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Expression Pattern of Scavenger Receptors and Amyloid-β Phagocytosis of Astrocytes and Microglia in Culture are Modified by Acidosis: Implications for Alzheimer's Disease

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Abstract. The pathological hallmarks of Alzheimer's disease (AD) are amyloid-β (Aβ) plaques, neurofibrillary tangles, and glia activation. The pathology also includes vascular amyloidosis and cerebrovascular disease. Vascular compromise can result in hypoperfusion, local tissue hypoxia, and acidosis. Activated microglia and astrocytes can phagocytose Aβ through membrane receptors that include scavenger receptors. Changes in glial cells induced by extracellular acidosis could play a role in the development of AD. Here, we assess whether extracellular acidosis changes glial cell properties relevant for Aβ clearance capacity. Incubation of glial cells on acidified culture medium (pH 6.9 or 6.5) for 24–48 h resulted in decreased cell diameter, with thinner branches in astrocytes, slight reduction in cell body size in microglia, a transient decrease in astrocyte adhesion to substrates, and a persistent decrease in microglia adhesion compared with control media (pH 7.4). Astrocyte Aβ phagocytosis decreased at pH 6.9 and 6.5, whereas microglia phagocytosis only transiently decreased in acidified media. Scavenger receptors class B member I (SR-BI) increased and scavenger receptors-macrophage receptors with collagenous structures (SR-MARCO) decreased in astrocytes cultured at pH 6.5. In contrast, in microglia exposed to pH 6.5, expression of SR-BI and SR-MARCO increased and fatty acid translocase (CD-36) decreased. In conclusion, the acidic environment changed the adhesiveness and morphology of both microglia and astrocytes, but only astrocytes showed a persistent decrease in Aβ clearance activity. Expression of scavenger receptors was affected differentially in microglia and astrocytes by acidosis. These changes in scavenger receptor patterns can affect the activation of glia and their contribution to neurodegeneration.

Keywords: Acidosis, amyloid-β peptide, glial activation, phagocytosis, scavenger receptors

INTRODUCTION

Alzheimer's disease (AD) is the degenerative disorder that most commonly causes dementia [1]. Risk factors for AD include aging, the ε4 allele of apolipoprotein E, atherosclerosis, diabetes, hyperlipidemia, high blood pressure, and neurovascular

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and cardiovascular diseases [2, 3]. Several of these risk factors can be associated with reduced cerebral perfusion leading to a hypoxic and acidic microenvironment, which in turn can result in oxidative stress, brain tissue damage, and AD progression [4–8]. Brain tissue acidification can be observed in normal processes like aging and in pathological conditions like ischemia and inflammation. It has been hypothesized that aging and ischemia increase cellular metabolism, accumulation of acidic metabolites, and inactivation of genes responsible for controlling pH, leading to acidification [9]. Acidification may induce cell death and promote development of AD or vascular dementia. In fact, enzymes associated with amyloid-β (Aβ) generation [10], like β-secretase and γ-secretase [11–13], and changes in amyloid-β protein precursor processing are induced by hypoxia-related increase in lactic acid [14] and aggregation of Aβ [15, 16]. Furthermore, Aβ degradation by insulin-degrading enzymes (IDE) could further decrease under acidic conditions given that the effect of low pH on enzyme formation, assembly, and stability may inactivate IDE [17].

A characteristic feature of inflammation is local acidosis. It has been suggested that acidic microenvironments inhibit immune function in certain respiratory conditions [18] and during neoplastic growth and invasion [19, 20]. So far, no pH data have been reported for chronic neuroinflammatory conditions of the central nervous system, but there are reports indicating that the brains of AD patients have decreased tissue pH in the frontal cortex and caudate nuclei [21, 22], in part due to a greater accumulation of lactate [21]. Acidosis also appears to induce changes in amyloidogenesis and contribute to neuronal death [10, 23]. Increased amyloidogenesis could worsen AD neurodegeneration because Aβ produces cytotoxic activation of microglia, which in turn produces oxygen radicals and cytokines [24]. Nevertheless, as we have shown previously, acidification has a neuroprotective effect for hippocampal neurons exposed to Aβ [25].

Local extracellular pH also fluctuates during normal brain functioning as a consequence of neuronal activity. Neuronal activity increases carbohydrate metabolism, increasing lactic acid, CO_2, and the release of synaptic vesicles containing protons that acidify the synaptic space. In addition, astrocyte and microglia pH regulation involves the contributions of carbonic anhydrase, the Na^+-HCO_3^- cotransporter, the Na^+/H^+ exchanger, and the Na^+-dependent or Na^+-independent Cl^-/HCO_3^- antiporters [26–30]. Acid-sensing or CO_2-sensing receptors are found in neurons and glia. TRP channels endow astrocytes with the capacity to sense CO_2 [31]. Astrocytes in the retrotrapezoid nucleus are capable of sensing increases in CO_2 and H+ and in response increase the cytoplasmic calcium concentration and releasing ATP [32]. Several mechanisms can account for the H+ and CO_2-sensitivity of astrocytes and microglia. Microglia express the voltage-sensitive proton channel Hv1, which under physiological conditions regulates intracellular pH and facilitates NADPH oxidase-dependent generation of reactive oxygen species [33]. Astrocytes and microglia can be depolarized by inhibition of inwardly rectifying potassium (Kir) channels, which contribute to extracellular potassium regulation [28, 34, 35]. Cx32 has been detected along with other connexions in activated microglia [28], and Cx26, Cx30, and Cx32 are expressed in astrocytes. These connexins have a carbamylation motif, a binding site for CO_2, the activation of which opens Cx hemichannels [36].

Microglia and astrocytes are the major players in neuroinflammation [37, 38]. In AD brains, microglia and astrocytes are activated at the Aβ deposition site closely associated with plaques. When activated, they increase their expression of inducible nitric oxide synthase (iNOS) and they secrete inflammatory molecules like interleukin-1β, MCP-1, and RANTES, among others [39–43], reviewed in [44]. Glial cell activation can be both deleterious and protective in neurodegenerative diseases, depending on the co-stimulants and temporal context [44–50]. Activated microglia and astrocytes can phagocytose Aβ *in vitro* [50, 51]. Aβ phagocytosis appears to be impaired in aged animals [52, 53]. Reduction of microglial cells results in an increased accumulation of Aβ in a murine AD model [47] and impairment of glia function by inflammatory conditions appears to contribute to AD pathogenesis by decreasing Aβ clearance [37, 44, 54–56] and increasing neurotoxicity [57]. Scavenger receptors (SR) are among the receptors mediating Aβ clearance by glial cells as well as glial cell activation [47, 50, 58, 59]. Glial cells express scavenger receptor class A (SR-A) [60] and SR-MARCO [61], class B member I (SR-BI) [62], and fatty acid translocase (CD36) [63], the receptor for advanced glycation end products [64], low density lipoprotein receptor-related protein [65], and mannose receptor [66]. Glial cell expression of scavenger receptors is modified by several factors, some depending on acute changes in cerebral homeostasis and on chronic changes like those produced by

hypoperfusion, aging, and chronic inflammation [37, 44, 50, 52, 67, 68].

Changes in SR levels secondary to extracellular acidification could be especially relevant for AD, because of potential changes on both Aβ clearance and their participation in the activation of glial cells. Several pattern recognition receptors including Toll-like receptors (TLR) [69] and SR [58, 70] appear to trigger the activation of specific inflammatory pathways according to the ligand they bind. To evaluate the effect of reduced extracellular pH on glial activity, we determined the expression of SR on astrocyte and microglial cell cultures maintained at pH 6.9 and 6.5, and assessed the effect of pH in Aβ clearance activity. First we assessed whether reduced pH had different effects in the binding of various substrates, indicating that it was affecting specific recognition and not general processes. Next we performed competition experiments in Aβ binding with SR ligands to reveal if SR were indeed mediating interaction with Aβ. Finally, we assessed whether extracellular acidification differentially affected astrocyte and microglia activation, glial cell adhesion, and Aβ phagocytic activity.

