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Functional Idiotypic Mimicry of an Adhesion- and Differentiation-Promoting Site on Acetylcholinesterase

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Abstract Acetylcholinesterase mediates cell adhesion and neurite outgrowth through a site associated with the peripheral anionic site (PAS). Monoclonal antibodies raised to this site block cell adhesion. We have raised anti-idiotypic antibodies to one of these antibodies. The anti-idiotypic antibodies recognized the immunogenic antibody and non-specific mouse IgG, but not acetylcholinesterase. Five antibodies (out of 143 clones, an incidence of 3.5%) were able to promote neurite outgrowth in human neuroblastoma cells in vitro in a similar manner to acetylcholinesterase itself, suggesting that these antibodies carry an internal image of the neuritogenic site. Two of the antibodies were significantly more effective ($P < 0.01$) than acetylcholinesterase in this regard. The antibodies also bound specifically to mouse laminin-1 and human collagen IV, as does acetylcholinesterase. This binding was displaced by unlabelled antibody, as well as by acetylcholinesterase itself, indicating competition with acetylcholinesterase. We have also investigated the development of anti-anti-idiotypic antibodies in mice in vivo, and have observed that four of these (out of 318 clones, an incidence of 1.26%) mimic the idiotypic antibody and abrogate adhesion in neuroblastoma cells. We have thus demonstrated functional mimicry of the neuritogenic site on acetylcholinesterase in anti-idiotypic antibodies, enhancement of this activity in one antibody, and mimicry of the idiotypic antibody site in anti-anti-idiotypic antibodies. Implications of these findings for differentiation-promoting cancer therapy are discussed. J. Cell. Biochem. 91: 999–1009, 2004.

Key words: acetylcholinesterase; peripheral anionic site; cell adhesion; idiotypic mimicry

Apart from its traditional role in synaptic neurotransmission, acetylcholinesterase (EC 3.1.1.7; AChE) is associated with cell adhesion and differentiation [Layer and Willbold, 1995] as well as apoptosis [Yang et al., 2002] of neural cells, and differentiation and apoptosis of haematopoietic cells [Soreq et al., 1994]. Deletions of the ACHE gene have been observed in myelodysplastic syndrome and acute amyeloblastic leukemia [Stephenson et al., 1996]. In the embryo, anatomical studies have shown that transient expression of AChE coincides with axonal growth from primitive neural cells [Drews, 1970]. Culture of neural cells in vitro with exogenous AChE [Jones et al., 1995; Small et al., 1995; Srivatsan and Peretz, 1997; Bataillé et al., 1998; Johnson and Moore, 2000; Day and Greenfield, 2002; Olivera et al., 2003] or transfection with AChE [Karpel et al., 1996; Koenigsberger et al., 1997; Sternfeld et al., 1998; Bigbee et al., 2000; De Jaco et al., 2002] promotes neurite outgrowth. This function is non-cholinergic, as indicated by the lack of effect of AChE active site inhibitors [Layer et al., 1993; Small et al., 1995; Johnson and Moore, 2000] and is instead mediated by a structural site, localized to the peripheral anionic site (PAS) [Johnson and Moore, 1999; Munoz et al., 1999]. The PAS is composed of five, largely aromatic residues, clustered around the entrance to the active site gorge. Associated with it are a number of omega loops. The site binds, but does not hydrolyze, acetylcholine as well as a variety of inhibitors. It is implicated in the allosteric modulation of catalysis at the active centre [Barak et al., 1994], interaction with the amyloid beta-peptide in the formation of

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amyloid deposits [De Ferrari et al., 2001], as well as binding the basement membrane components laminin-1 and collagen IV [Johnson and Moore, 2003].

We have previously observed that treatment of neuroblastoma cells with PAS-reacting monoclonal antibodies results in a loss of cell–substrate adhesion, followed by apoptotic cell death [Johnson and Moore, 2000], results consistent with anoikis. Similar, albeit less dramatic, results are found with AChE inhibitors, such as BW284c51 and propidium that bind to the PAS [Layer et al., 1993; Sharma et al., 2001]. These results suggest that the AChE PAS, or structures resembling it, are essential for the adhesion and differentiation of neural cells. The identification of the site invited the question of whether it could be used to induce differentiation and apoptosis of neural tumours, such as neuroblastoma, in vivo. Differentiation-inducing agents, such as retinoids, vitamin D, and short-chain fatty acids, have been found useful in inducing cell maturation, but have significant toxicity on long-term administration [Miller, 1998; Evans and Kaye, 1999]. AChE also promotes differentiation and apoptosis in hematopoietic precursors [Soreq et al., 1994]; although neither the mechanism nor the site of action have been identified, it is possible that these are similar or identical to those found in neural cells. This could extend the usefulness of the therapy to leukemias. It is obviously impossible to use AChE itself, nor is it possible to isolate the site as it is conformational rather than continuous. One alternative is rational drug design, based on a detailed structural knowledge of the site. Another possibility is the presentation of the site in a different, neutral, context, using idiotypic mimicry.

