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Characterization of Amyloid-β Granules in the Hippocampus of SAMP8 Mice

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Abstract. The senescence accelerated mouse-prone 8 (SAMP8) strain of mice is an experimental model of accelerated senescence that has also been proposed as a model of Alzheimer's disease as it shares several features with this dementia. We have recently reported amyloid-β (Aβ) granules in the hippocampus of SAMP8 mice, which contain $A\beta_{42}$ and $A\beta_{40}$ peptides and other amyloid-β protein precursor fragments. These granules appear clustered mainly in the *stratum radiatum* of the CA1 region and increase in number and size with age. Here we performed several studies to examine whether the Aβ granules in the hippocampus of SAMP8 mice contain other proteins characteristic of neuropathological aggregates, such as tau, MAP2, and α-synuclein. Moreover, we examined whether the Aβ granules in the hippocampus correspond to heparan sulphate proteoglycan (HSPG) positive granules previously described in this animal model. The results showed that Aβ granules correspond to the HSPG granular structures, being syndecan-2, a protein involved in the remodeling of dendritic spines, the type of HSPG found. Tau and MAP2, but not α-synuclein depositions, were also found in Aβ aggregates. Granules do not appear to have an astrocytic origin, since although some Aβ clusters are associated with astrocyte processes, most clusters are not. On the other hand, the presence of tau, MAP2, and NeuN in Aβ granules suggests a neuronal origin. As the components identified in Aβ granules are characteristic of the aggregates present in some neurodegenerative diseases, the SAMP8 model seems to be appropriate for the study of the processes involved in these pathologies.

Keywords: Alzheimer's disease, amyloid-β, hippocampus, HSPG, MAP2, SAMP8, syndecan, tau

INTRODUCTION

The senescence accelerated mouse prone 8 (SAMP8) is a strain of mice with a characteristic accelerated aging process and reduced lifespan. At few months of age, SAMP8 mice share similar characteristics with aged humans, such as lordosis, hair loss, and reduced physical activity [1, 2]. It has recently been proposed as a neurodegeneration model [3] due to impairments in learning tasks, as well as altered emotions and abnormality of the circadian rhythm [4], spongy degeneration [5], neuronal cell loss [6], and gliosis [7] in the brain. Notably, SAMP8 also show other characteristics seen in Alzheimer's disease (AD) patients, such as learning and memory deficits [7, 8], brain microvessel defects [9], blood-brain barrier dysfunction [10, 11], alteration of the cholinergic system [12], and other neurotransmitter changes [13–15]. Treatment of SAMP8 mice with antisense oligonucleotides directed to the amyloid-β (Aβ) region of amyloid-β protein precursor (AβPP) decrease the expression of AβPP and reverses the deficits in learning and memory that appear in aged SAMP8 mice [16]. This reversion can be mediated by decreasing the free radical-mediated oxidative stress [17].

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Deficits in learning and memory can also be reduced by immunotherapy with antibodies to Aβ protein [18, 19], and it has been demonstrated that this therapy induce acetylcholine increases in the hippocampus of aged SAMP8 [20]. Given such characteristics, it is perhaps not surprising that SAMP8 have also been proposed as an animal model of AD [21, 22].

In a recent study, we reported that SAMP8 animals show Aβ depositions in the hippocampus that increase in number and extent with age, while ICR-CD1 mice only showed an increase of these depositions from 12 months of age onwards [23]. The Aβ deposits are composed of clustered granules that contain $A\beta_{42}$, $A\beta_{40}$, and other Aβ protein precursor fragments. Aβ deposition observed in the hippocampus of SAMP8 animals emulates granular structures previously described in this strain, which stain with periodic acid Schiff (PAS) [24]. PAS staining is mainly used to label structures containing a high proportion of carbohydrate macromolecules such as glycogen, glycoprotein and proteoglycans. The PAS granular structures (PGS) present in SAMP8 mice show this positive staining due to the presence of heparan sulfate proteoglycan (HSPG), because these granules can be immunostained with antibodies against HSPG [25]. Temporal and spatial evolution of Aβ granules and PGS are similar. Both appear at early ages, around 3 months, and increase with age. Their distribution in clusters and their localization, mainly in the *stratum radiatum* of CA1, are also coincident. Either Aβ granules or PGS can also be found in healthy control animals, but at older ages.

HSPG and Aβ proteins are characteristic constituents of various abnormal protein aggregates present in neurodegenerative diseases, e.g., senile plaques and neurofibrillary tangles in AD. The presence of proteins or peptides that can organize in fibrillar structures and form insoluble aggregates, like Aβ, is a distinctive feature of most non-infectious neurodegenerative diseases [26]. The main constituents of these aggregates are Aβ peptide, tau, or synuclein proteins, although these components can coexist in these structures, and the aggregate composition is complex due to the secondary addition of other proteins or molecules [27].

