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The Anti-Amyloid-β Monoclonal Antibody 4G8 Recognizes a Generic Sequence-Independent Epitope Associated with α-Synuclein and Islet Amyloid Polypeptide Amyloid Fibrils

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Abstract. Recently we reported that several monoclonal antibodies that recognize linear segments of amyloid-β (Aβ) also recognize amyloid fibrils, but not monomers of unrelated sequences, indicating that recognition of a linear sequence segment is not a reliable indicator of sequence specificity. We asked whether any of the commonly used commercially available Aβ antibodies also recognize fibrils of unrelated sequence. Here we report that 4G8, which recognizes residues 18–23 of the Aβ sequence and is widely believed to be sequence-specific, also recognizes fibrils formed from α-synuclein and islet amyloid polypeptide (IAPP). The recognition of amyloid fibrils is aggregation-dependent because 4G8 does not recognize α-synuclein or IAPP monomer. 4G8 also stains fibrillar α-synuclein aggregates in human multiple system atrophy brain where it colocalizes with anti-α-synuclein monoclonal antibody LB509 immunoreactivity. We also found that LB509 recognizes Aβ fibrils, but not monomer, indicating that generic epitope-reactive antibodies are also produced in response to α-synuclein immunization. Taken together, our results indicate that generic fibril conformational epitope specificity may be a pervasive property among monoclonal antibodies raised against amyloid-forming antigens and that the specificity of their immunoreactivity should be rigorously established and otherwise interpreted with caution.

Keywords: Amyloid, amyloid-beta, conformation, monoclonal antibody, IAPP, synuclein

INTRODUCTION

The amyloid-β (Aβ) peptide is a major component of the extracellular plaques found in Alzheimer's disease (AD) brain and is implicated as a causative agent in the pathology of this neurodegenerative disorder [1]. As a result, there are hundreds of polyclonal and monoclonal Aβ antibodies in use today. Many of these antibodies are reported to be Aβ sequence specific [2–4] because they recognize specific peptide segments of Aβ. Other antibodies recognize generic conformational epitopes that are specific to a particular aggregation state, but are independent of the specific aggregated sequence [5–11]. For example, A11 polyclonal antibodies specifically recognize

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antiparallel β-sheet Aβ prefibrillar oligomers (PFOs), along with PFOs of other amyloid peptides sharing a common generic epitope arising from antiparallel β-sheet aggregates [5, 12]. Polyclonal rabbit serum OC recognizes parallel β-sheet Aβ fibrils and fibrillar oligomers, along with amyloid fibrils of other peptides sharing common epitopes arising from parallel, in-register β-sheet structure [7, 13]. Recently, we cloned 23 unique monoclonal antibodies from $A\beta_{42}$ fibril vaccinated rabbits producing OC serum [14]. We observed that most of these antibodies appear to recognize linear epitopes on Aβ peptide sequence arrays [14], but several of the antibodies that recognize linear Aβ segments also recognize generic epitopes associated with amyloid fibrils formed from other unrelated amyloidogenic sequences, such as α-synuclein and IAPP. This indicates that recognition of a specific segment of the Aβ sequence is not a reliable indicator of sequence specificity and raised the question of whether other Aβ monoclonals also recognize other types of amyloid fibrils. 6E10 and 4G8 are two widely-used Aβ antibodies believed to be sequence-specific on the basis of their ability to react with short linear segments of Aβ [2]. Thus, we set out to determine whether these antibodies may also react in a conformation-dependent manner. Here we report that the monoclonal antibody 4G8 reacts with amyloid fibrils formed by α-synuclein and IAPP *in vitro* in an aggregation-dependent manner. 4G8 also reacts with α-synuclein on western blots and with pathologic α-synuclein aggregates in human brain sections from multiple system atrophy (MSA) patients. We also observe that LB509, an α-synuclein monoclonal antibody, reacts in an aggregation-dependent manner with Aβ fibrils *in vitro*. The data presented here indicate that recognition of generic, sequence-independent conformation-specific epitopes is a common property of antibodies raised against amyloid aggregates and a reflection of the tendency of amyloidogenic peptides and proteins to form β-sheet structures with generic amyloid conformations. The results also indicate that the specificity of anti-amyloid antibodies should be rigorously characterized with a panel of unrelated amyloid fibrils and oligomers. Otherwise, claims of sequence specificity should be viewed with caution.