According to the idiotypic network theory of Jerne [1974], the immune system responds to foreign substances as a regulatory network composed of idiotypes and their anti-idiotypes. Antibodies may thus be elicited against the variable regions of other antibodies. Of particular interest are those that recognize the antigen-combining site (idiotype) of the eliciting antibody (the Ab1). These secondary (anti-idiotypic or Ab2) antibodies may have, in their combining sites, structures complementary to the idiotype of the Ab1, and thus may resemble the original antigen. Such antibodies are said to bear the "internal image" of the antigen. The concept of internal image does not imply that the Ab2 carries an exact replica of the antigenic site [Bona, 1996]. It is more likely to represent an image of the epitope, with perhaps only a few vital contact residues. Because internal image anti-idiotypic antibodies may elicit similar biological responses as the antigen, they have proved useful in identifying receptors and ligands [McGuigan, 1994], mimicking the effects of hormones [Garcia et al., 1992; Pan et al., 1995], as "vaccines" for the treatment of viral, bacterial, and fungal infections [Beninati et al., 2001] and as an immunotherapy for various cancers [Bhattacharya-Chatterjee et al., 2001; Wang et al., 2001]. In these cases, the anti-idiotypic antibodies mimic tumor antigens, allowing the development of an immune response against the tumor.

Our aim in this study was to present the adhesion- and differentiation-mediating site in the context of an immunoglobulin, and to investigate its potential as a differentiation-inducing therapy in neuroblastoma cells in vitro. We have raised anti-idiotypic (Ab2) antibodies to an anti-AChE antibody (Ab1) that recognizes the site, and investigated their ability to induce differentiation of neuroblastoma cells in vitro. We have also investigated whether these Ab2s produce Ab3s, or anti-anti-idiotypic antibodies, in vivo.

MATERIALS AND METHODS

Reagents

The following reagents were obtained from Sigma: human erythrocyte AChE (cholinesterase, acetyl, C5400), recombinant human AChE (C1682), Freund's Complete and Incomplete adjuvants, Protein-A-Sepharose 4B, serum- and protein-free hybridoma medium, mouse IgG and IgM, biotinylated anti-mouse IgG and IgM, streptavidin peroxidase, mouse laminin-1, human collagen IV, poly-L-lysine, bovine serum albumin, and the N-hydroxysuccinimide biotin ester. Tissue culture medium Dulbecco's MEM and RPMI) and fetal bovine serum were obtained from Delta. 2,2′-azinobis-3-ethylbenzthiazoline sulfonic acid (ABTS) was obtained from Boehringer Mannheim.

Immunization of Mice

Monoclonal antibody (MAb) E8 reacts with the PAS of human AChE; it is also catalytic and blocks cell adhesion [Johnson and Moore, 1995]. The antibody was purified from ascites fluid by standard protocols [Harlow and Lane, 1988] on

protein-A-Sepharose 4B, using low salt buffers, and appeared homogeneous on SDS–PAGE. Fifty micrograms of antibody, in Freund's complete adjuvant, was injected into each of six BALB/c mice. The initial immunization was subcutaneous and subsequent immunizations (at 3 week intervals and in incomplete adjuvant) were intraperitoneal. Mice were sacrificed after 9–10 weeks. The care and handling of animals was approved by the Animal Research Ethics Committee of the University of Stellenbosch.

Culture of Hybridomas and Purification of Antibodies

Spleen lymphocytes were fused with mouse myeloma cells of the non-secreting SP2/0-Ag14 line, according to standard protocols [Harlow and Lane, 1988]. Hybridomas were screened by ELISA for the production of IgM and IgG and anti-AChE antibodies (see Immunoassays below). Positive hybridomas were cloned twice by limiting dilution onto mouse spleen cell feeder layers and the cultures expanded. The last two passages before harvesting of antibody were conducted in serum- and protein-free tissue culture medium. Cells were washed twice with phosphate-buffered saline at subculture. IgG was purified on protein-A-sepharose 4B, using low salt buffers [Harlow and Lane, 1988]. No significant IgM antibodies were found. The IgG-containing fractions were identified and quantitated by measuring their absorbance at 280 nm, and ELISA using biotinylated anti-mouse IgG as probe.

Culture and Differentiation of Neuroblastoma Cells

Cell line. The human neuroblastoma cell line N2α was obtained from Highveld Biological, Johannesburg, South Africa. Cells were cultured in Dulbecco's MEM with 10% fetal bovine serum, at 5% CO_2 in 250 ml tissue culture flasks (Nunc, Denmark).

Assessment of neurite outgrowth. Coverslips were pre-coated for 1.5 h at 37°C with 0, 10, 20, 30, or 40 μg/ml AChE or IgG in serum-free medium in 6 well plates (Nunc, Denmark). These values are based on previous experiments [Johnson and Moore, 2000]. After incubation, the solution was suctioned off, the coverslips washed twice with phosphate buffered saline, and cells plated at 2×10^4/ml in serum-free medium. Cells were grown for 48 h, fixed (50% methanol, 50% acetone, 5 min at room temperature, and washed three times in phosphate buffered saline) and examined by phase contrast microscopy. Images of cells and neurites were recorded, and at least 100 cells assessed for each experiment. Processes extending from the cells were measured, and cells were considered to be differentiated or "with neurites" if the processes were equal to at least twice the diameter of the cell body [Shea et al., 1991]. The lengths of neurites, taken from the cell body to the base of the growth cone, were also measured from photographic images.