MATERIALS AND METHODS

Chemicals and reagents

Poly-L-lysine, fucoidan, polyinosinic acid (Poly-I) and polycytidylic acid (Poly-C), bovine serum albumin (BSA), lidocaine, and trypsin EDTA were purchased from Sigma. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin were obtained from Invitrogen. Tissue culture dishes and plastic ware were from Falcon (Franklin Lakes, NJ) and Nalgene Nunc (Rochester, NY). The cytotoxicity detection kit (LDH) was from Roche Applied Science. Other reagents were purchased from Sigma or Merck. The $A\beta_{1-42}$ was a generous gift from Dr. Heinz Döbeli (Hoffmann-La Roche, Switzerland). All animal experimentation was performed according to the protocol submitted to and approved by the Animal Research Ethics Committee of the Pontificia Universidad Católica de Chile, and in compliance with the current Chilean law.

Primary glial cell culture

Astrocytes and microglial cell cultures were prepared from the brains of 1–2-day-old neonatal rats [71]. Cortices were minced and incubated with 0.25% trypsin-EDTA in Hanks' solution (0.4 g/l KCl, 0.06 g/l KH_2PO_4, 0.048 g/l Na_2HPO_4, 8 g/l NaCl, 1 g/l D-glucose, and 3.5 g/l $NaHCO_3$) for 15 min and mechanically dissociated. Cells (one brain per flask) were seeded in 75-cm^2 culture flasks coated with poly-L-lysine (Sigma) in complete medium (DMEM/F-12, 10% FBS, 100 units/ml penicillin and 100 μg/ml streptomycin) and incubated in a water-saturated atmosphere with 5% CO_2 at 37°C. After 14–28 days of culture, flasks were treated with 12 mM lidocaine and shaken at 37°C for 10 min to detach microglia. Astrocytes were obtained by trypsinization. Cells were pre-plated for 1 h; cells that were not adherent were recovered, counted, and seeded for experimental use. This procedure yielded highly enriched astrocyte cultures (95% or more) and microglial cell cultures over 99% pure. Cell typing was done by labeling fixed cultures with fluorescein isothiocyanate (FITC)-conjugated lectin *Griffonia simplicifolia* (1 : 200; Sigma), which recognizes microglia and an antibody against glial fibrillary acidic protein (GFAP; Dako, Denmark) to identify astrocytes.

Cell adhesion assay at various values of pH

The 96-well tissue culture plate was left uncoated (plastic) or coated with one of the following substrates: 50 μg/ml of poly-L-lysine, 1 μg/ml BSA, 50 μg/ml collagen IV or 10% gelatin, or 25 μg/ml melibiose-BSA (mel-BSA) for 1 h, in Dulbecco's modified Eagle's medium and nutrient mixture F-12 (DMEM-F12), with 10% of fetal bovine serum, and then washed with distilled water and air-dried under sterile conditions. For the adhesion assay, 3×10^4 cells/well were seeded in a 96-well plate with 100 μl of DMEM-F12 equilibrated at various values of pH (7.4; 6.9; 6.5) and placed in an incubator at 37°C for 24 h. To evaluate viability of detached cells, floating cell were recovered and plated at pH 7.4 for an additional 24 h to evaluate their adhesion. The number of cells that were adherent was determined by quantifying the LDH released by the cells after exposure to non-ionic detergent by the Cytotoxicity LDH assay™ (Roche Molecular Biochemicals) according to the manufacturer's instructions. A standard curve was generated by plating $0.5–30 \times 10^3$ cells, which were processed in parallel with each LDH assay. For quantification, adhesion in various matrices at different pH levels was compared with the adhesion of astrocytes and microglia to poly-L-lysine at pH 7.4.

For the SR-A ligand competition assay, glial cells were preincubated in suspension in DMEM-F12+1 mg/ml BSA with the various ligands for scavenger receptors: 200 μg/ml fucoidan, mel-BSA, or poly-I, or 200 μg/ml of the control ligand poly-C, with gentle agitation at 37°C for 1 h. After preincubation, the cells were plated on Aβ-coated wells as previously described.

Aβ phagocytosis

Glass coverslips were placed in 24-well plates and coated with 500 μl of 50 μg/ml poly-L-lysine at 37°C for 24 h, and washed three times with distilled water. Then, 10 μl of 10 μg/ml Cy3-Aβ was placed on the coverslips, and air-dried under sterile conditions. Purified astrocytes and microglia were plated at a density of 3×10^4 cells/well. The phagocytosis assay was run in DMEM-F-12 at varied pH (7.4; 6.9 or 6.5) for 5 h, 24 h or 5 days for microglia, and 24 h, 48 h, or 6 days for astrocytes. After the phagocytosis assay, cells were immunolabeled for cell identity markers or endosomal markers (EEA1; early endosome antigen 1) and Cy3-Aβ (Red) to assess phagocytosis. For quantification, 8 fields per cell culture of 5 independent experiments were photographed and the cells that took up Aβ were quantified with the program Image J (NIH).

Immunofluorescence

After the Aβ phagocytosis assay, astrocytes and microglia were washed with PBS with 1 mM Ca^{2+}, and fixed in 2% p-formaldehyde at 20°C for 15 min. Cells were permeabilized with 0.2% Triton X-100 in PBS for 15 min, blocked with 10% goat serum in PBS and incubated at 4°C overnight with rabbit anti-GFAP (1 : 200; Dako), rabbit human anti iNOS (1 : 200; Santa Cruz), monoclonal human anti-EEA1 (1 : 200; kindly provided by Dr. Alfonso Gonzalez, PUC School of Medicine), or Alexa-488-conjugated lectin from *Griffonnia simplicifolia* (1 : 200; Molecular Probes, Inc., Eugene, OR). Except for samples labeled with lectin, coverslips were washed in PBS and incubated with the corresponding secondary antibody, either anti-rabbit Alexa 488 (1 : 100; Molecular Probes) or mouse anti-human Alexa 546 (1 : 200; Origen) in blocking solution at room temperature for 2.5 h. Nuclei were stained with 0.1 μg/ml Hoechst 33258 (B2883; Sigma). Coverslips were washed in PBS and water and mounted in low fluorescence mounting medium (Dako).

Immunoblot analysis

After cells were exposed to the experimental conditions, they were harvested and lysed in ice-cold lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, and protease and phosphatase inhibitors). Protein concentration was determined by the BCA method. The same amount of protein for each sample was added to sample buffer (80 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 2% 2-mercapthoethanol, 0.02% Coomassie Blue G250) and heated for 5 min. SDS-PAGE was performed in 10 and 12% (w/v) polyacrylamide gels and transferred to a PVDF membrane (Thermo Scientific). The membrane was incubated with the primary antibody in 5% milk-PBST buffer: anti-SR-A antibody (1 : 1,000, R&D), anti-SR-B antibody (1 : 2,000; Novus Biologicals), anti-SR-MARCO antibodies (1 : 1,000, Serotec) or anti-CD36 antibody (1 : 1,000; Santa Cruz Biotechnology) overnight. Primary antibodies were rinsed and membranes incubated with the appropriate secondary antibody conjugated with horseradish peroxidase (1 : 5,000 in blocking buffer) for 2 h. Signals were detected by enhanced chemiluminescence in accordance with the manufacturer's instructions. Positive controls were obtained from adrenal gland (SR-B), liver (SR-A and SR-MARCO), and heart (CD36). Quantification of bands from independent immunoblotting groups were subjected to a densitometry analysis using the Image J software (NIH).

Statistical analysis

Values are expressed as mean ± SD or SEM as indicated. Statistical analysis was performed with an ANOVA, followed by the Newmann–Keul test. Statistical significance was established for $p < 0.05$.