MATERIALS AND METHODS

$A\beta_{40}$ and $A\beta_{42}$ aggregation reactions

$A\beta_{40}$ and $A\beta_{42}$ were aggregated under condition B over a 7-day time course as previously described [14]. Briefly, 0.5 mg of the lyophilized peptide was resuspended in 333.33 μl hexafluoroisopropanol (HFIP) and incubated for 15 min. This solution was then diluted with 1.33 ml deionized water (dH_2O) and the tube containing the solution was covered with a punctured cap and placed under a hood; this solution was continuously stirred during the aggregation time course using a stir plate. The solution contained 0.02% sodium azide (NaN_3).

α-synuclein aggregation reaction

We aggregated α-synuclein (a gift from Ralph Langen) over a 7-day time course. In order to initiate the aggregation reaction, we resuspended 0.3 mg of the lyophilized polypeptide in 400 μl of HFIP in an Eppendorf tube and allowed it to incubate for 15 min at room temperature (R/T). We then diluted this solution using 1,600 μl dH_2O containing 0.02% NaN_3, covered the tube containing the solution with a punctured cap, and placed the tube under a hood; this solution was continuously stirred during the aggregation period using a stir plate.

IAPP aggregation reaction

Lyophilized IAPP was aggregated over a 7-day time course. 30 μl of 100 mM NaOH was added to 0.3 mg of IAPP and the mixture was incubated at R/T for 25 min. We then added 1 ml phosphate buffered saline (PBS) containing 0.02% NaN_3 to the solution and allowed the mixture to aggregate at R/T over a 7-day time period. All aggregation reaction samples were OC positive and A11 negative at day 3 and day 7, indicating that they are fibrillar in nature except for IAPP at time zero, which was immunoreactive for both OC and A11, indicating that it contains both fibrillar and prefibrillar aggregates.

Dot blot assay

1 μl of each sample was pipetted onto a Whatman nitrocellulose membrane (GE Healthcare, Pittsburgh, PA) at each time point. This volume corresponded to 300–375 ng of Aβ, 150–187.5 ng of α-synuclein, and 300 ng of IAPP. After the last sample was deposited, the membranes were allowed to air-dry. Non-specific binding was blocked by incubation in 10% non-fat dry milk in Tris-buffered saline containing 0.1% Tween-20 (TBS-T) for 1 h at R/T. The membranes were then incubated with 6E10 and 4G8 (Covance, Princeton, NJ) or LB509 (Invitrogen, Frederick, MD) antibodies overnight at 4°C. The antibodies were diluted

1:10,000 in 5% non-fat dry milk in TBS-T. After three 5-min washes in TBS-T, the membranes were incubated in horseradish peroxidase (HRP)-conjugated goat anti-mouse immunoglobulin G (IgG) (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) for 1 h at R/T. Secondary antibody was diluted 1:10,000 in 5% non-fat dry milk in TBS-T. The blots were then washed 3×5 min in TBS-T and reactive dots were visualized using the enhanced chemiluminscence (ECL) protocol (GE Life Sciences, Pittsburgh, PA). Images of the membranes were obtained using a Nikon D700 (Nikon Inc., Melville, NY) camera as described previously [15]. Representative results from 3 independent experiments are shown. For dilution series experiments, the fibrillar preparations were further diluted as indicated in either dH_2O (for Aβ and α-synuclein), or PBS (for IAPP) immediately before deposition onto the nitrocellulose membrane. 1 μl of each resulting solution was spotted (2 μl of the 1X sample was used to obtain the 2X sample).

Western blots

10 μl of each sample from the appropriate time points were mixed with 2X loading buffer (125 mM Tris pH 6.8, 4% SDS, 16% glycerol, 10% 2-mercaptoethanol, bromophenol blue). The resulting 20 μl mixtures were loaded onto an 18-well 4%–12% precast Triton Gradient eXtended (TGX) gel (Life Science, Hercules, CA). The gel was then run at 250 V and the resolved proteins were transferred onto a nitrocellulose membrane at 350 mA for 45 min. Non-specific binding was blocked by incubating the membrane in 10% non-fat dry milk in TBS-T for 1 h at R/T. The membrane was then incubated in the appropriate primary antibody overnight at 4°C. After three 5-min washes in TBS-T, the membranes were incubated with anti-mouse secondary antibody for 1 h at R/T. Following three 5-min washes in TBS-T, the results were visualized using the ECL protocol and images of the results were obtained using a Nikon D700 camera as previously described for the dot blot experiments. Representative results from three independent experiments are shown.