Assessment of adhesion. Cells (2×10^4/ml) were plated with 0, 10, 20, 30, or 40 μg/ml AChE or IgG in serum- and protein-free medium in 96 well plates. After 48 h, unattached cells were removed from triplicate wells by pipetting, and retained. Each well was then rinsed with 0.2 ml phosphate buffered saline, and the rinsing solution combined with the unattached cells and counted in a hemocytometer (Improved Neubauer). The remaining, attached, cells were removed with Versene and counted. These two values give the total numbers of unattached and attached cells in the well, respectively. The number of attached cells was expressed as a percentage of the total (attached + unattached) and described as the "percentage adherent." The viability of both attached and unattached cells was assessed at the time of counting by their ability to exclude 0.25% trypan blue. This was expressed as a mean value for the total number of cells.

Immunoassays

Immunoassays for the detection of antibody specificity were conducted by the ELISA sandwich method, using 10 μg/ml MAb E8, nonspecific mouse IgG and IgM, and recombinant human AChE as plate coating. Biotinylated anti-mouse IgG and IgM, streptavidin peroxidase, and H_2O_2/ABTS were used for detection. The blocking solution was 5% bovine serum albumin in phosphate buffered saline, which was also used for dilution of reagents. The washing solution was 0.1% Triton X-100 in phosphate buffered saline.

Binding to Laminin-1 and Collagen IV

Purified IgG and recombinant human AChE were biotinylated by the method of Bhakdi et al. [1989] using the *N*-hydroxysuccinimide biotin ester. No reduction in the AChE enzyme activity was noted after biotinylation, suggesting

conformational integrity of the enzyme. Similarly, no differences were observed between the binding of biotinylated or non-biotinylated Abs to laminin and collagen, measured by anti-mouse IgG. Laminin and collagen, as well as poly-L-lysine and BSA as controls were used at concentrations of 1.25–20 μg/ml in 50 mM $NaHCO_3$, pH 8.0, buffer to coat microtiter plates (Costar) overnight at 4°C. After blocking, biotinylated antibodies or AChE (as positive control) were added at dilutions of 150 ng/ml–20 μg/ml (2–266 nM IgG, based on a molecular weight of 150 kDa and allowing for two binding sites; 2–286 nM AChE, based on a molecular weight of 70 kDa for one catalytic subunit) and incubated for 3.5 h at room temperature. The buffer in all cases was 0.1 M Tris, pH 7.4. The amount of IgG or AChE bound was probed with streptavidin-peroxidase, and the H_2O_2/ABTS substrate/detector solution, and the absorbance at 405 nm measured. Nonlinear regression analysis of binding data was performed with the program SigmaPlot™.

As a measure of the specificity of AChE binding, the ability of unlabelled IgG or AChE to compete with the labeled proteins was assessed. Serial dilutions of unlabelled IgG or AChE (12.5–400 ng/ml; 92.8 pM–2.95 nM IgG; 180 pM–5.72 nM AChE) were incubated, simultaneously, with the concentrations of biotinylated IgG or AChE described above.

Statistics

Binding data was analyzed by non-linear regression using SigmaPlot. Statistical significance was calculated with Student's *t*-test, for two-tailed samples, using the same program.

RESULTS

The Antibodies

The Ab1 MAb E8, which was used as immunogen, was raised against human erythrocyte AChE, and recognizes the PAS [Johnson and Moore, 1999]. It is also catalytic, with esterase-like activity, and the catalytic site and idiotype are indistinguishable [Johnson and Moore, 2002].

The Ab2s, anti-idiotypic antibodies, were the result of immunization with MAb E8 for a relatively short period of time (9–10 weeks). This is sufficient for the development of an immune response to the antigen, but not sufficient for the development of Ab3s, the next step in the idiotypic cascade [Shoenfeld, 1994]. A total of 143 antibody-producing clones were obtained from two fusions. Potential AChE mimics were isolated by their ability to inhibit the catalytic activity of the Ab1 and thus to recognize the idiotype; five antibodies fulfilled these criteria, an incidence of 3.49% (Fig. 1A). These antibodies recognized MAb E8 and mouse IgG, but not AChE.

The Ab3s (anti-anti-idiotypic) resulted from immunization with MAb E8 for a relatively long (>6 months) period of time, allowing for the development of both Ab2s and Ab3s in vivo. Three hundred and eighteen clones were produced from two fusions. The antibodies produced by these clones were analyzed for their ability to recognize MAb E8. Antibodies that recognized MAb E8 were classified as Ab2 (92 clones); those that did not, as Ab3 (226 clones). Many of the Ab3s recognized AChE (Fig. 1B). Four of the Ab3s were found to be catalytic (an incidence of 1.26%): these have been characterized and described elsewhere [Johnson and Moore, 2002].

Effects of Ab2s and Ab3s on Cell Adhesion and Neurite Outgrowth in Neuroblastoma Cells

The five Ab2s that inhibited MAb E8 all showed a significant ability to promote neurite outgrowth in human neuroblastoma cells in vitro (Fig. 2 and Table I) when compared to cells cultured either with no antibody or a non-specific antibody. The neurite promoting ability of two of the antibodies (MAbs 17B and 11C) was somewhat less than that of AChE (34.15 ± 1.61 and $27.62 \pm 1.00\%$ cells exhibiting neurite outgrowth, respectively, compared to $38.57 \pm 2.95\%$ for cells cultured with AChE). However, while MAb 210G induced 40.42 ± 1.01 cells to grow neurites, the figures for MAbs 211E and 29H were 86.47 ± 3.18 and $52.32 \pm 2.32\%$ respectively, significantly higher than AChE ($P < 0.01$; $n = 4$, in all cases). Cells incubated with MAbs 211E, 29H, 210G, and 17B also showed neurite lengths significantly longer than controls; in cells incubated with MAbs 211E and 29H, neurite lengths were significantly greater than those induced by AChE.