Tau protein is a microtubule associated protein (MAP). While normal tau promotes assembly and stabilizes microtubules, abnormally hyperphosphorylated tau sequesters normal tau, MAP1 and MAP2, and disrupts microtubules. The abnormal hyperphosphorylation of tau promotes its misfolding, decrease in turnover and self-assembly into tangles of paired helical, and/or straight filaments. Disruption of microtubules by the non-fibrillized abnormally hyperphosphorylated tau, as well as its aggregation as neurofibrillary tangles, probably impairs axoplasmic flow and leads to slow progressive retrograde degeneration and loss of connectivity of the affected neurons. The abnormal hyperphosphorylation of tau is seen as neurofibrillary tangles in neuropil threads and dystrophic neurites and is apparently required for the clinical expression of AD. In related tauopathies this abnormal hyperphosphorylation leads to dementia in the absence of amyloid plaques [28].

Alpha-synuclein is predominantly a neuronal protein, but it is also found in glial cells. In neurons, α-synuclein localizes mainly in the presynaptic terminals, significantly interacts with tubulin [29] and may act as a potential microtubule-associated protein like tau [30]. There is growing evidence that α-synuclein is involved in the functioning of the neuronal Golgi apparatus and vesicle trafficking [31]. In pathological conditions α-synuclein can aggregate to form insoluble fibrils, which are the main component of the Lewy bodies seen in brains from patients with Parkinson's disease and dementia patients. Tubulin, MAP5 and neurofibrillary proteins can also contribute to the formation of these structures. On the other hand, α-synuclein is also the main component of the glial cytoplasmatic inclusions, which can also contain tau and MAP2 as accompanying proteins, among others [32].

The origin and functional significance of PGS in SAMP8 animals remains unclear and, although some degenerative process is suggested by the ultrastructural characteristics, neither the mechanism of this degenerative process nor the cellular compartment where it occurs are known yet. At light-microscopic level, a close anatomical relationship between granules and astrocytes has been established [33]. However, the fibrillar material may be a byproduct of neuronal phagocytosis by astrocytes. Although neuronal phagocytosis is primarily undertaken by microglia, astroglial proliferation and phagocytosis of debris have been reported in the degenerating brain. Studies at the electron microscope revealed that only 5% of the granules were associated with filament bundles [25], what challenges their astrocyte origin. Moreover, it has been reported that similar granules can represent enlarged presynaptic terminals or elements postsynaptic to axon terminals [34], supporting the idea of neuronal origin.

One aim of the present study was to examine whether Aβ clusters in the hippocampus of SAMP8 mice correspond to HSPG positive granules. As the most predominant HSPGs in the central nervous system are

syndecan-2 and perlecan, the former related to the maturation of dendritic spines and the latter to the basal membranes of the brain blood vessels, the presence of these two components was examined. Moreover, we also explored the possible presence of tau, MAP2 and α-synuclein in these aggregated structures at three different time-points. Finally, we studied the spatial relationship between the amyloid granules in the hippocampus of SAMP8 mice and both astrocytes and neurons.

MATERIAL AND METHODS

Animals

Male SAMP8 mice aged 3, 6, and 12 months were used. They were kept in standard conditions of temperature ($22 \pm 2^{\circ}$C) and 12:12-h light-dark cycles (300 lux/0 lux). Throughout the study, they had access to food and water *ad libitum*. Studies were performed in accordance with the institutional guidelines for the care and use of laboratory animals established by the Ethical Committee for Animal Experimentation at the University of Barcelona.

Brain processing

The animals were anaesthetized (i.p.) with 80 mg/Kg of sodium pentobarbital. The thoracic cavity was opened and the animals received an intracardiac gravity-dependent perfusion of 50 mL of phosphate buffered saline (PBS, pH = 7.2). After perfusion, brains were dissected, frozen by immersion in isopentane chilled in dry ice and stored at -80°C until sectioning. Thereafter, frozen brains were embedded in OCT cryostat-embedding compound (Tissue-Tek, Torrance, CA), cut into 20-μm-thick sections on a cryostat (Leyca Microsystems, Germany) at -22°C, and placed on slides. Sections of the central zone of the hippocampus (at about bregma −2.30) were selected according to a mouse brain atlas [35]. Slides containing brain sections were fixed with acetone for 10 min at 4°C, allowed to dry at room temperature and then stored at -20°C until staining.

Immunohistochemistry

Slides were brought to room temperature before being rehydrated with PBS for 5 min. Sections were then blocked and permeabilized with PBS containing 1% BSA (Bovine Serum Albumin, Sigma-Aldrich, Madrid, Spain) and 0.1% Triton-X-100 (Sigma-Aldrich) for 20 min. After two 5-min washes in PBS, brain sections were incubated with the primary antibody (see below) overnight in the dark at room temperature. Slides were washed again and then incubated for 1 h at room temperature in the dark with the secondary antibody (see below). After washing again, nuclear staining was performed by incubating slides in Hoechst (H-33258, Fluka, Madrid, Spain) at 2 μg/ml in PBS for 10 min at room temperature in the dark. Finally, slides were washed and coverslipped with Prolong Gold antifade reagent (Invitrogen, Carlsbad, CA). Staining controls were performed by incubating with PBS instead of the primary antibody or both primary and secondary antibodies. In double stainings, controls for cross reactivity of the antibodies were also performed.