Preadsorption of 4G8 antibody

4G8 antibody was incubated with a 40x molar excess of either α-synuclein or IAPP fibrils from the 7-day time points of the aggregation reactions described above for 2 h at R/T. The antibody was then diluted to 1:10,000 in 5% non-fat dry milk in TBS-T. The 4G8-only antibody solution was prepared in the same manner as the preadsorped solutions, but without α-synuclein or IAPP incubation. Fibrillar preparations of $A\beta_{40}$, $A\beta_{42}$, IAPP, and α-synuclein were probed with antibody solutions containing 4G8, 4G8 pre-incubated with α-synuclein, or 4G8 pre-incubated with IAPP in a western blot experiment.

Immunohistochemistry

Frozen postmortem brain tissue from the cerebellum of an MSA patient with minimal AD-type pathology was obtained from the New York Brain Bank (NYBB)-Sample ID: T-3569, Age: 70 years, Gender: male, Cold Postmortem Interval (PMI): 5:25, Frozen PMI: 15:25, Diagnosis: MSA. The brain tissue was simultaneously thawed and fixed in 4% paraformaldehyde overnight at 4°C. 40 μm-thick sections of fixed tissue were obtained using a Vibratome Series 1000 vibrating microtome (The Vibratome Company, St. Louis, MO). Sections were stored in PBS containing 0.02% NaN_3 at 4°C. Endogenous peroxidase activity was quenched by incubating the sections in 3% hydrogen peroxide and 3% methanol in TBS for 30 min at R/T. The sections were then blocked in 2% bovine serum albumin (BSA) (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) and 0.1% Triton X-100 (Tx) in TBS for 1 h at R/T. Tissues were incubated with 1 mg/L of the appropriate primary antibody (4G8, 6E10, or LB509) diluted in blocking solution overnight at 4°C. The sections were then washed 2×5 min with 0.1% Tx in TBS and blocked for 30 min in blocking buffer. Following the blocking step, the tissues were incubated with horse anti-mouse secondary antibody (Vector Laboratories, Inc., Burlingame, CA) for 1 h at R/T. Anti-mouse secondary antibody was diluted 1:200 in blocking buffer containing a 1:75 dilution of normal horse serum (Vector Laboratories, Inc., Burlingame, CA). An ABC peroxidase kit and 3,3'-diaminobenzidine (DAB) substrate kit (Vector Laboratories, Inc., Burlingame, CA) were used to detect the biotinylated secondary antibodies. Following the DAB incubation, the tissue sections were washed 5×5 min in TBS, mounted, and allowed to air-dry. The sections were then dehydrated using sequential 3-min incubations in 50%, 70%, and 95% ethanol, followed by a 15-min incubation in 100% ethanol. The sections were then cover-slipped with DePeX (EMS, Hatfield, PA), and visualized using an Olympus BH-2 light microscope (Olympus America Inc., Center Valley, PA). The omission of either the primary or the secondary antibody was used as the negative control and resulted in no DAB staining.

Immunofluorescence

40-μm-thick fixed sections from the cerebellum of an MSA patient (sample ID T-3569 described above) were washed 2 × 5 min in TBS and permeabilized for 15 min using 0.1% Triton X-100 in TBS. Following a 30 min blocking step in 2% BSA +0.1% Tx in TBS, the sections were incubated for 24 h in LB509 at 1.5 μg/ml. The sections were then washed 2 × 5 min using 0.1% Triton X-100 in TBS, blocked for 30 min, and incubated with Alexa-488-conjugated anti-mouse antibody (Life Technologies, Carlsbad, CA) for 2 h. The secondary antibody concentration was 10 μg/ml. Following 3 × 5 min washes and 30 min of blocking, the sections were incubated with biotinylated 4G8 antibody (Biolegend, San Diego, CA) at 1.5 μg/ml for 24 h. Following 2 × 5 min washes and 30 min of blocking, the sections were incubated with streptavidin Alexa-647 secondary antibody (Invitrogen, Grand Island, NY) at 10 μg/ml for 2 h. The sections were then washed 3 × 5 min, incubated with DAPI for 15 min, washed 3 × 5 min, and mounted using ProLong Gold Antifade reagent (Life Technologies, Carlsbad, CA). For staining with ThioflavinS (Sigma-Aldrich, St. Louis, MO), the sections were incubated for 8 min with 1% ThioflavinS, washed 2 × 3 min in 80% ethanol, 2 × 3 min in 95% ethanol, and 3 × 5 min in water prior to mounting.