Most Ab3s had no effect on neuroblastoma cells (percentage adherent cells >95%; neurite outgrowth in $8.51 \pm 1.75\%$ of cells; $n = 29$); however, the four catalytic Ab3s (MAbs 13B9C, 13B9F, 12D11F, 13A) showed a significant inhibition of cell adhesion (Table I). No enhancement

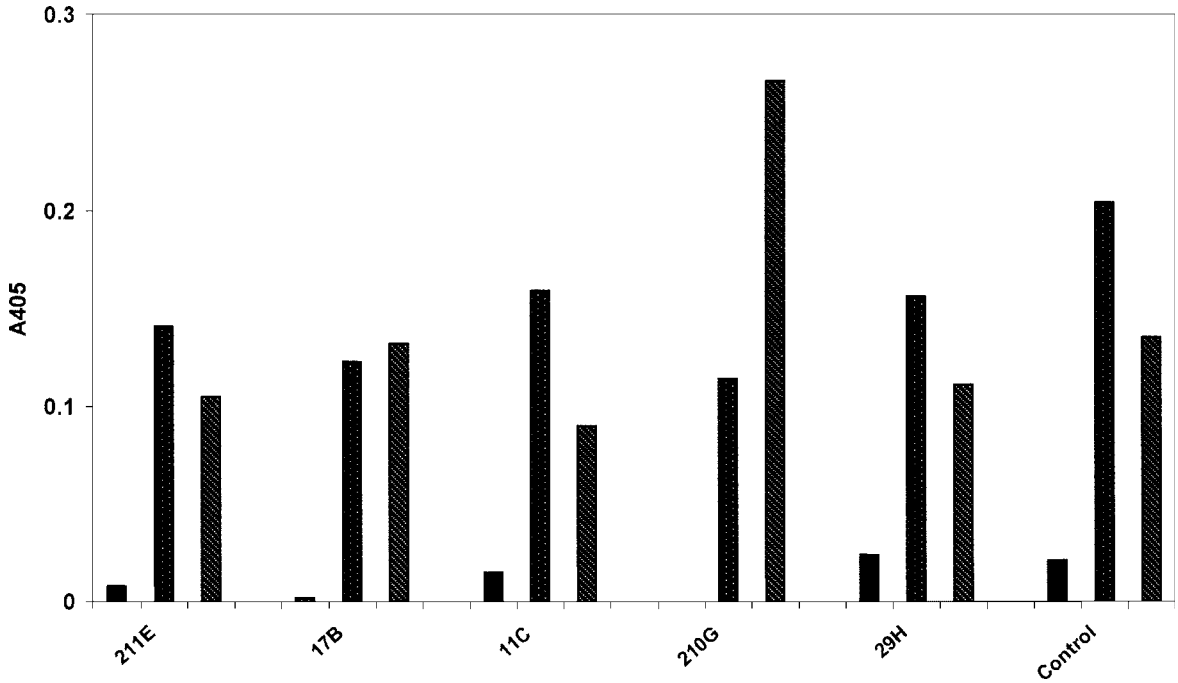

Fig. 1. Recognition of AChE, MAb E8, and mouse IgG by Ab2s. Microtitre plates were coated with 2 μg ml^{-1} (100 ng $well^{-1}$) recombinant human AChE (column 1), MAb E8 (column 2), or mouse IgG (column 3), respectively. Antibodies were added at 375 ng $well^{-1}$. The amount of antibody bound was probed with biotinylated anti-mouse IgG, streptavidin peroxidase, and H_2O_2/ABTS color reagent. Values are given as A405. Background values (in the absence of MAb) were, 0.115 ± 0.002; $n = 8$ in all cases.

of neurite outgrowth by these antibodies was seen.

Binding of Ab2s to Laminin-1 and Collagen IV

The five antibodies were found to bind both laminin-1 (Fig. 3A) and collagen IV (Fig. 3B). No significant binding to either poly-L-lysine or BSA was observed (data not shown). K_d values are shown in Table II. The binding is displaced by addition of unlabelled antibody (data not shown). The five Ab2s also compete with AChE for binding (Fig. 4A,B).