Several primary antibodies were used: mouse monoclonal antibody 4G8, directed against human Aβ (amino acid residues 17–24 of the Aβ peptides, Sigma-Aldrich); mouse monoclonal antibody against tau protein (Millipore, Billeica, MA), which detects all phosphorylated and non-phosphorylated isoforms of tau; polyclonal antibody goat anti-syndecan-2 (Santa Cruz Biotechnology, Santa Cruz, CA); rat monoclonal antibody to Heparan Sulphate Proteoglycan or perlecan (Abcam, Cambridge, UK); rabbit polyclonal antibody against MAP2 (Millipore); rabbit monoclonal antibody to laminin (Sigma-Aldrich); chicken polyclonal antibody to glial fibrillary acidic protein or GFAP (Millipore), which was used to localize astrocytes; rabbit polyclonal antibody to AKT1 (Abcam); sheep polyclonal antibody to α-synuclein (Abcam); and mouse monoclonal antibody raised against a protein called neuronal nuclei protein (NeuN, Millipore).

AlexaFluor 555 donkey anti-goat IgG, AlexaFluor 488, 555 or 660 donkey anti-mouse IgG, AlexaFluor 555 donkey anti-rabbit IgG, AlexaFluor 555 donkey anti-rat IgG and AlexaFluor 488 goat anti-chicken IgG (Invitrogen) were used as secondary antibodies.

Image acquisition

Images of fluorescence were taken with a fluorescence laser microscope (BX41, Olympus, Germany) and stored in tif format. All images for each set of experiments were acquired with the same microscope, laser and software settings. Some images for fine colocalization analysis were obtained with a confocal scanning laser microscope (TCS/SP2, Leica Microsystems, Germany). Image treatment and analysis were performed by means of the Image J program (National Institute of Health, USA).

RESULTS

Amyloid 4G8$^+$ granules stain with syndecan-2 but not with perlecan

In order to discern whether the previously described granules containing Aβ and different fragments of its precursor protein correspond to the HSPG positive granules described by Kuo et al. [25], double immunohistochemical stainings on brains from 6-month-old SAMP8 mice were performed with the 4G8 antibody, which recognizes the 17–24 amino acid residues of Aβ peptides, and antibodies to perlecan or syndecan-2, which are the main HSPG in brain.

Some representative images from these stainings can be observed in Fig. 1. Staining with 4G8 shows the presence of clustered granules in the hippocampus of SAMP8 mice, with the characteristics already published [23]. The staining also shows the neuronal cell bodies and some blood vessels, the walls of which can also contain Aβ accumulation.

Clustered granular structures can also be observed when staining with antibodies to syndecan-2, and the 4G8 and syndecan-2 double staining shows a good granular colocalization (Fig. 1A), thus indicating that the granules contain both components and that Aβ granules correspond to HSPG positive granules.

When staining with 4G8 and perlecan, different results were obtained. The antibodies against perlecan label blood vessels, which is not surprising as perlecan is a component of the basal lamina of vessel wall, but amyloid granules are not stained (Fig. 1B). Thus, this 4G8-perlecan combination of antibodies shows clustered granules stained with 4G8 and blood vessels stained by perlecan. In some cases, blood vessels also stained with 4G8, but perlecan staining did not appear in amyloid granules. On the other hand, this double staining shows that there is no direct relationship between the localization of blood vessels and clusters, because the latter can be found both proximal and distal to blood vessels.

Control staining performed using only secondary antibodies or those of cross reactivity did not show any staining of these structures, thus confirming the specificity of the stainings.

Amyloid granules contain tau and MAP2 proteins

In a second set of experiments performed on brains from 6 month old SAMP8 mice, we examined the presence of other characteristic components of insoluble aggregates that appear in brains that present neurodegenerative pathology.

The presence of tau in amyloid granules was analyzed by double staining with antibodies to tau and syndecan-2. This double staining shows that all syndecan-2 positive granules are also positive for tau antibodies, thus demonstrating that the tau protein is present in amyloid granules (Fig. 2A). On the other hand, not all tau positive granules are stained with the antibodies against syndecan-2 (Fig. 2A inset), but only the largest granules.

In order to determine the presence of MAP2 in the granules, double staining with antibodies directed against this protein and 4G8 was performed. In the hippocampal regions where there are clusters of 4G8-positive granules, there is also granular staining for MAP2 (Fig. 2B). More detailed examination of these stainings, however, indicates that the colocalization of the two proteins is not complete. Although some granules, generally the largest ones, are labeled with both antibodies, MAP2 staining predominates in some and 4G8 prevails in others. Moreover, some granules are only stained with 4G8 or the antibodies to MAP2 (Fig. 2B Inset).

When combining MAP2 and tau staining, tau protein seems to be predominant in the granules, as already seen when double-staining with syndecan-2 and tau (Fig. 2C). Almost all the granules that are labeled with MAP2 are also positive for tau, while some tau$^+$ granules are not stained with MAP2 (Fig. 2C Inset). Colocalization studies with tau and MAP2 show that there are more granules stained with tau than with MAP2, and that all granules stained with MAP2 are also positive for the tau staining (Fig. 2C).

Laminin staining gave no positive label in the granular aggregates, although the antibody clearly labeled blood vessels, as expected as it stained the basal lamina. On the other hand, antibodies against α-synuclein did not stain granules or other structures in the hippocampus of SAMP8 mice (data not shown).