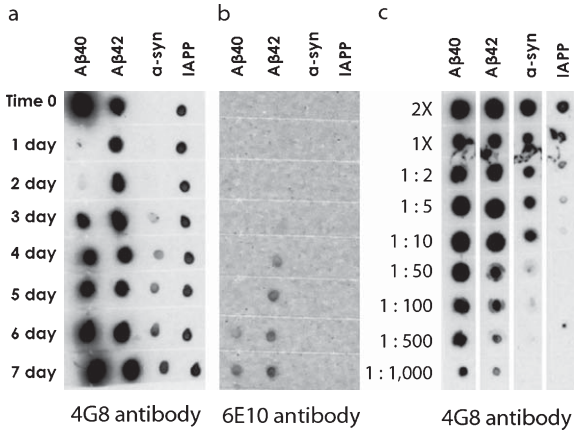

Fig. 1. Dot blot demonstrating time- and aggregation-dependent 4G8 reactivity with α-synculein aggregates. $A\beta_{40}$, $A\beta_{42}$, α-synuclein, and IAPP were aggregated as described in the Materials and Methods section over a 7-day time course. Samples from each reaction were deposited onto a nitrocellulose membrane at time 0 and at 24-h intervals until the 7-day time point and probed with 4G8 (a) and 6E10 (b). c) Dot blot indicating reactivity of 4G8 with dilution series of $A\beta_{40}$, $A\beta_{42}$, α-synuclein, and IAPP fibrils. The amounts of protein deposited are as follows: $A\beta_{40}$ and $A\beta_{42}$ - 1x (375 ng), α-synuclein - 1x (187.5 ng), IAPP - 1x (300 ng).

RESULTS

In order to determine whether 4G8 and 6E10 react in a sequence-specific or generic conformation-dependent manner with amyloid aggregates formed by peptides other than Aβ, we aggregated $A\beta_{40}$, $A\beta_{42}$, α-synuclein, and IAPP over a 7-day time period and analyzed the resulting aggregates at time 0, and the 3-day and 7-day time points using western blot and dot blot assays. We observed that 4G8 reacted with both α-synuclein and IAPP aggregates in the dot blot experiment, in addition to reacting with the Aβ preparations. Interestingly, 4G8 reactivity with $A\beta_{40}$ was biphasic: high at time 0, decreased at days 1 and 2, and then increased, indicating that the immunoreactivity changes with time of incubation. The immunoreactivity of α-synuclein aggregates was clearly aggregation time dependent, whereas the $A\beta_{42}$ and IAPP preparations were positive beginning at time 0 and remained so during the 7-day time course (Fig. 1a). No reactivity against α-synuclein and IAPP aggregates was observed for 6E10, consistent with the interpretation that it recognizes a sequence-specific epitope (Fig. 1b). Western blot results from the same samples more clearly display the aggregation-dependent changes in 4G8 reactivity in the Aβ and α-synuclein preparations, with stronger reactivity across a wide range of aggregate sizes at later time points (Fig. 2a, b). IAPP samples did not show any 4G8 reactivity in the western blots, indicating that the epitope is sensitive to SDS (Fig. 2b). In this regard, 4G8 is similar to several anti-Aβ monoclonals that we have described recently [14]. It is important to note that while 4G8 clearly recognized the α-synuclein samples in a conformational manner in dot blot and western blot assays, it reacted with the Aβ preparations more robustly in the same assays. While 4G8 western blot reactivity with α-synuclein is clearly demonstrated in a high-exposure image (Fig. 2a), a western blot containing samples from the Aβ preparations, as well as the α-synuclein and IAPP reactions illustrates the dramatic difference in the strength of reactivity of 4G8 with the Aβ preparations and its reactivity with the α-synuclein samples (Fig. 2b). In fact probing a dilution series of the aggregated $A\beta_{40}$, $A\beta_{42}$, α-synuclein, and IAPP samples with 4G8 indicates that the lower limit of detection for Aβ is less than 0.375 ng, while they are 1.875 ng and 30 ng respectively for α-synuclein and IAPP (Fig. 3c). 4G8 also displays a clearly lower intensity of staining for $A\beta_{40}$ compared to $A\beta_{42}$ (Figs. 2b, 3), even though they both contain the same linear epitope, suggesting that the difference is due to the greater propensity of $A\beta_{42}$ to aggregate. No 6E10