RESULTS

Astrocytes exposed to an acidic environment showed differential adhesion to various substrates

We evaluated the effect of an acidic environment on the adhesion of astrocytes to various substrates. Changes in astrocyte pH-dependent adhesion were different depending on the type of adhesion surface. Adhesion was maximal on poly-L-lysine at pH 7.4 (Fig. 1A). Adhesion was moderate on plastic, gelatin, and mel-BSA (50%) and poor for BSA

(<10%). Adhesion to BSA and plastic did not change significantly at acidic pH. Astrocytes adhesion to poly-L-lysine, gelatin, and mel-BSA was sensitive to pH changes. However, whereas adhesion to poly-L-lysine and gelatin decreased to 80% and 10%, respectively ($p < 0.001$) with acidification, adhesion to mel-BSA increased by almost 200% when cells were cultured in an acidic environment ($p < 0.001$; Fig. 1B). These results show that acidic environments have different effects on adhesion to various substrates. For the experiments related to adhesion, the adhesion on poly-L-lysine was considered to represent 100% adhesion. The substrates BSA and collagen IV were non-adherent for astrocytes and microglia, respectively [50].

Extracellular acidosis decreased glial cell adhesion but not cell viability

Glial cells presented morphological changes and decreased cell adhesion when exposed to an acidic environment. Astrocytes at physiological pH (pH 7.4-7.4) had extensive cytoplasm and robust extensions (Fig. 2). However, when changed to an acidic environment for a further 24 h (pH 7.4-6.5), they showed thinner extensions with decreased cell diameter compared with the control condition, compatible with the morphology of activated astrocytes. In contrast, astrocytes that were initially exposed to an acidic environment for 48 h and then transferred to a physiological pH environment for 24 h (pH 6.5-7-4) reverted to their normal morphology. iNOS immunodetection to evaluate glia inflammatory activation revealed that iNOS immunolabeling was slightly higher in astrocytes cultured in acidosis than in those cultured at pH 7.4.

Microglia showed very mild morphological differences when exposed to acidic pH. At physiological pH (7.4-7.4), microglia were round shaped, and after exposure to an acidic environment (7.4-6.5), they were smaller than those under the control condition. When the culture medium was changed to physiological pH for 24 h (6.5-7-4), microglia morphology returned to that under control conditions. Acidosis was not effective as an inflammatory activator of microglia, since iNOS labeling was similar at the different pH levels (Fig. 2).

The adhesion assays showed that astrocyte adhesiveness decreased by 25% in an acidic environment compared with controls ($p < 0.001$). This loss in adhesiveness was transiently observed at 24 h of culture (Fig. 3A), whereas the adhesion assessed at 48 h was

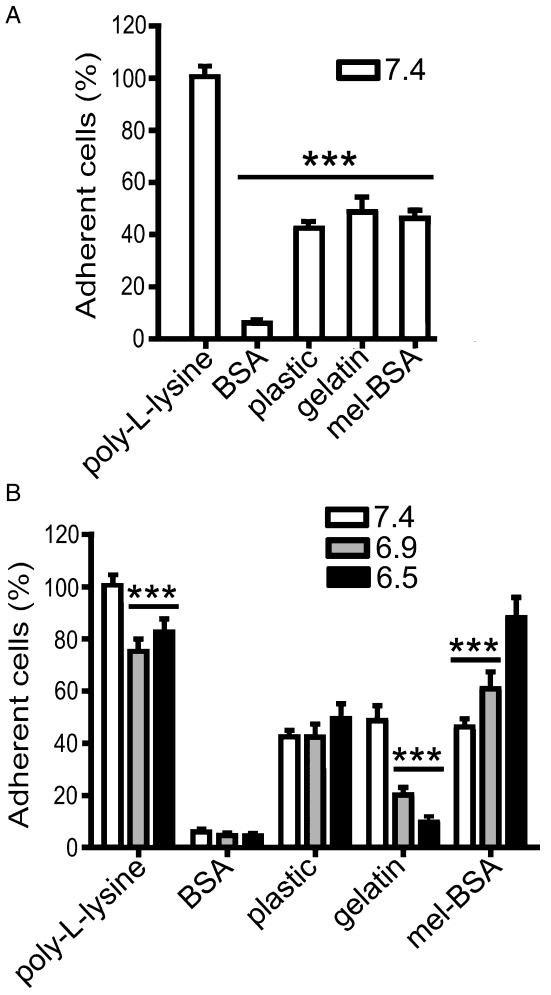

Fig. 1. Acidic environments affected astrocyte adhesion depending on the adhesion substrate. A) Astrocyte adhesion to different substrates was measured under physiological conditions (pH 7.4). After 24 h of culture, astrocytes showed the greatest adhesion when poly-L-lysine was used as a substrate, which was considered 100% adhesion. Data are expressed as the mean ± SEM of the percentage of adhesion on poly-L-lysine at pH 7.4, of 3-to-7 independent experiments in quadruplicate. ***$p < 0.001$. B) Astrocyte adhesion to various substrate matrices was evaluated after 24 h of culture in environments with different acidity levels. The mean of adhesion on poly-L-lysine at pH 7.4 was considered 100%. Bovine serum albumin (BSA) was the least adherent substrate, whereas plastic, gelatin and mel-BSA had moderate adhesion of around 50% of that of poly-L-lysine at pH 7.4. Adhesion to poly-L-lysine and gelatin decreased with acidification, whereas adhesion to mel-BSA increased in a low pH environment. Adhesion to BSA and plastic showed no changes with acidification. Data are expressed as the mean ± SEM of the percentage of adhesion to poly-L-lysine at pH 7.4, of 3-to-7 independent experiments in quadruplicate. ***$p < 0.001$.

similar at all pH conditions. In contrast, microglial cell adhesion decreased by 40% after 24 h under acidic conditions ($p < 0.001$) and was further reduced