The staining of tau, syndecan, 4G8 and MAP2 has also been studied in the hippocampus of SAMP8 mice aged 3 and 12 months. As can be observed in Fig. 3A and 3B, and according to what has been already published about Aβ staining in this strain [22], at 3 months of age there are few clusters and those contain only some granules, while at 12 months the number of clusters is markedly higher than at younger ages, these clusters contain numerous granules and they extend throughout the hippocampus. At 3 months of age double staining with tau and syndecan-2 (Fig. 3A) and tau and MAP2 (Fig. 3E) show that there are some

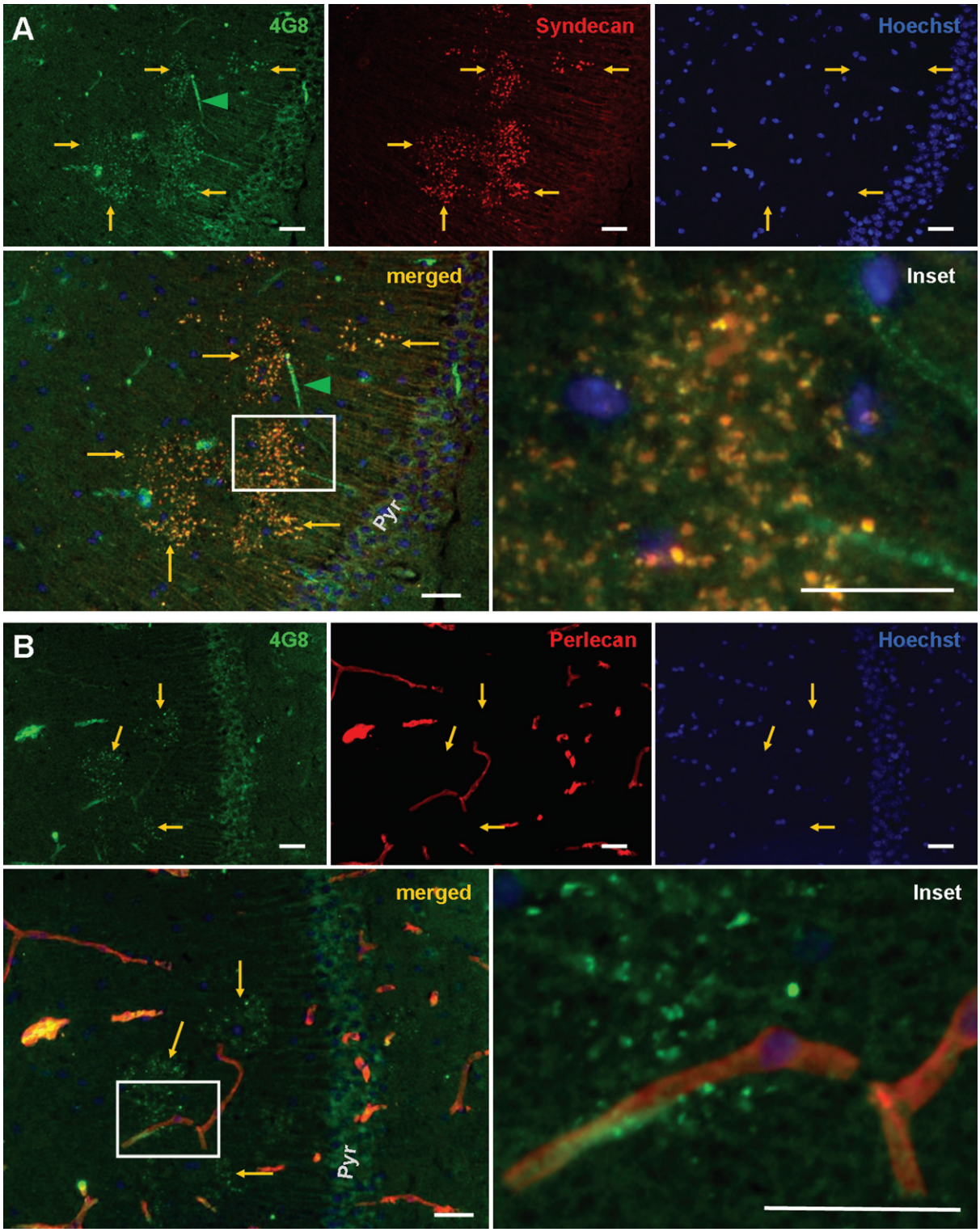

Fig. 1. Presence of Aβ and HSPG in hippocampal granules of 6-month-old SAMP8 mice. A) Staining with 4G8 (Aβ, green) and syndecan-2 (red). B) Staining with 4G8 (green) and perlecan (red). Hoechst staining (blue) corresponds to cellular nuclei. Colocalization of green and red corresponds to yellow color. Colocalization occurs between Aβ and syndecan-2, but not between Aβ and perlecan. Arrows indicate clusters of granules. Arrowheads indicate blood vessels. Scale bars: 50 μm. Pyr: pyramidal layer.