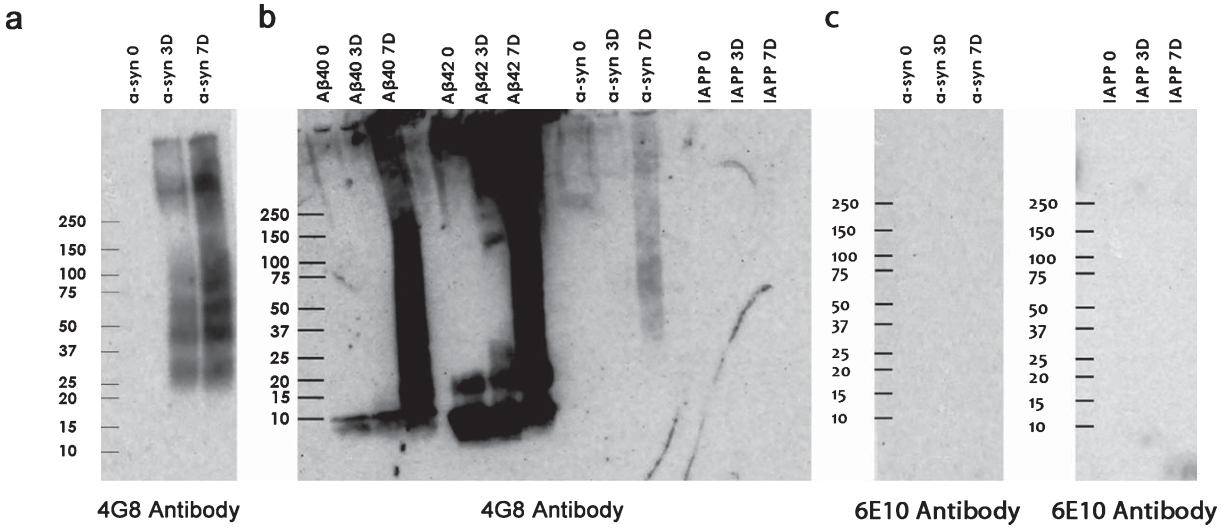

Fig. 2. Western blot demonstrating time- and aggregation-dependent 4G8 reactivity with α-synuclein and Aβ aggregates. α-synuclein was aggregated over a 7-day time course as described in the Materials and Methods section and samples from time 0, and the 3-day and 7-day times points were subjected to SDS-PAGE and probed using 4G8 (a) or 6E10 (c). A western blot experiment comparing samples from the time 0, and the 3-day and 7-day time points of the $A\beta_{40}$, $A\beta_{42}$, α-synuclein, and IAPP aggregation reactions was performed to demonstrate relative 4G8 reactivity with the different preparations (b).

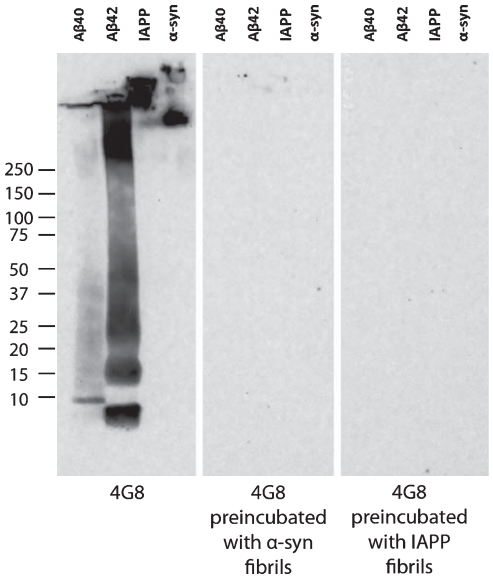

Fig. 3. α-synuclein and IAPP fibrils adsorb 4G8 immunoreactivity. 4G8 was preincubated with α-synuclein and IAPP fibrils as described in Materials and Methods and then used to probe western blots of preformed $A\beta_{40}$, $A\beta_{42}$, α-synuclein, and IAPP fibrils. Preincubation with either α-synuclein (middle panel) or IAPP (right panel) completely blocked 4G8 immunoreactivity.

immunoreactivity was observed for α-synuclein and IAPP at any time point (Fig. 2c).

To unequivocally establish whether the same population of 4G8 antibody molecules binds to Aβ, α-synuclein, and IAPP, we examined whether pre-incubation of 4G8 with α-synuclein and IAPP fibrils blocks 4G8 immunoreactivity on western blots. As shown in Fig. 3, pre-incubation with either α-synuclein or IAPP fibrils blocked the 4G8 immunoreactivity of $A\beta_{40}$, $A\beta_{42}$, α-synuclein, and IAPP, indicating that all of the Aβ immunoreactivity can be blocked by adsorption with α-synuclein and IAPP fibrils.