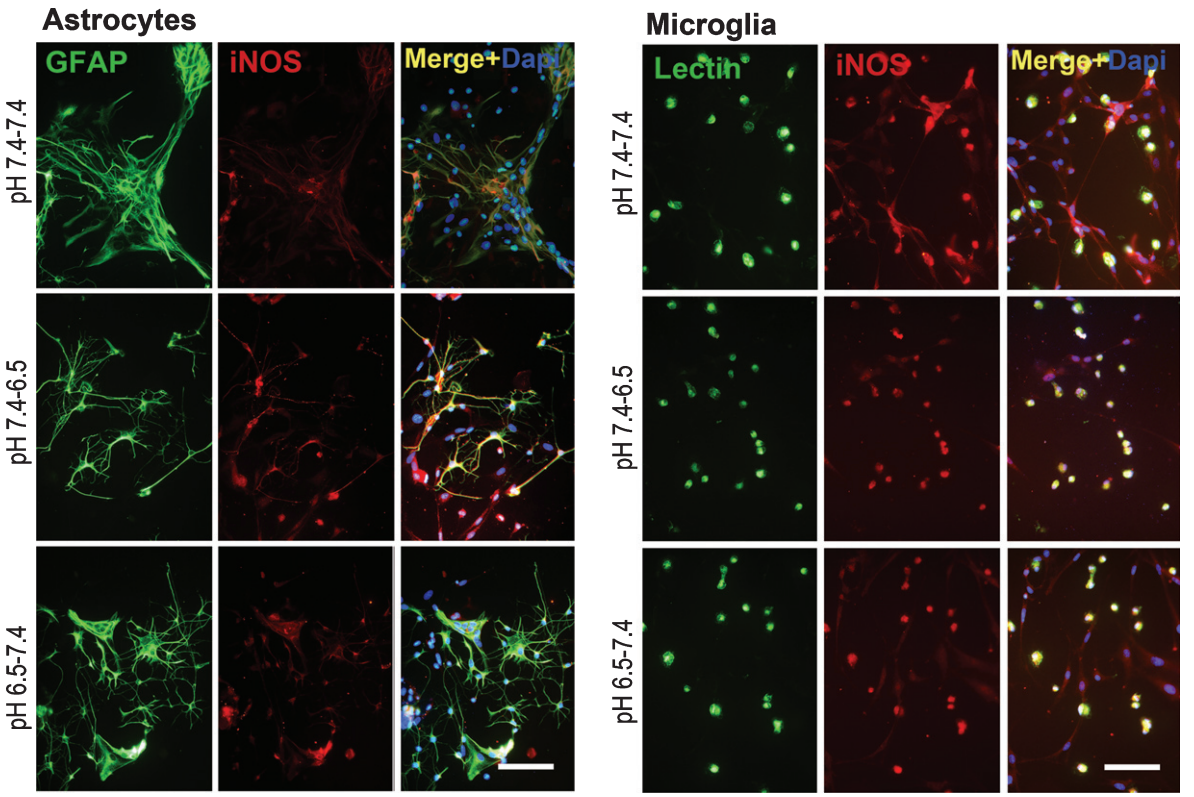

Fig. 2. Acidic environments affected the morphology and expression of iNOS by glial cells. Glial cells showed morphological changes when exposed to moderate acidosis. Microphotography of astrocyte cultures maintained at pH 7.4 or 6.5 for 48 h and later incubated at pH 7.4 or 6.5 for an additional 24 h. Immunolabeling of glial cells exposed to different values of pH with antibodies against GFAP (FITC; astrocyte identity marker) or FITC-conjugated lectin (microglia identity marker) and iNOS (Rho) as a glia activation marker. Nucleus stained with DAPI (blue). Astrocytes had fine filamentous cell processes in an acidic environment, suggesting their activation. The effect was reversible, with astrocytes recovering their previous morphology when exposed again to a pH 7.4 environment. There was a mild increase in the presence of iNOS in cells exposed to an acidic environment. Microglia exposed to an acidic environment were smaller than those in cultures maintained at pH 7.4. There was no effect on iNOS immunolabeling. Bar = 30 μm.

by 70% after 48 h of culture in an acidic environment ($p < 0.001$; Fig. 3A).

To evaluate if reduced adhesiveness at low pH depended on changes in the adhesion mechanism of glia or on reduced cell viability, detached glial cells from cultures at acidic pH were recovered and cultured at physiological conditions (pH 7.4) for 24 h. These glial cells recovered adhesiveness at pH 7.4, presenting similar viability to that of cells maintained at pH 7.4 (Fig. 3B).

Adhesion of astrocytes and microglia to surfaces coated with Aβ was mediated by SRs

Microglia and astrocytes adhesion to Aβ was concentration dependent. Glial cell adhesion to non-fibrillary Aβ (nfAβ) and fibrillary Aβ (fAβ) was similar up to 1 μg/ml Aβ, whereas at 2.5 μg/ml Aβ, adhesion to fAβ decreased by 30% compared with nfAβ (Fig. 4A,B). Adhesion of astrocytes was 104±3.7% for nfAβ and 77±4% for fAβ (Fig. 4A). Adhesion of microglia reached a maximum of 94±4.3% for nfAβ and 63±3% for fAβ. Decreased adhesion of glial cells to fAβ could depend on the cytotoxicity of fAβ, or on the inflammatory activation of glial cells.

We assessed the ability of different SR ligands to inhibit the adhesion of glial cells to 1 μg/ml Aβ, including fucoidan, a polysaccharide that inhibits SR class A, Poly-I and mel-BSA (chemically modified protein), which binds all SR. The three ligands were capable of inhibiting astrocyte (Fig. 4C) and microglia adhesion (Fig. 4D) to fAβ. Fucoidan inhibited astrocyte adhesion to fAβ by 75±5% and microglial adhesion by 90%±3. Mel-BSA and Poly-I inhibited astrocyte adhesion by 85% and microglia

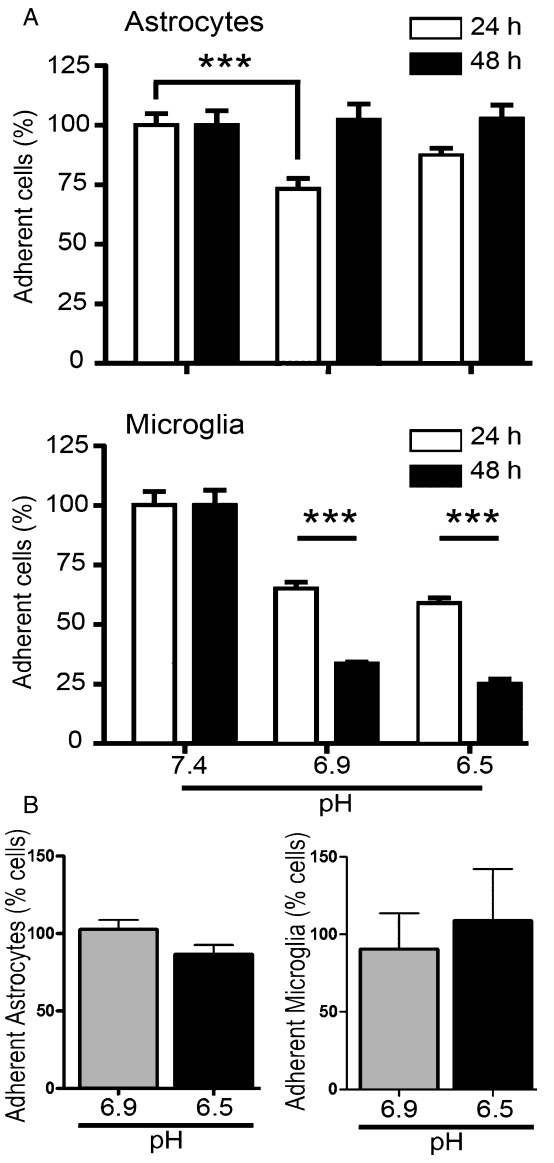

Fig. 3. Acidic environments had differential effects on the adhesion of astrocytes and microglia, but no effect on their viability. A) Cells were quantified by the LDH assay (quantification of LDH released after treatment of cells with non-ionic detergent). Astrocytes and microglia that adhered to poly-L-lysine coated plates at pH 7.4 (control condition; 100% of adhesion), 6.9 and 6.5 for 24 h and 48 h. Cell counts were lower at pH 6.9 and 6.5 than at pH 7.4. Adhesion data are expressed as % of adhesion observed at pH 7.4 (mean ± SEM) of 4 independent experiments, each in triplicate. $^{***}p < 0.001$. B) Glial cells detached under acidic environment were viable. Cells detached from cultures were kept under acidic conditions for 24 h and re-plated in media at pH 7.4. Astrocytes and microglia that were adherent were quantified with the LDH assay. Data are expressed as the mean ± SEM of the percentage of LDH concentration at the control condition at pH 7.4 of 4 independent experiments, each in triplicate.

adhesion by more than 90% ($p < 0.001$). Poly-C (negative control) did not inhibit astrocytes or microglia adhesion to fAβ. Our results indicate that the adhesion of glial cells to an otherwise non-adhesive substrate containing Aβ is mediated by SR.

Aβ phagocytosis by glial cells decreased in an acidic environment

Differences in adhesion at different pH values may affect Aβ uptake, which in turn probably affect the capacity of glial cells to clear Aβ. The effect of acidosis on phagocytosis was assessed for various durations (from 5 h to 6 days). Astrocyte Aβ uptake was 30 to 40% lower at pH 6.9 and 6.5 than at pH 7.4 ($p < 0.001$) at 24–48 h in phagocytosis assays. Aβ uptake was still significantly lower in the 6-day assays at pH 6.5 (Fig. 5A). Aβ uptake by microglia was 25 and 35% lower at pH 6.9 and 6.5 ($p < 0.01$) respectively, in the 5 h assay. In the 24 h assay, Aβ uptake at pH 6.9 was similar to the phagocytosis observed at pH 7.4, but a 10% decrease persisted at pH 6.5 ($p < 0.05$). However, Aβ phagocytosis was not affected by pH in the 5-day phagocytosis assays (Fig. 5B), indicating that acidic environments have only transient effects on Aβ uptake by microglia.