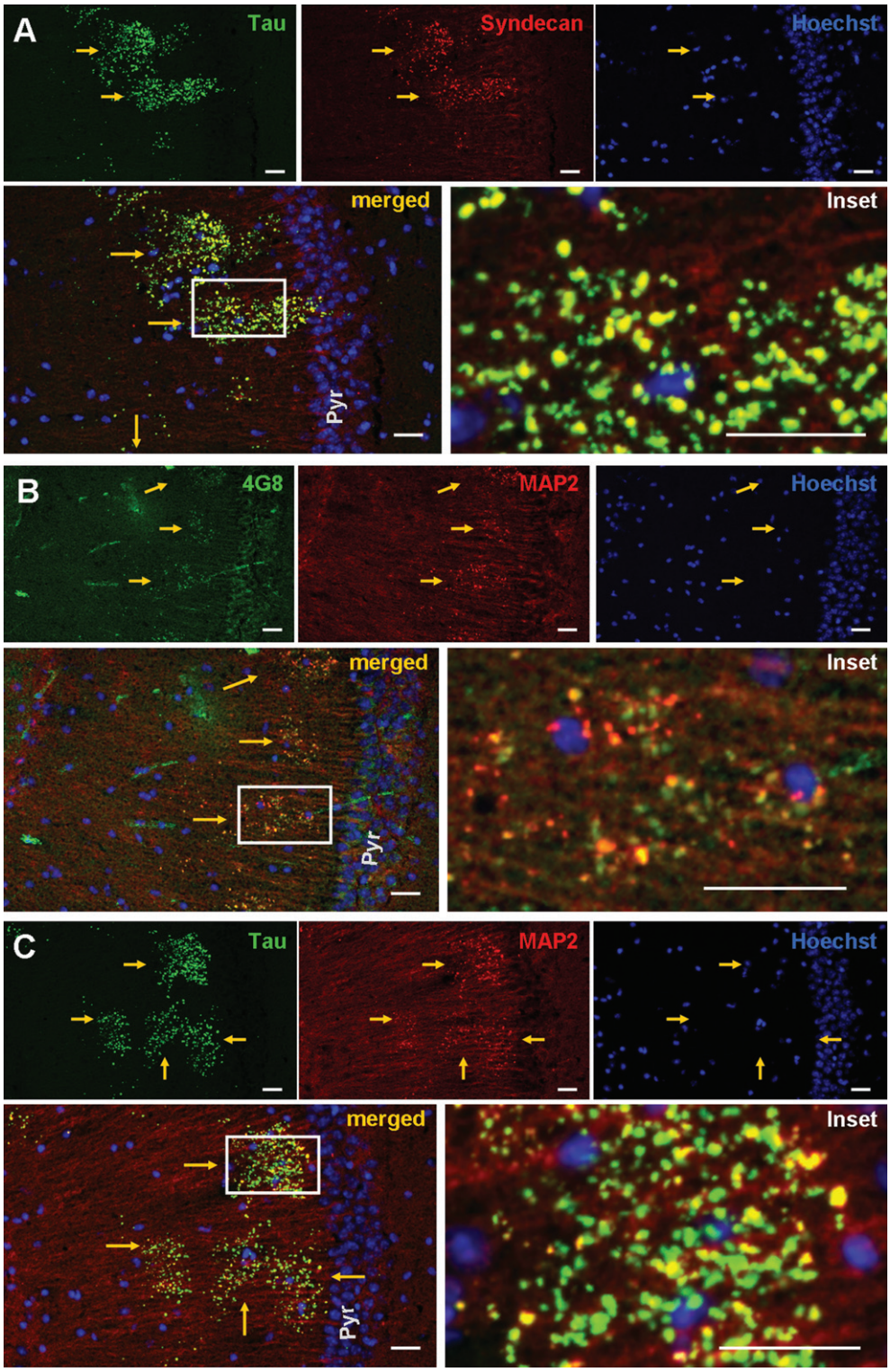

Fig. 2. Protein composition of hippocampal granules of 6-month-old SAMP8 mice. A) Staining with tau (green) and syndecan-2 (red). B) Staining with 4G8 (green) and MAP2 (red). C) Staining with tau (green) and MAP2 (red). Hoechst staining (blue) corresponds to cellular nuclei. Colocalization of green and red corresponds to yellow color. Arrows indicate clusters of granules. Scale bars: 50 μm. Pyr: pyramidal layer.