4G8 recognizes pathological α-synuclein aggregates in MSA brain

After observing clear evidence of 4G8 reactivity against α-synuclein aggregates *in vitro*, we asked whether it would be able to recognize α-synuclein in pathologic aggregates in human brain. In order to answer this question, we performed immunohistochemistry on postmortem brain sections from five different individuals diagnosed with MSA using antibody 4G8. MSA brains were chosen because they exhibit minimal Aβ amyloid plaque pathology compared to other synucleinopathies. We used a human α-synuclein-specific antibody, LB509, as a positive control, and the Aβ-specific antibody 6E10, along with the omission of the primary antibody incubation step as negative controls. We observed that the staining pattern displayed by 4G8 closely resembles the reactivity pattern obtained with LB509 (Fig. 4a). Unlike 4G8, antibody 6E10 did not recognize α-synuclein

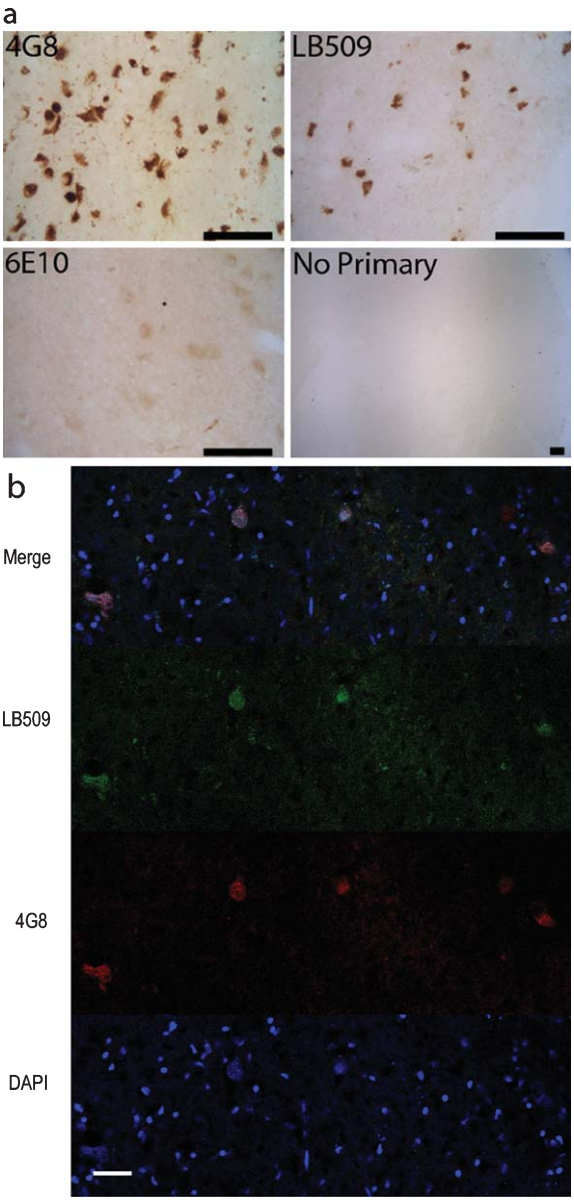

Fig. 4. 4G8 reacts with pathologic α-synuclein aggregates in MSA brain. a) 40-μm-thick sections from postmortem MSA brains were histochemically stained as described in Materials and Methods using 4G8, 6E10, and LB509. Staining without the use of a primary antibody was used as a negative control. Objective magnification is 20X for the antibody staining and 4X for the no primary control. Scale bar = 20 μm. b) Representative image of 4G8 and LB509 co-immunostaining experiment showing that both antibodies co-localize to the same intracellular inclusions. Scale bar = 50 μm.

pathology present in these brain sections, supporting the idea that the reactivity pattern obtained with 4G8 is indeed due to binding to α-synuclein aggregates and not AβPP or Aβ, aggregates of which would have also been recognized by 6E10. In order to confirm that the staining pattern observed with 4G8 in MSA brain tissue is specific to α-synuclein amyloid aggregates, we performed co-immunofluorescence experiments with 4G8 and LB509. Our data indicate that 4G8 and LB509 co-localize to the same pathological inclusions (Fig. 4b), demonstrating that the material bound by 4G8 is α-synuclein.