Acidification changed the expression pattern of scavenger receptors depending on glial cell type

The protein levels of some scavenger receptors in astrocytes were modified by an acidic environment (Fig. 6). There were no significant changes in SR-A (Fig. 6A) or CD-36 (Fig. 6D) expression by astrocytes at acidic conditions. SR-MARCO expression was unaffected at pH 6.9, but decreased by 25% at pH 6.5 ($p < 0.05$; Fig. 6B). SR-BI expression was 50% higher at pH 6.9 and pH 6.5 than under the control condition ($p < 0.05$; Fig. 6C).

The presence of scavenger receptors in microglia was also modified by an acidic environment (Fig. 7). Whereas no significant changes were observed in SR-A levels under acidic conditions (Fig. 7A), SR-MARCO increased by 55% at pH 6.9 and by 50% at pH 6.5 ($p < 0.01$; Fig. 7B), and SR-BI increased by up to 30% at pH 6.5 compared with the control condition ($p < 0.001$; Fig. 7C). In contrast, CD-36 expression decreased by 20 to 30% after incubation of microglia under acidic conditions ($p < 0.01$; Fig. 7D).

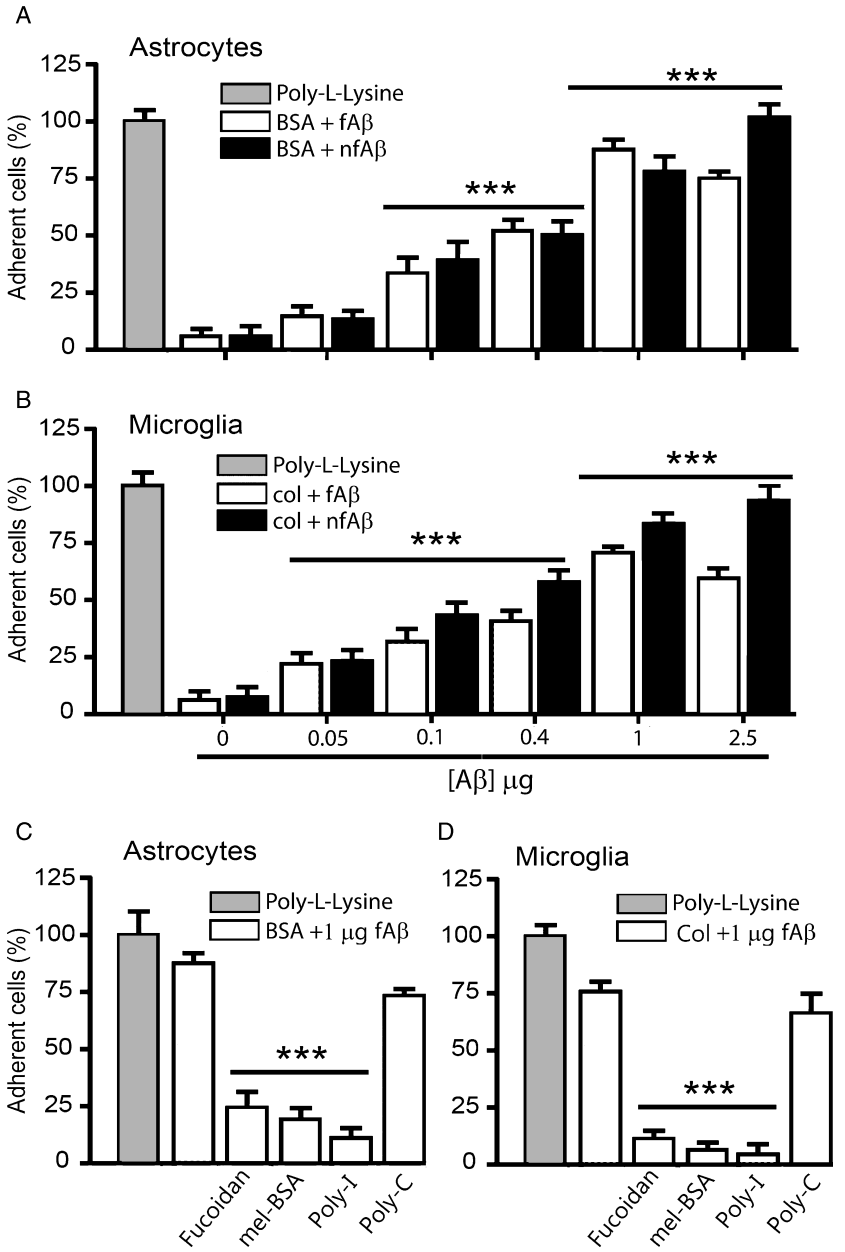

Fig. 4. Adhesion of astrocytes and microglia to a non-adhesive substrate depended on the concentration of Aβ and was mediated by scavenger receptors. Atrocytes (A) and microglia (B) adhered to fibrillary Aβ (fAβ) and non-fibrillary Aβ (nfAβ) in a concentration dependent manner. Competing ligands for SR inhibit adhesion of astrocytes (C) and microglia (D) to the non-adherent substrate containing 1 μg fAβ. Glial cells were pre-incubated with SR-competing ligands: 200 μg/ml fucoidan, mel-BSA, or poly-I, or the control ligand poly-C, with gentle agitation at 37°C for 1 h. The three SR ligands inhibited adhesion of glial cells to Aβ, whereas exposure to Poly-C did not affect adhesion. Adherent cells were quantified by the LDH assay (quantification of LDH released after treatment of cells with non-ionic detergent). 100% of astrocytes and microglia adhered to poly-L-lysine coated plates at pH 7.4 correspond to 100%. Adhesion data are expressed as % of adhesion to poly-L-lysine coated plates (mean ± SEM) of 4 independent experiments, each in triplicate. $^{***}p < 0.001$.

Aβ phagocytosed by glial cells was observed in the early endosome compartments

In astrocytes and microglia, Cy3-Aβ and early endosomes labeled with EEA1 antibody shared the same plane localization in confocal analysis. The localization was similar at pH 6.5 and pH 7.4 (Fig. 8). Z-scan images taken to verify Aβ uptake revealed that the Cy3-Aβ was internalized by glial cells and not bound to the cell surface (Fig. 8).