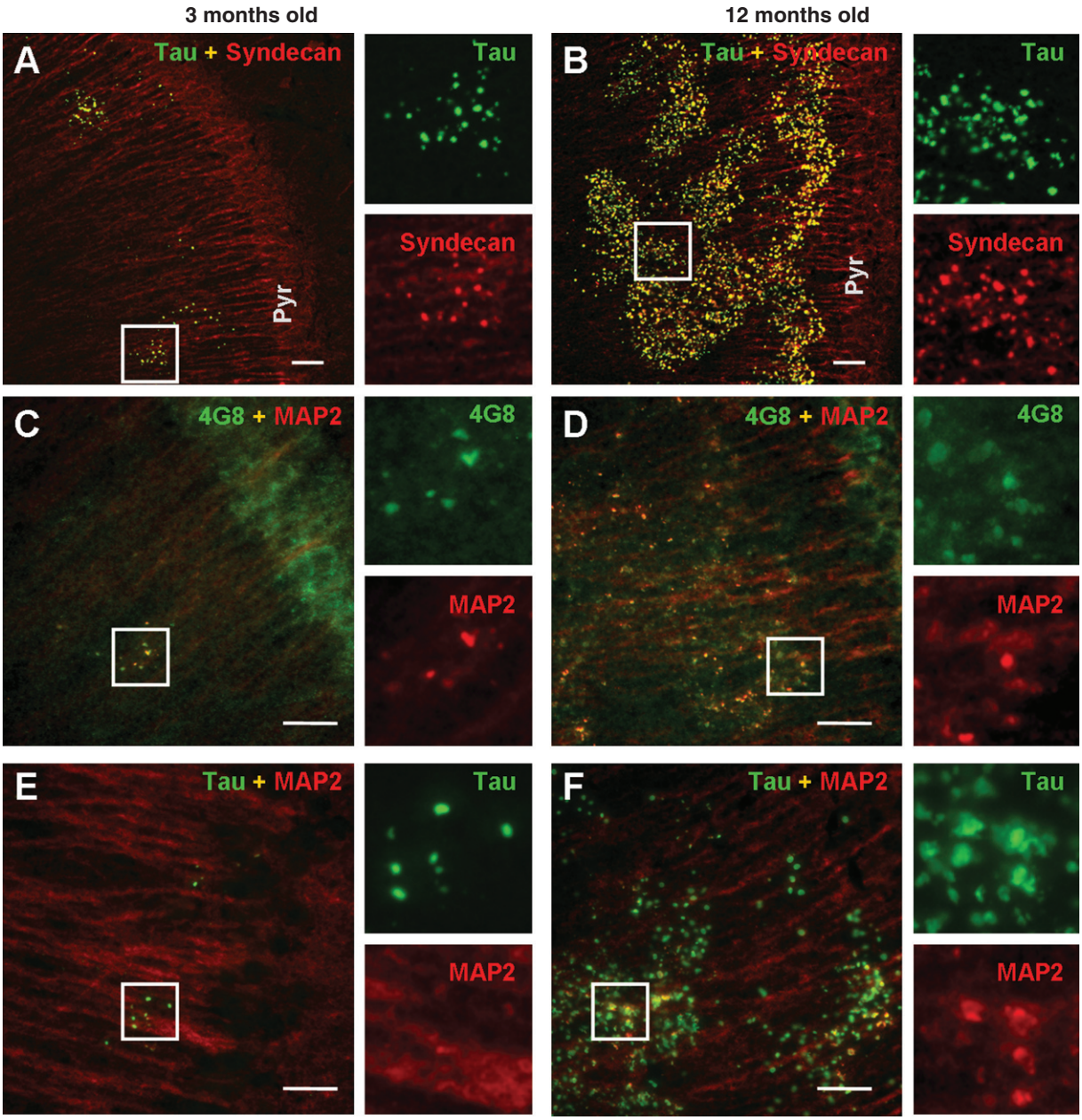

Fig. 3. Protein composition of hippocampal granules of 3- and 12-month-old SAMP8 mice. A and B) Staining with tau (green) and syndecan-2 (red). C and D) Staining with 4G8 (green) and MAP2 (red). E and F) Staining with tau (green) and MAP2 (red). Colocalization of green and red corresponds to yellow color. A, C and E: 3-month old; B, D and F) 12-month old. Scale bars: 50 μm. Pyr: pyramidal layer.

double-stained granules and other granules that are only stained with tau, but there are not granules only stained with syndecan-2 or MAP2. In the 4G8 and MAP2 double staining (Fig. 3C) some granules are only stained with one of the two components, MAP2 or 4G8, and others are double stained. At 12 months of age we also observed that some granules double stained with syndecan-2 and tau and other granules double stained with MAP2 and tau, while some granules only stains with tau (Fig. 3B, 3F). Moreover, when staining with 4G8 and MAP2, we also observed that some granules are only stained with one of the two components, MAP2 or 4G8, and others are double stained (Fig. 3D). Thus, in all the studied ages, a predominance of tau protein is observed over the other stained proteins.

Cellular types related with the amyloid granules

In the last set of studies performed on brains from 6-month-old SAMP8 mice, the relationship between amyloid granules and astrocytes or some constitutive parts of hippocampal neurons was explored.

Double staining of amyloid aggregates with antibodies against MAP2 and GFAP, a constitutive protein present in reactive astrocytes, showed several cases of clusters stained with MAP2 located in regions where no GFAP reactivity was found (Fig. 4A). In some other cases, however, an astrocyte is placed near or overlapping the deposits (Fig. 4B). In these latter situations, there is some spatial relationship between amyloid granules and the astrocyte processes. Although colocalization of GFAP and amyloid staining is minimal, most of the granules in the cluster seem to be adhered to the astrocyte processes (Fig. 4C). In some cases, a ring of GFAP staining can be observed around amyloid granules, which suggest that the astrocyte processes encompass the granule (Fig. 4C, arrowhead, single labeling in C1 and C2).