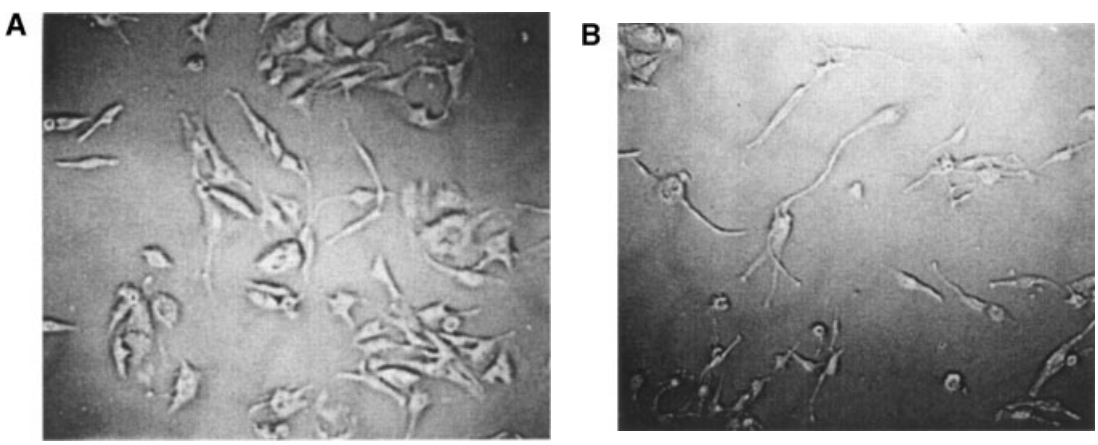

Fig. 2. Phase-contrast micrographs of N2α neuroblastoma cells after 48 h in culture. **A**: Control (non-specific MAb); (**B**) cells cultured with 40 μg/ml AChE. **C**: Cells cultured on 40 μg/ml MAb 211E.

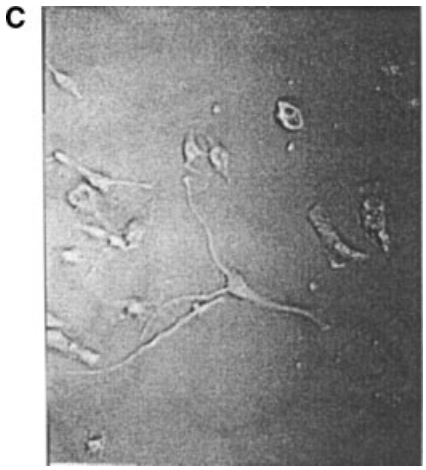

Fig. 2. (*Continued*)

DISCUSSION

We have demonstrated functional mimicry of AChE's adhesion-mediating site in the anti-idiotypic antibodies, shown by their ability to promote neurite outgrowth significantly better than controls (either no antibody, or non-specific antibodies), approaching or surpassing AChE's ability to do so. Considerable variation, however, was observed in their neuritogenic capability. It is also noteworthy that the antibodies all recognize non-specific mouse IgG to varying degrees, indicating that they are not wholly idiotype-specific. We have previously described idiotypic mimicry of MAb E8 in anti-anti-idiotypic Abs [Johnson and Moore, 2002]: it was interesting that, in the catalytic mimics, while there were obvious similarities in function (ester hydrolysis), there appeared to be significant differences in the active site structures, as deduced from their kinetic behavior and reaction with different substrates and inhibitors. The crystallographic structures of a number of antigen-anti-Id combinations have been solved; it is noteworthy that in no cases were the anti-idiotypic antibodies internal images replicas of the antigenic sites [e.g., Fields et al., 1995; Goldbaum et al., 1997]. Instead, considerable structural variation was found, in some cases with little or no sequence identity. This is not unforeseen, as the process of antigen selection operates on functional, rather than structural, conformations [Lescar et al., 1995]. It appears that side chain mobility of the epitope residues contributes to confer steric and electrostatic complementarity to differently shaped binding sites, allowing functional mimicry to occur [Lescar et al., 1995]. These idiotypic structural variations undoubtedly account for the variability observed in the behavior of the antibodies.

The CDRs of immunoglobulins are composed of open-loop structures, and it is probable that these form the structural basis for molecular mimicry [Ban et al., 1994]. It follows that loop structures on antigens are more easily mimicked than alpha-helices. The presence of loops associated with the PAS is thus likely to account for the strong antigenic character of this site and the relative ease with which it is mimicked. It would also appear that these PAS-associated loops have a predisposition towards aggrega-

TABLE I. Effects of Antibodies on Neuroblastoma Cell Adhesion and Neurite Outgrowth

Ab classification	MAb	% adherent cells	% neurites	Neurite length (μm)	% viability
Ab1	E8	8.0 ± 3.6^{a}	—[c,d]	—[c,d]	26.3
Ab2s	17B	>95	34.15 ± 1.61^{b}	159.06 ± 6.71^{c}	>95
	211E	>95	$86.47 \pm 3.18^{c,d}$	$203.79 \pm 4.95^{c,d}$	>95
	29H	>95	$52.32 \pm 3.32^{c,d}$	$187.66 \pm 9.25^{c,d}$	>95
	210G	>95	40.42 ± 1.02^{c}	165.71 ± 5.03^{c}	>95
	11C	>95	27.62 ± 1.00^{b}	148.28 ± 7.13	>95
Ab3s	13B9C	51.4 ± 5.7^{a}	—[c,d]	—[c,d]	69.5
	13B9F	15.0 ± 2.9^{a}	—[c,d]	—[c,d]	31.2
	12D11F	63.4 ± 5.1^{a}	—[c,d]	—[c,d]	92.7
	13A	9.2 ± 3.7^{a}	—[c,d]	—[c,d]	45.5
Controls	No Ab	>95	8.69 ± 0.12	141.22 ± 6.77	>95
	MAb E413D8	>95	9.02 ± 1.11	153.29 ± 3.68	>95
	Non-specific IgG	>95	8.85 ± 0.75	144.87 ± 4.45	>95
	AChE	>95	37.23 ± 2.57	166.23 ± 8.01	>95

An anti-AChE MAb that does not recognize the PAS [36]. The figures given are for 40 μg/ml IgG or AChE. Cells were incubated for 48 h.
[a]Significantly different from control (no Ab or non-specific Ab) or Ab2; $P < 0.01$.
[b]As for (a); $P < 0.02$.
[c]Significantly different from control (no Ab or non-specific Ab); $P < 0.01$.
[d]Significantly different from AChE; $P > 0.01$.

