAb2 could bear an inaccurate image of the receptor-binding epitope.

Moreover, the paratope of mAb1 could be an inaccurate copy of a GM1-binding site of CT. If so, mAb2, although bearing an internal image of CT, would also not exactly mimic the natural receptor-binding epitope of CT. These considerations suggest that it would be preferable to produce another mAb2, with a higher affinity for mAb1 and GM1. This could prove very difficult, as this mAb2 could then react with a self-antigen (GM1 is present, albeit at very variable densities [1,4], on membranes of most mammalian cells).

The significant anti-CT Ab3 response elicited by mAb2, despite its low affinity, suggests that a better mAb2 could be a good vaccine candidate, as well as a prophylactic agent, by competition with CT or LT for the GM1 receptor. *In vivo* blocking of GM1 receptors by CT-B has already been tested with some success in mice [33], rabbits [4,5,34] and humans [35]. Such prophylaxis could decrease the risk of severe disease among infected family members and provide a new direction in the prevention of cholera and related diarrhoeas, as discussed by Glass *et al.* [35]. However, due to fast human enterocyte turnover (72 h) and the amount (5·0 μg) of CT needed to induce severe diarrhoea in volunteers, GM1-blocking agents would have to be used frequently in large doses. The feasibility of mass production of MoAbs renders this possibility attractive; the appropriate mAb2 used as 'blocking agent' entirely protected monkeys against emesis and diarrhoea upon duodenal challenge with staphylococcal enterotoxin B [36].

The molecular mimickry between CT and our mAb2 could be due to a homology of amino acid sequence. The nucleotide-deduced amino acid sequence of the complementary determining regions of both V_H and V_L of mAb2 can easily be obtained. One of these regions could have a short sequence stretch identical to a stretch in CT-B. Such homology was found in three antigenic systems: reovirus type 3 [37], synthetic glutamic-alanine-tyrosine polypeptide or GAT [38], and the thyrotropin system (Taub *et al.*, cited in Nisonoff [39]). However, this homology is not frequent and may require linear, non-conformational epitopes. The GM1-binding epitope(s) of CT is not yet well defined (for review see [40,41]); in particular, it is not known if, in CT and CT-B, the GM1-binding epitope is conformational or linear.

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