When combining MAP2 and NeuN staining, a neuronal marker apparently restricted to neuronal nuclei, perikarya and some proximal neuronal processes in both fetal and adult brain, a clear NeuN staining can be observed in the hippocampus pyramidal region, in which the cell bodies of pyramidal neurons are located (Fig. 4D). Granular clusters stained with both NeuN and MAP2 can also be observed (Fig. 4D, single labeling in D1 and D2). Detailed visualization of the granules indicates that they contain different proportions of both constituents. In some granules there is double staining, but in others MAP2 or NeuN staining clearly predominates.

Finally, amyloid granule staining was also combined with the labeling of AKT protein, which allows visualization of some neurites, i.e., axons and dendrites, from neurons in the hippocampus of SAMP8 animals. This double staining revealed $4G8^{+}$ amyloid granules and part of the neuronal network in the neuropil of the hippocampus (Fig. 4E). Detailed visualization of this staining does not reveal any relationship between axons or dendrites stained with AKT and the presence of amyloid granules (Fig. 4F).

DISCUSSION

Besides the Aβ components already described [23], the granules in the hippocampus of SAMP8 mice contain tau, MAP2, and syndecan-2. All these components are characteristic of the aggregates of some neurodegenerative diseases. The coexistence of Aβ peptides and tau protein is frequently observed in neurodegenerative diseases [36]. Aβ can also interact with syndecan-2, related with the formation of synaptic contacts [37], and both can be found in amyloid plaques of AD patients [38]. Moreover, tau and MAP2, which in physiological conditions stabilize the neurofilaments of axons and dendrites, have been found together in neurofibrillary tangles [28]. The presence of these components, as well as that of NeuN, a characteristic protein of neurons, suggests a neuronal origin for the granules and some neurodegenerative process for its formation.

The staining with antibodies against α-synuclein demonstrated no positivity in the aggregates found in the hippocampus of SAMP8 animals. This protein is present in Lewy body disease [39, 40], Parkinson's disease [32], and other α-synucleinopathies [41]. The absence of α-synuclein in SAMP8 aggregates and the presence of tau and Aβ suggest that these granules are closer to the depositions of taupathies and amyloidopathies rather than those of α-synucleinopathies. However, an increase in α-synuclein levels has been reported in SAMP8 animals aged 5 and 10 months, although these results were obtained using homogenized brain tissue and Western-blot techniques [42, 43].

We also determined here the presence of both Aβ and tau in granular structures. Considering that both proteins can be the main components of the aggregates in neurodegenerative diseases, there is a need for a deeper study of these two components in these amyloid granules from SAMP8 animals, in order to determine whether this model support the Aβ cascade hypothesis, the tau hypothesis, both, or neither. It has been described that Aβ initiates the hyperphosphorylation of tau, but other studies indicated that hyperphosphorylated tau is the element that initiates the pathological process [36]. Our results indicate, already at three months of age, that the granules show a predominance of tau over Aβ and all other components tested. At 6 and 12 months of age, the number of clusters and the number of granules in the clusters increase, but remains the predominance of tau over all the other components tested. These facts point out that tau protein has an important role in the formation of the granules and its presence could be related with the subjacent neurodegenerative process. However, it has to be taken into account that current thinking on AD pathology includes the concept that hallmark lesions may be nontoxic and may represent a host response to an upstream pathophysiological process [44], and thus the observed