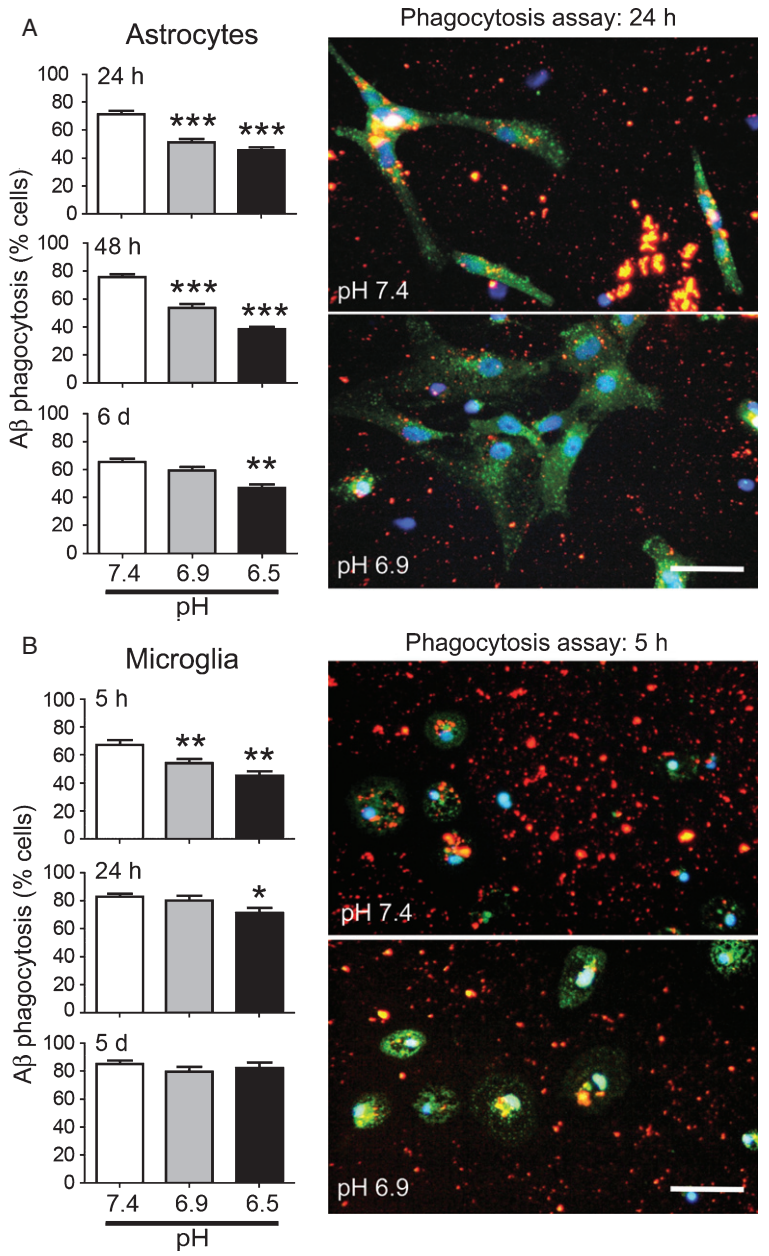

Fig. 5. Moderate acidosis decreased Aβ phagocytosis by glial cells. The phagocytosis assay of Cy3- conjugated Aβ (in red) by astrocytes (A) and microglia (B) exposed to pH 7.4, 6.9, and 6.5 for varying lengths of time. Astrocytes were labeled with anti-GFAP (FITC, in green) and microglia were labeled with FITC-conjugated lectin. Reduced phagocytosis by astrocytes and microglia was observed at an acidic pH level. However, whereas phagocytosis by astrocytes persisted at a reduced level at day 6 of the assay, the phagocytic capability of microglia recovered to basal levels by day 5 of the assay. Data are the number of the cells taking up Cy3-Aβ expressed as the mean ± SEM of 5 independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. The right panels show immunolabeling of glial cells exposed to different pH with antibodies against EEA1 (FITC; an early endosome marker) and Cy3-Aβ (Red) to assess phagocytosis. Nuclei stained with DAPI (blue). Bar = 25 μm.

DISCUSSION

Deposition of Aβ as senile plaques is a well-described pathological hallmark of AD and neuroinflammation appears to have a major participation in the disease progress [72]. Microglia can phagocytose Aβ [51, 73] and have both a protective and a deleterious effect. The neuroinflammatory

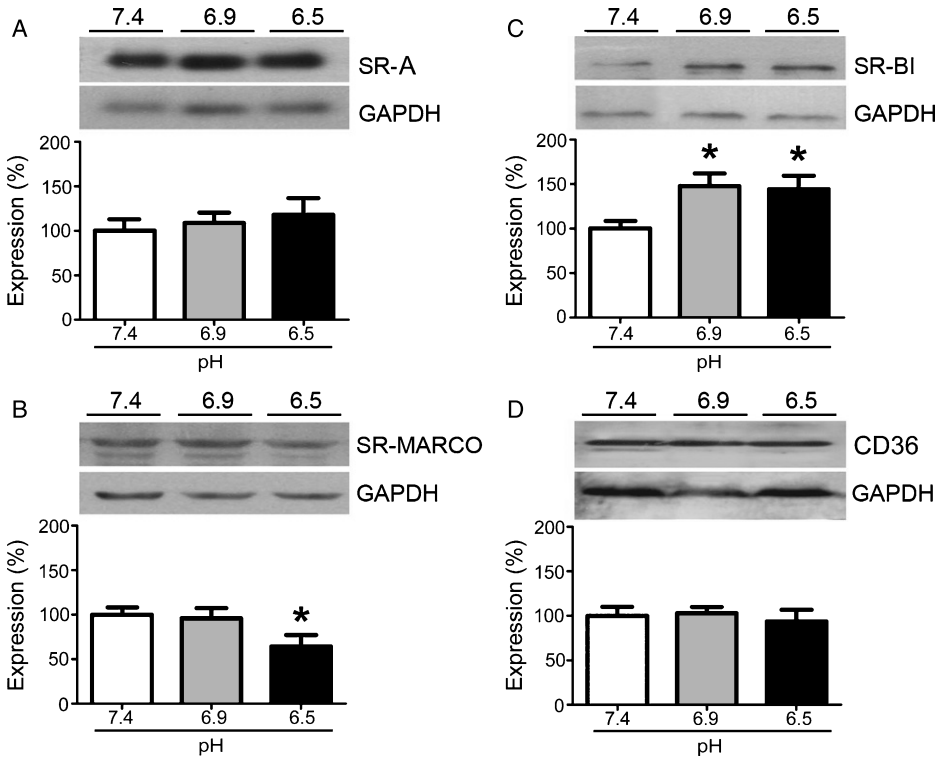

Fig. 6. Receptor-dependent effect of an acidic environment on scavenger receptor expression by astrocytes in culture. Representative western blots of scavenger receptors and their densitometry analysis normalized by GAPDH as load control of SR-A (A), SR-MARCO (B), SR- BI (C) and CD36 (D). The amount of the SR under control conditions (pH 7.4) was considered 100% of receptor expression. Acidosis increased SR-BI and decreased SR-MARCO, without inducing changes in SR-A and CD36 expression. Data are the mean ± SEM of 5 independent experiments. $^{*}p < 0.05$.

response mediated by activated microglia and astrocytes results in increased concentrations of cytokines, chemokines, nitric oxide, and reactive oxidative species [74–76], all of which are found to be increased in the brain of AD patients [77]. If noxious stimuli fail to be resolved, and a persistent inflammatory condition develops, microglia acquire a cytotoxic activation that could favor the progression of neurodegeneration [57].

We have previously observed that SR are present and participate in the interaction of glial cells with Aβ [50, 59], and regulate inflammatory activation [70]. Here, we show that under acidic conditions like those observed under conditions of impaired perfusion and brain ischemia [4, 5, 78], the properties of phagocytosis, adhesion, and expression of scavenger receptors of glial cells change, leading to impairment of Aβ clearance. Transient acidification also occurs in response to acute sub-lethal ischemic injury, a condition that can be observed in age-dependent pathophysiological changes. In this sense, acidification could constitute a preconditioning factor for other potentially noxious stimuli or inflammation and Aβ.

Diabetes has been associated as a risk factor for AD [79]. The proposed mechanisms for the association include augmented oxidative stress [80], changes in the inflammatory response [81], cerebrovascular pathology mediated damage [82], and also insulin resistance as a process that impairs brain function [83]. Acidosis may also be a diabetes-associated mechanism favoring AD onset and progression. It has been described that diabetic ketoacidosis leads to glial activation in the brain [84], and reports on a murine model indicate that it could result in decreased pH in the hippocampus [85].