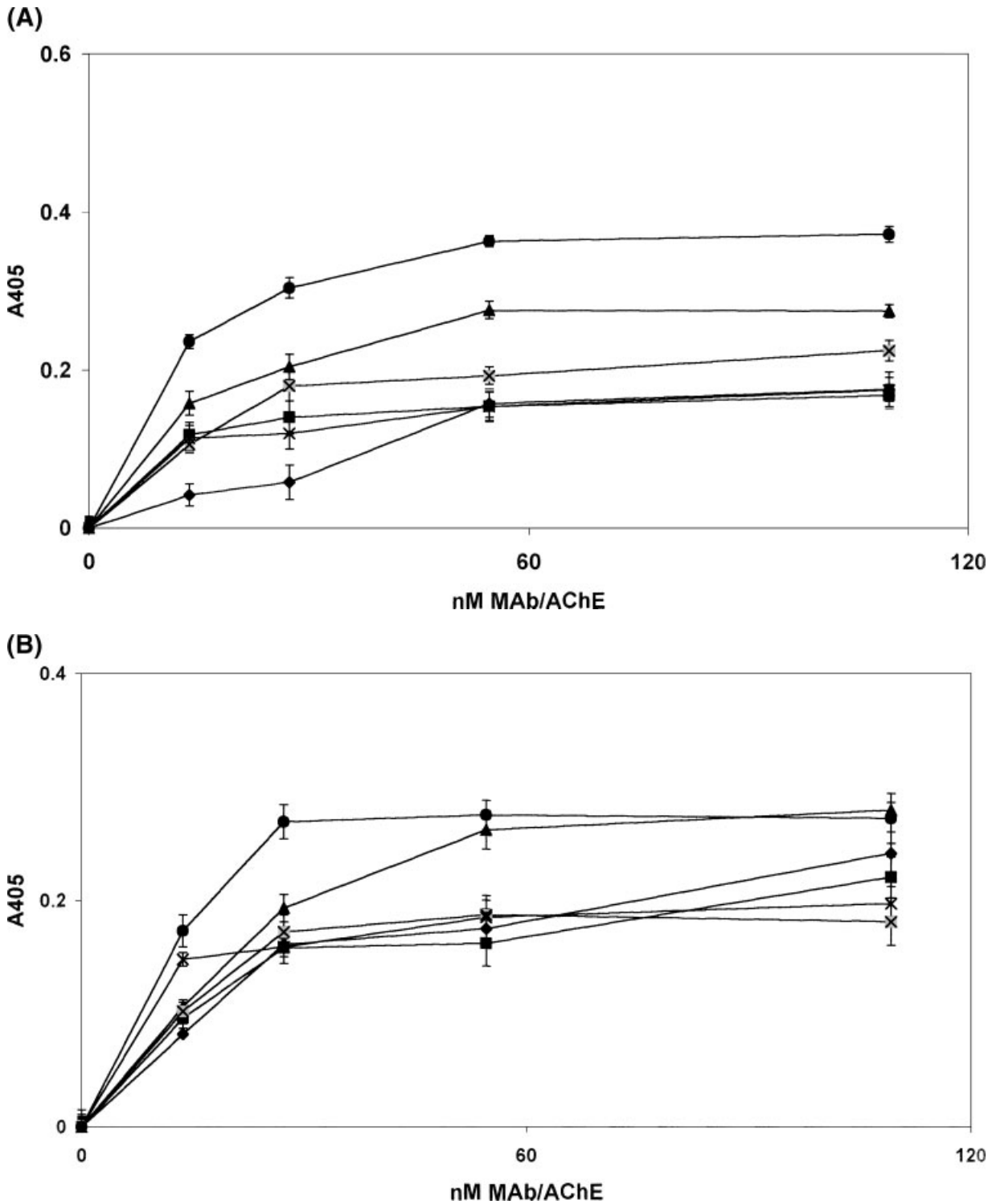

Fig. 3. Binding of biotinylated MAbs and AChE to laminin-1 and collagen IV. **A**: Binding of MAbs and AChE to laminin-1 as a function of MAb and AChE concentration. **B**: Binding of MAbs and AChE to collagen IV as a function of MAb and AChE concentration. Microtiter plates were coated with 10 μg/ml laminin-1 or collagen IV in 50 mM $NaHCO_3$, pH 8, overnight at 4°C. After blocking (1% BSA on phosphate-buffered saline for 2 h at 22°C), varying concentrations of biotinylated MAb or AChE (0–10 μg/ml; 0–133 nM IgG, or 143 nM AChE) were added and incubated for 3.5 h at 22°C. The buffer was 0.1 M Tris, pH 7.4. The amount of MAb or AChE bound was probed with streptavidin-peroxidase, H_2O_2/ABTS, and absorbance measured at 405 nm (n = 4). MAb 17B, ◆–◆; MAb 29H, ■–■; MAb 210G, △–△; MAb 211E, X–X; MAb 11C, ⧓⧓; AChE, ●-●.