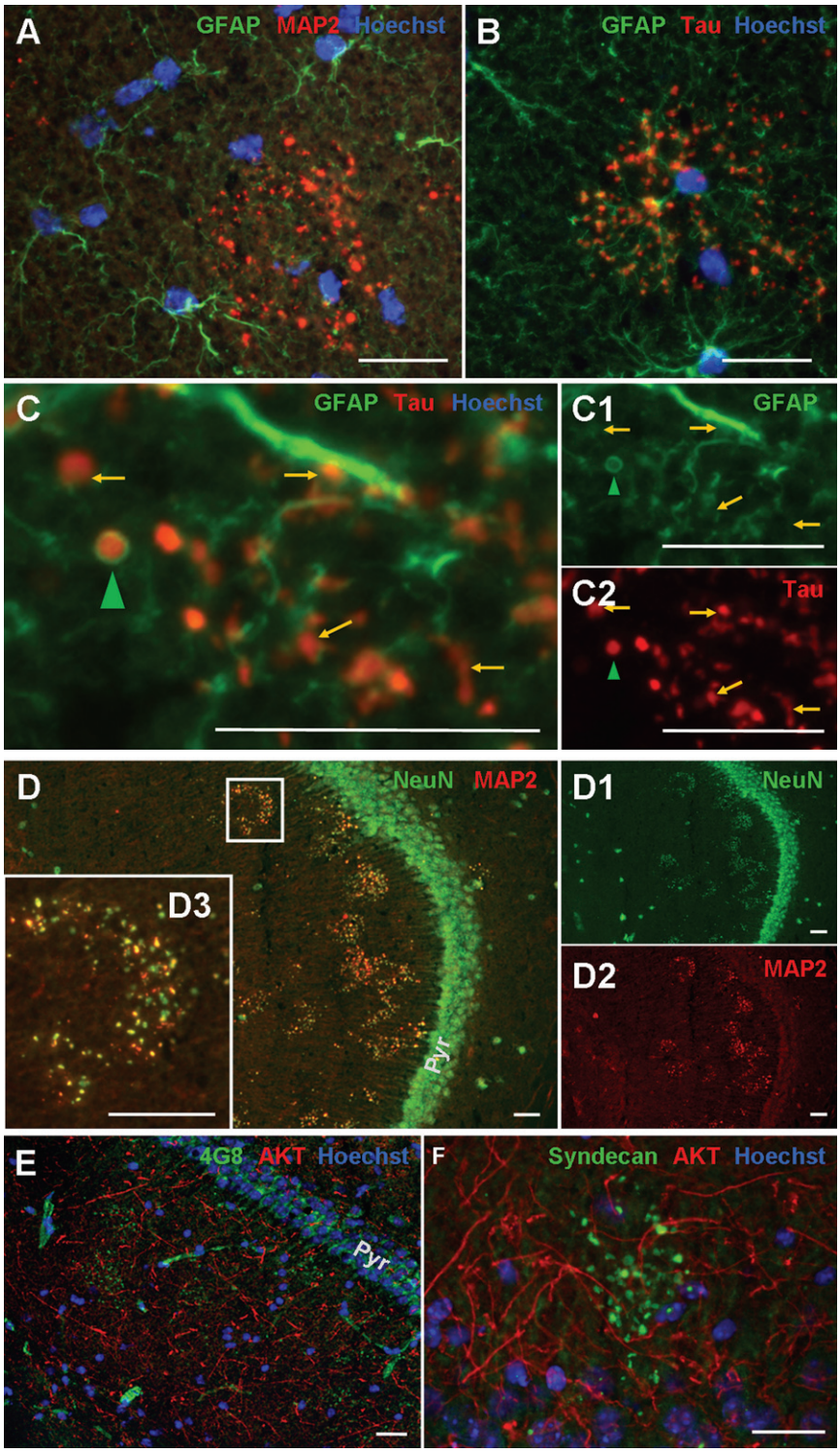

Fig. 4. Relationship of cellular types and hippocampal aggregates from 6-month-old SAMP8 mice. A) Staining with GFAP (green) and MAP2 (red). B) Staining with GFAP (green) and tau (red). C) Staining with GFAP (green) and tau (red). Single labeling is shown in C1 and C2. D) Staining with NeuN (green) and MAP2 (red). Single labellings are shown in D1 and D2. D3) Magnification of inset in D. E) Staining with 4G8 (green) and AKT-1 (red). F) Staining with syndecan-2 (green) and AKT-1 (red). Hoechst staining (blue) corresponds to cellular nuclei. Colocalization of green and red corresponds to yellow color. Arrows indicate clusters of granules. Scale bars: 50 μm. Pyr: pyramidal layer.

granules and the presence of tau could represent only a final stage but not the responsible of the pathological processes that are being produced.

Here we have studied tau protein as total tau and it would be interesting to ascertain which types of tau are present in the granules, as hyperphosphorylated tau is involved in multiple neurodegenerative diseases, ultimately promoting the degeneration of affected neurons [45]. Indeed, the increase in hyperphosphorylated tau protein in SAMP8 mice has been found in the hippocampus by Western-blot techniques [46]. The abnormal tau protein can sequester, among others, MAP2 protein [47], which may explain the presence of MAP2 in the granules of the hippocampus of SAMP8 mice.

Although the components found in the granules of SAMP8 mice seem to indicate a neuronal origin, we have also observed in some cases that the region occupied by a cluster of granules is also occupied by a determinate astrocyte. However, not all the aggregates are close to reactive astrocytes, thus a reactive astrocyte is not essential for the presence of these clusters. Moreover, when clusters and astrocytes are close, the astrocyte processes are adjacent to the granules, even surrounding them, but the granules do not appear to constitute a part of the astrocyte process. The presence of reactive astrocytes close to amyloid aggregates could thus be related to a reactive process, in which astrogliosis is induced in response to injury, like an alteration of synaptic transmission and cytotoxicity or extracellular accumulation of fibrillar material [48].

Our results also indicate that amyloid granules present in the hippocampus of SAMP8 mice correspond to the PAS granular structures that contain HSPG described previously by Kuo et al. [25], being syndecan-2 the HSPG. Kuo and colleagues, in ultrastructural studies of the PGS stained with HSPG antibodies, detected some deformed cellular organules and remains of cellular membranes surrounding the PGS. Although it is not yet established, these membranes could correspond to residual degenerative parts of neurons.

Although we could not relate the granules with AKT-1 staining, it has to be pointed out that AKT-1 staining only shows a minor part of the neuropil, and recent studies suggest that activation of AKT inhibits toxicity of Aβ and formation of neurofibrillary tangles, leading to protection of neurons against apoptosis [49].

In summary, we conclude that SAMP8 mice present complex aggregates formed by multiple proteins that are distinctive of neurodegenerative diseases. The presence of MAP2, syndecan-2, NeuN, and especially tau protein and Aβ peptides, points to SAMP8 mice as a potential animal model in which to study the process of senescence and neurodegenerative diseases.

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Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=799).

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