The α-synuclein antibody LB509 recognizes Aβ aggregates in an aggregation-dependent manner

Since we found that 4G8 reacts in a conformation- and aggregation-dependent manner with α-synuclein aggregates, we wondered whether the α-synuclein monoclonal antibody LB509 would also react with Aβ aggregates in the same manner. In order to answer this question, we performed western blot and dot blot experiments on the $A\beta_{40}$, $A\beta_{42}$, α-synuclein, and IAPP aggregates described previously using LB509 as the primary antibody. We observed that LB509 reacts with $A\beta_{40}$ and $A\beta_{42}$ in an aggregation-dependent manner in dot blot experiments, albeit at a much lower intensity compared to its reactivity with the α-synuclein preparation. The IAPP preparation was not recognized at any time point by LB509 (Fig. 5a). We observed $A\beta_{40}$ and $A\beta_{42}$ reactivity at the 7-day time point in the western blot experiment. As in the dot blot experiment, Aβ reactivity is weaker than reactivity against the α-synuclein preparation, and the IAPP aggregates failed to react with LB509 (Fig. 5b).

DISCUSSION

The aggregation of amyloid-forming proteins is a central feature of many degenerative diseases [16], including but not limited to AD [17], Parkinson's disease [18], Huntington's disease [19], prion diseases [20], type 2 diabetes [21], and light-chain amyloidosis [22]. Not surprisingly, a large number of polyclonal and monoclonal antibodies have been developed as experimental tools for these proteins and as therapeutics for diseases related to their misfolding and aggregation. 4G8 and 6E10 are two routinely used antibodies against Aβ and are widely believed to be sequence-specific because they react with specific short peptide sequences contained within Aβ [2]. We found that 4G8 also reacts with IAPP and α-synuclein aggregates in a conformation- and aggregation-dependent manner *in vitro* and in MSA brain *in vivo*. While both 4G8 and 6E10 recognize linear segments of the Aβ peptide, 4G8 is also able to react with an aggregation-dependent, generic amyloid epitope present in the aggregates of

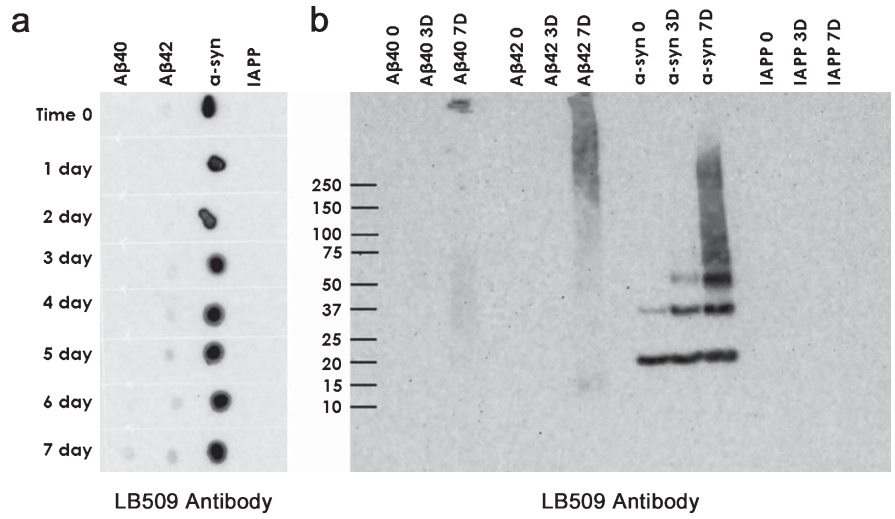

Fig. 5. α-synuclein-specific antibody LB509 recognizes Aβ in a time- and aggregation-dependent manner *in vitro*. $A\beta_{40}$, $A\beta_{42}$, α-synuclein, and IAPP were aggregated over a 7-day time course as described previously. Samples from each reaction were deposited onto a nitrocellulose membrane at time 0 and at 24-h intervals ending at the 7-day time point and the membrane was probed with LB509 (a). In addition, samples from each reaction from the time 0, and the 3-day and 7-day time points were subjected to SDS-PAGE and probed with LB509 in western blot experiments (b).