TABLE II. K_d Values for Ab2 Binding to Laminin and Collagen

Ab	Ab-laminin	Ab-collagen
17B	35.2009 ± 6.2600	21.7581 ± 4.3840
29H	5.3145 ± 0.5811	11.1593 ± 5.4412
211E	3.2597 ± 0.0851	2.0799 ± 0.0813
210G	11.7917 ± 5.3078	10.6891 ± 4.1058
11C	16.4766 ± 9.4381	10.9526 ± 3.7134
AChE	2.7987 ± 4.9651	3.2239 ± 2.6340

Results are given as means and standard errors (n = 7 for all).

tion: resemblance between a PAS-associated loop and aggregating proteins, in particular, the amyloid β-peptide and the prion proteins, has been noted [Bourne et al., 1999], and interaction between another loop and the β-peptide has been demonstrated [De Ferrari et al., 2001].

It is possible that the enhanced effect of the antibodies is due to a better "fit" with the ligand, resulting in more efficient signaling, or, alternatively, to interference with a negative signaling pathway. We found that the association constants of MAbs 211E and 29H, for binding to laminin and collagen, were not an improvement on those of AChE, despite the antibodies' superior neuritogenic ability. This suggests interference with a negative signal, and points to possible routes for the investigation of signaling pathways triggered by AChE.

A further interesting point is that of functional redundancy. Functional homologues of AChE almost certainly exist, as indicated by the survival of the AChE knockout mouse [Xie et al., 2000]. Grifman et al. [1998] have observed functional redundancy between AChE and the structurally homologous neuroligin, with both proteins binding to β-neurexin in the mammalian brain. Certain domains of β-neurexin are homologous with laminin, which may account for the interaction with AChE with both molecules. Crosstalk and redundancy are well-known in signaling molecules. The AChE PAS itself is known to interact with a number of molecules: laminin and collagen [Johnson and Moore, 2002], the amyloid β-peptide [De Ferrari et al., 2001], AChE itself [Bourne et al.,

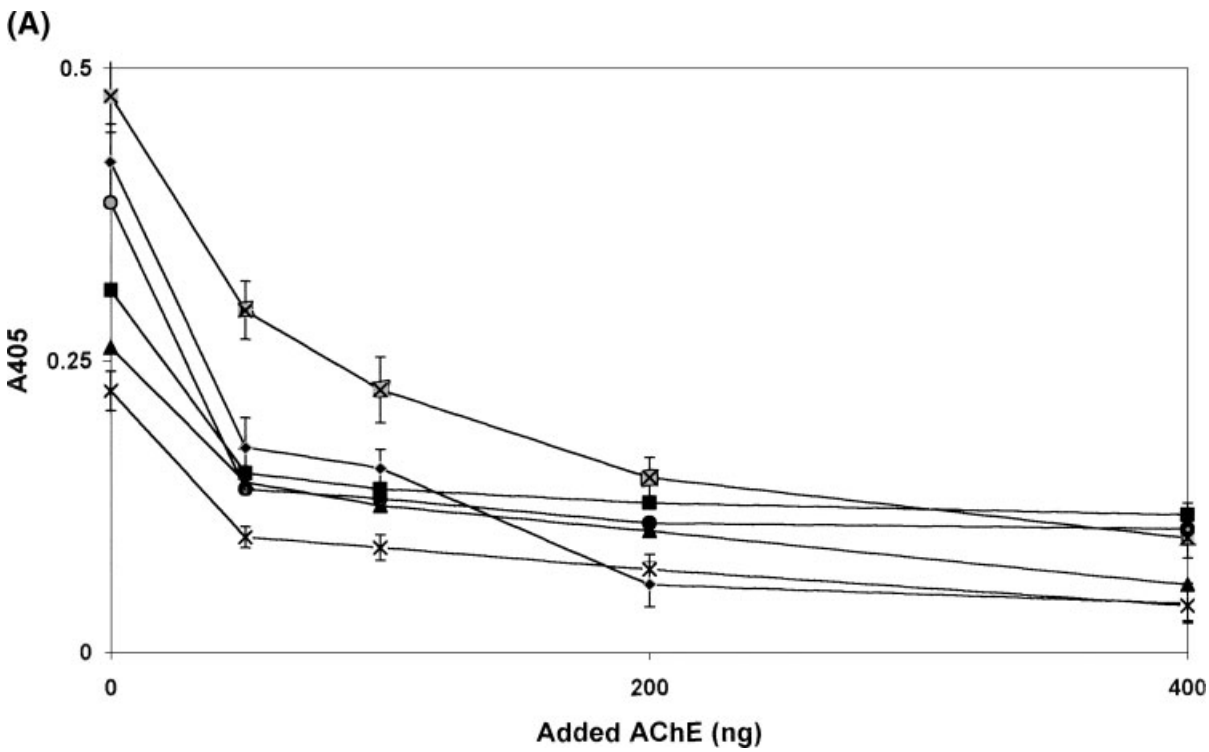

Fig. 4. Competition between MAbs and AChE for binding to laminin-1 and collagen IV. **A**: Competition for binding to laminin-1. **B**: Competition for binding to collagen IV. Microtiter plates were coated and blocked as in Figure 3. Biotinylated MAb or AChE was added at a concentration of 10 μg/ml (0.5 μg/well) simultaneously with varying concentrations of unlabelled AChE (0–10 μg/ml; 0–143 nM) and incubated for 3.5 h at 22°C. The amount of biotinylated MAb or AChE bound was probed as in Figure 3 (n = 4). MAb 17B, ◆–◆; MAb 29H, ■–■; MAb 210G, △–△; MAb 211E, X–X; MAb 11C, ⧓⧓; AChE, ●-●.