Acidosis at both tissue and cell levels has been detected in the nervous system during diseases such as stroke, traumatic brain injury, epilepsy, Parkinson's disease, and AD [86, 87]. In ischemia, acidosis is an important component of pathological events leading to brain damage [4, 5]. Moderate ischemia can result in a fall of parenchymal pH to around 6.6 without clear evidence of irreversible cell damage

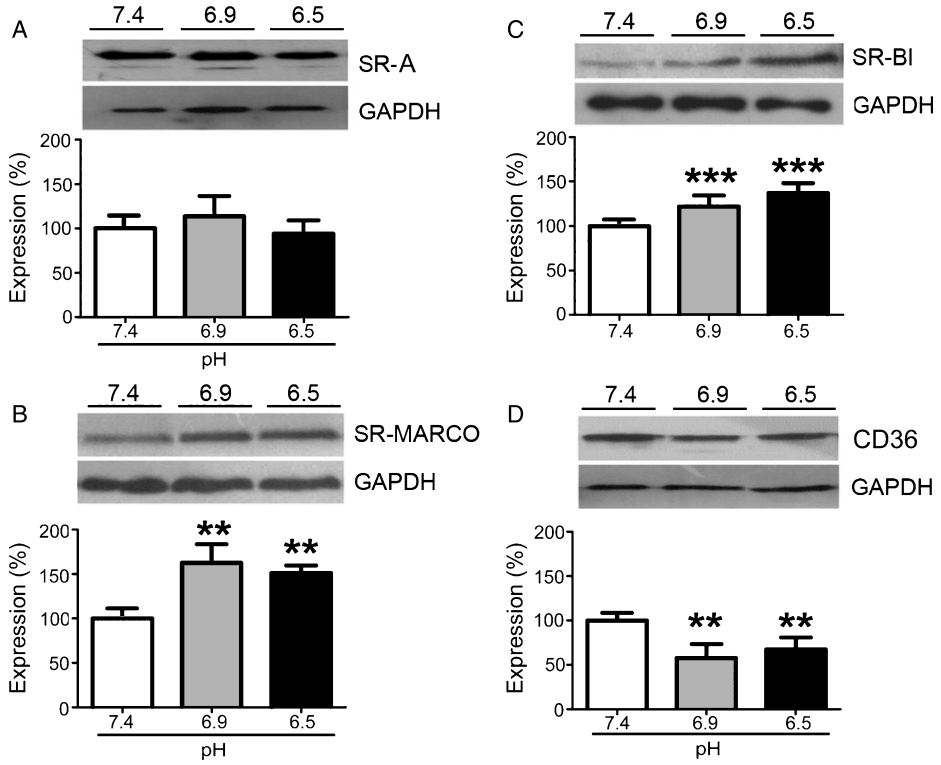

Fig. 7. Receptor-dependent effect of an acidic environment on scavenger receptor expression by microglia in culture. Representative western blots of scavenger receptors and their densitometry analysis, normalized by GAPDH as load control, of SR-A (A), SR-MARCO (B), SR-BI (C), and CD36 (D). The levels of SR under the control condition (pH 7.4) were considered 100%. Acidosis increased SR-BI and SR-MARCO and decreased CD36 levels, with no change for SR-A. Data are the mean ± SEM of the quantification of 5 independent experiments. $^{**}p < 0.01$, $^{***}p < 0.001$.

[88], although there are reports that cholinergic neurons die when pH is lowered to 6.8 [23]. In severe ischemia, anaerobic glycolysis leads to accumulation of acids, causing pH to decrease to around 6.0 [88], a condition associated with cell death and irreversible damage. Both ischemia lesions and brain acidosis have been reported in AD [21, 23]. In AD brains, the lysosomal enzyme asparaginyl endopeptidase, is selectively activated and translocated from neuronal lysosomes to the cytoplasm. Asparaginyl endopeptidase cleaves and inhibits key phosphatases, such as the protein phosphatase 2A, which appears to be involved in the abnormal hyperphosphorylation of tau [89]. Prolonged acidosis may in fact contribute to the dysregulation of Aβ and subsequent plaque deposition and cell death of cholinergic neurons. Under acidic conditions (pH 6.0), cholinergic neurons degenerate in brain slices, an effect that is accompanied by aggregation of Aβ peptides [90]. Furthermore, acidosis enhances iron-catalyzed production of reactive oxygen species [91], which in turn favors Aβ aggregation and neurodegenerative changes.

In AD, acidosis also increases potentially amyloidogenic enzymes such as β- and γ-secretase [9, 10], whereas acidosis reduces IDE activity, which is involved in the clearance of Aβ [92, 93]. These changes as a whole could lead to an overproduction of Aβ and promote Aβ accumulation induced by acidosis, facilitating the development of AD.

We observed that decreased adhesion and morphological changes induced by low pH were reversible, returning to basal levels when cells were again plated at physiological pH. In astrocytes, reduction of adhesiveness was transient. In contrast, microglial cell adhesion appeared to be more sensitive to acidification than that of astrocytes. Considering that microglia are naturally moving cells, decreased adhesion could be associated with greater mobility toward injured regions. However, this could also result in impaired recognition of environmental signals. Our *in vitro* approach has the advantage of allowing us to

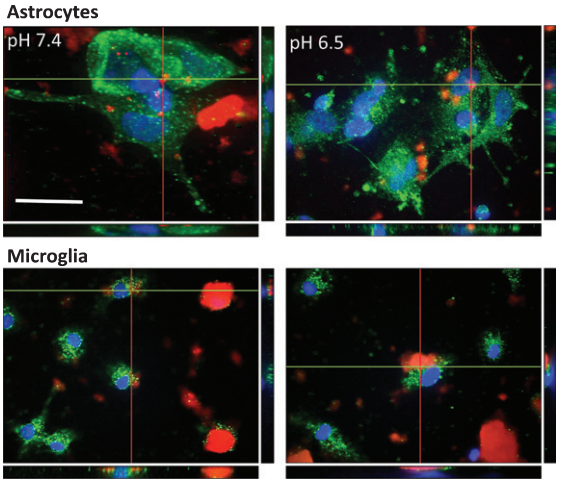

Fig. 8. Aβ was phagocytosed by glial cells via the endosomal pathway. Confocal images of immunolabeling assays of Cy3-Aβ phagocytosed by astrocytes and microglia exposed to pH 7.4 (left) and 6.5 (right) for 48 h. Uptake of Cy3-Aβ (Red) and early endosomes labeled with antibodies against the marker EEA1 (green) were in the same z-plane compartment of the cell, suggesting Cy3-Aβ incorporation into the cell. Nuclei are labeled with DAPI (blue). Planes YZ and XZ are also shown. Green and red lines indicate corresponding points on the orthogonal planes, showing localization of the label within the pictured cell. Bar = 20 μm

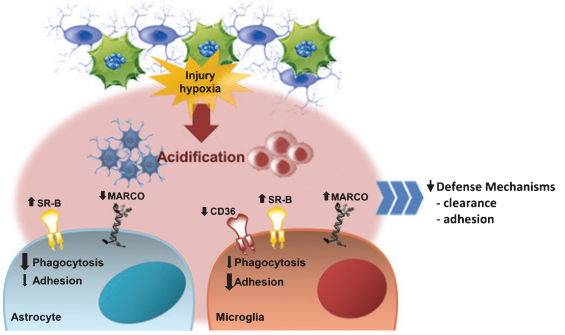

Fig. 9. Working summary of changes in SR expression, adhesion and the phagocytic capacity of glial cells induced by exposure to acidic environments. An acidic environment had differential effects on the expression of diverse scavenger receptors (SR) by astrocytes and microglial cells, resulting in different functional effects. The adhesion of astrocytes transiently decreased in the acidic environments, returning to control level by 48 h, whereas adhesion of microglia decreased progressively and persistently. This might allow microglia to facilitate migration to an injury site. On the other hand, astrocyte-mediated phagocytosis of Aβ was persistently inhibited in an acidic environment, whereas phagocytosis by microglia decreased transiently but was not affected by pH over long-term exposure.

expose cells to well defined stimuli, but on the other hand, the complex regulatory signaling that is present in the normal *in vivo* situation is not available.

Changes in glial cell adhesion to substrates associated with acidification were also matrix dependent. The best adhesion was obtained with poly-L-lysine, a cationic polymer associated by epsilon C-bond rather a normal peptide bond (α-C). On other matrices, like gelatin, which is 90% collagen, adhesiveness worsened in an acidic environment, which could depend on protonation of proteins. In contrast, for mel-BSA matrix, which is a BSA modified by negative charges, adhesion increased in an acidic environment. Neutral matrices like BSA were not changed under acidified conditions.