Table 1
Primary sequences for Aβ, α-synuclein, and IAPP, and the apparent linear epitopes for 4G8 and 6E10

Name of Protein	Amino Acid Sequence
Aβ	DAE FRHDSGY EVHHQKL VFFAED VGSNKGAIIGLMVGGVVIAT
α-synuclein	MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVATVAEKTKEQVTNVGGAVV TGVTAVAQKTVEGAGSIAAATGFVKKDQLGKNEEGAPQE GILEDMPVDP DNEAYEMPSE EGYQDYEPEA
IAPP	MGILKLQVFLIVLSVALNHLKATPIESHQVEKRKCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY GKRNAVEVLK REPLNYLPL

The apparent linear epitope for 4G8 is highlighted in red, while that of 6E10 is highlighted in blue [14]. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/JAD-150696)

α-synuclein and IAPP, which lack the primary amino acid sequence proposed to be the linear epitope for 4G8. For comparison, the sequences for Aβ, α-synuclein, and IAPP, along with the apparent linear epitopes for 4G8 and 6E10 are summarized in Table 1. LB509, which is believed to be α-synuclein-specific, also reacts with aggregated Aβ in a conformation-dependent fashion. In this way, 4G8 and LB509 behave in a similar manner to conformation-specific mOC and A11 monoclonal antibodies developed in our lab. That is, they recognize generic sequence-independent epitopes that arise as a consequence of peptide aggregation and amyloid formation [12, 14]. This indicates that generic epitope immunoreactivity is a pervasive feature of antibodies raised against amyloidogenic antigens.

Although the structural bases for the generic epitopes are not known at atomic resolution, some inferences about their plausible structures may be drawn from what is known about fibril and oligomer structure. Conformation-specific antibodies seem to fall into two broad categories: Antibodies, like those found in OC serum, that react with fibrils that are known to be parallel, in-register β-sheets, and antibodies like A11, which recognize antiparallel β-sheets [12, 13, 23, 24]. Parallel, in-register β-sheets have homogeneous tracts of amino acid side chains, known as "spines" that run parallel to the fibril axis, while antiparallel β-sheets, β-barrels, and β-solenoids have a pattern of alternating side chains [25, 26]. Wherever a particular amino acid side chain occurs within a parallel, in-register sheet or where a pair of amino acids occurs in the same type of spine structure, they will be represented on the fibril surface regardless of what the underlying sequence is that contains these amino acids. Thus, these surface spines could represent the generic epitopes that are associated with amyloid aggregates. Just as a large number of consumer products can be

specified by a product bar code, a large number of protein sequences can form β-sheet amyloid structures, but the immune system only sees the common structural elements, like the different "bars" rather than the underlying sequence that gives the bar code a unique pattern.

Lack of sequence specificity is generally viewed as a negative property because it leads to cross reactivity with unwanted targets and leads to ambiguity as to the molecular nature of immunoreactivity. The case of sequence independent recognition of common or generic epitopes by anti-amyloid antibodies presents a significantly different situation. Because the generic epitopes are associated with the adoption of amyloid structure, recognition of multiple amyloids may be a useful feature rather than an unwanted target. Since many amyloids are related to disease, the only unwanted targets would be the functional amyloids where the amyloid structure is related to the normal function of the protein. Besides providing a broad spectrum of therapeutic activity, antibodies that recognize generic epitopes have proven to be useful in discovering previously unknown associations between amyloid accumulation and several diseases, such as idiopathic cardiomyopathy [27], preeclampsia [28], and atrial fibrillation [29], and in identifying native amyloids [30].

These data further reinforce the idea that recognition of a linear peptide segment is not a reliable indicator of sequence specificity. For instance, the fact that 4G8 recognizes a linear epitope of $A\beta_{18-23}$ absent from the $A\beta_{25-35}$ peptide has been used to support the idea that increased 4G8 reactivity in the brains of $A\beta_{25-35}$-injected rats is due to increased AβPP production [31]. However, based on our results, it is entirely possible that in this case, 4G8 is recognizing a fibrillar conformation adopted by the exogenous $A\beta_{25-35}$. Alternatively, the increased 4G8 reactivity may be due to more of the endogenous Aβ adopting the specific fibrillar conformation recognized by 4G8 in the presence of $A\beta_{25-35}$. Thus, to completely characterize antibody specificity and rule out the recognition of generic amyloid epitopes, a panel of several oligomeric and fibrillar preparations of amyloids should also be examined. In the absence of a thorough characterization of antibody specificity, the results should be interpreted with caution regarding the molecular nature of the reactive species. Together, the data in this study highlight an important caveat in the use of antibodies raised against amyloid proteins, especially if their reactivity is presumed to be specific for the total population of a particular protein.

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Authors' disclosures available online (http://j-alz.com/manuscript-disclosures/15-0696r1).

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