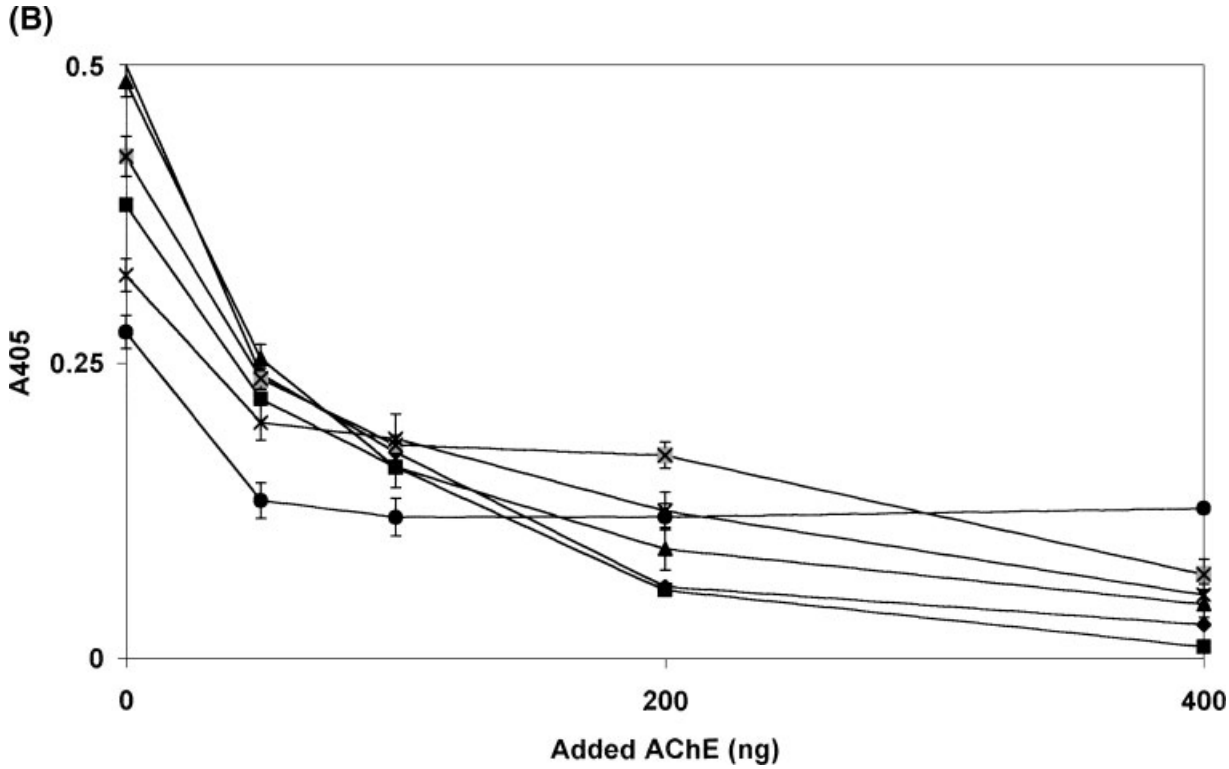

Fig. 4. (*Continued*)

1999], β-neurexin [Grifman et al., 1998], as well as PAS-binding inhibitors. This promiscuity suggests redundancy. It is thus feasible that the PAS may resemble a site on other molecules, for instance the integrins, known to be receptors for both laminin and collagen. It follows that AChE's adhesion and neuritogenic functions may not be primary, but may act as fine-tuning devices.

We have previously observed that AChE-like catalytic antibodies, whether Ab1 or Ab3, are cytotoxic [Johnson and Moore, 2000, 2002]. These antibodies recognize a site associated with the PAS, suggesting that their action is through blockade of the adhesion site. The viability measurements, also, suggest that adhesion is lost prior to cell death. This is consistent with anoikis [Frisch and Francis, 1994], apoptosis induced by inadequate or inappropriate cell–matrix interactions. It is apparent from the work presented here that Ab3s are formed in vivo, and that a small, but significant number of these are catalytic. If anti-idiotypic antibodies were to be used as an in vivo therapy, the probable generation of such cytotoxic antibodies is a concern. While such antibodies might promote the apoptosis of tumor cells, they also recognize the PAS and thus inactivate AChE, which could produce neurological and myasthenic symptoms. The use of the AChE site as a differentiation-inducing therapy might therefore be better accomplished through structural analysis of the sites of AChE and the antibodies, leading to rational drug design. Comparison of the sites, particularly those of the two antibodies with enhanced neuritogenic ability, would be informative, and could also lead to identification of possible "signaling homologues" of AChE.

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