The effect of acidic environments on Aβ phagocytosis by glial cells is especially interesting. Acidification permanently reduced the capacity of astrocytes for Aβ phagocytosis. In contrast, the phagocytic capacity of microglia is only transiently affected by acidification. In the context of an injury, microglia are activated earlier and move more easily to the lesion site than do astrocytes [94]. To orchestrate their response, glial cells have a large battery of membrane receptors, including SR and TLRs [94]. Although astrocytes express receptors similar to those of microglia, microglia appear to be more efficient at Aβ clearance. The difference can be due to the mobility of microglia in reaching Aβ plaques or to the fact that astrocytes express lower levels of TLRs and SR [95]. Low levels or the absence of certain receptors could explain differences in Aβ phagocytosis by glial cells, as well as its modulation by acidic environments. However, given the elevated number of astrocytes, their participation in phagocytosis could be highly relevant for the homeostasis of the brain.

Although our results clearly indicate the negative impact of acidic environments on binding to and phagocytosis of Aβ, we did not establish a clear correlation between the changes in the SR expression in astrocytes and microglia and the functional effect. We propose that the pattern of expression of the various SR is relevant for overall cell activation. We have previously shown that the absence of SR-A results in the dysregulation of inflammatory signaling and the response of microglia [58, 70, 96]. Furthermore, it has recently been shown that macrophages are modulated by environmental conditions depending on the expression of surface receptors, including SR [97]. The absence of SR-A, SR-B, and CD36 has been associated with increased levels of inflammatory cytokines [98–100] in various tissues and injury models, suggesting a role for SR in the production and regulation of diverse cytokines and other inflammatory mediators [101–105]. The absence of SR

significantly increases induced inflammation, contributing to inflammation-induced tissue injury [99, 100]. In peritoneal macrophages and macrophage cell lines, the induction of TNFα, IL1β, and NO production by SR ligands appears to depend on the activation of mitogen-activated protein kinases (MAPKs) and NFκB signaling pathways [106–108].

It is particular important to study the expression of specific receptors that can be involved in Aβ phagocytosis and microglia activation because brain pH is lower in AD patients than in healthy individuals [109]. Acidosis also occurs after ischemia and is associated with neuronal injury [110, 111]. It is unknown if acidosis, in addition to affecting the clearance properties of glial cells, induces other phenotypic cellular changes that enhance neurodegeneration.

SR activation can result in ligand internalization and production of extracellular superoxide by microglia [62]. The class B scavenger receptor CD36 mediates free radical production and tissue injury in cerebral ischemia [112]. Both SR-A [113] and SR-BI [114] mediate adhesion and endocytosis of fibrillary Aβ by microglia. Interestingly, SR-A, SR-BI, and CD36 [59, 63] participate in the production of radical species by microglia in response to Aβ fibrils and other SR ligands [96]. SR-MARCO, an inducible member of the class A scavenger receptor family, has also been implicated in the adhesion of microglia and astrocytes to Aβ [50] and in the mediation of cytoskeleton rearrangements in fibroblast and microglia [115, 116].

There were no significant differences in the relative abundance of SR-A or SR-CD36 in astrocytes. However, SR-MARCO decreased and SR-Bs increased, both significantly, in response to acidic environments, which could explain the persistent decrease in Aβ phagocytosis by astrocytes cultured in acidic environments. In contrast, despite the notable change in cell adhesion when exposed to acidic media, microglia increased relative abundance of SR-BI and SR-MARCO and a slight decrease in CD36. Expression of SR-A in microglia is upregulated in the brains of patients with AD [117], and microglia also upregulate scavenger receptors in response to injury and to cytokines [118]. However, we did not find a significant increase in SR-A in response to acidification for 48 h. It is possible that repeated or longer lasting exposure to acidic environments is required for the modification of SR-A expression.

In conclusion, we have shown here that when glial cells are exposed to acidic extracellular conditions, they modify SR expression, inducing several phenotypic changes, including morphological changes to a more rounded shape, reduced adhesion to substrates, and impaired Aβ phagocytic capability. Modification of SR was a complex response, whereas SR-BI expression increased in glial cells under acidic conditions; SR-MARCO expression increased in microglia but decreased in astrocytes. Changes in SR expression patterns could explain key phenotypic changes observed in acidosis: reduced Aβ phagocytosis and reduced adhesion by astrocytes and microglia (Fig. 9), leading to decreased uptake of Aβ; thus favoring its accumulation. Since several age-related pathologies, including ischemia, trauma, and cardiorespiratory pathology induce tissue acidosis, the study of the changes induced by acidosis in glial cells provides a better understanding of the immune behavior of glial cells associated with an injury. In addition, given the role played by SR in neurodegenerative diseases such as AD, understanding how the environment modifies SR expression and function in glial cells can provide key data in the search for better therapies based on SR function.

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REFERENCES

[1] Mattson MP (2004) Pathways towards and away from Alzheimer's disease. *Nature* **430**, 631-639.

[2] Hasegawa T, Ukai W, Jo DG, Xu X, Mattson MP, Nakagawa M, Araki W, Saito T, Yamada T (2005) Homocysteic acid induces intraneuronal accumulation of neurotoxic Abeta42: Implications for the pathogenesis of Alzheimer's disease. *J Neurosci Res* **80**, 869-876.

[3] Zerbinatti CV, Wahrle SE, Kim H, Cam JA, Bales K, Paul SM, Holtzman DM, Bu G (2006) Apolipoprotein E and low density lipoprotein receptor-related protein facilitate intraneuronal Abeta42 accumulation in amyloid model mice. *J Biol Chem* **281**, 36180-36186.

[4] Siesjo BK (1988) Acidosis and ischemic brain damage. *Neurochem Pathol* **9**, 31-88.

[5] Siesjo BK (1992) Pathophysiology and treatment of focal cerebral ischemia. Part I: Pathophysiology. *J Neurosurg* **77**, 169-184.
[6] Misonou H, Morishima-Kawashima M, Ihara Y (2000) Oxidative stress induces intracellular accumulation of amyloid beta-protein (Abeta) in human neuroblastoma cells. *Biochemistry* **39**, 6951-6959.
[7] Iadecola C (2004) Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nat Rev Neurosci* **5**, 347-360.
[8] Humpel C, Marksteiner J (2005) Cerebrovascular damage as a cause for Alzheimer's disease. *Curr Neurovasc Res* **2**, 341-347.
[9] Fang B, Wang D, Huang M, Yu G, Li H (2010) Hypothesis on the relationship between the change in intracellular pH and incidence of sporadic Alzheimer's disease or vascular dementia. *Int J Neurosci* **120**, 591-595.
[10] Brewer GJ (1997) Effects of acidosis on the distribution of processing of the beta-amyloid precursor protein in cultured hippocampal neurons. *Mol Chem Neuropathol* **31**, 171-186.
[11] Yang LB, Lindholm K, Yan R, Citron M, Xia W, Yang XL, Beach T, Sue L, Wong P, Price D, Li R, Shen Y (2003) Elevated beta-secretase expression and enzymatic activity detected in sporadic Alzheimer disease. *Nat Med* **9**, 3-4.
[12] Zohar O, Cavallaro S, D'Agata V, Alkon DL (2003) Quantification and distribution of beta-secretase alternative splice variants in the rat and human brain. *Brain Res Mol Brain Res* **115**, 63-68.
[13] Hussain I, Powell D, Howlett DR, Tew DG, Meek TD, Chapman C, Gloger IS, Murphy KE, Southan CD, Ryan DM, Smith TS, Simmons DL, Walsh FS, Dingwall C, Christie G (1999) Identification of a novel aspartic protease (Asp 2) as beta-secretase. *Mol Cell Neurosci* **14**, 419-427.
[14] Xiang Y, Xu G, Weigel-Van Aken KA (2010) Lactic acid induces aberrant amyloid precursor protein processing by promoting its interaction with endoplasmic reticulum chaperone proteins. *PLoS One* **5**, e13820.
[15] Atwood CS, Moir RD, Huang X, Scarpa RC, Bacarra NM, Romano DM, Hartshorn MA, Tanzi RE, Bush AI (1998) Dramatic aggregation of Alzheimer Abeta by Cu(II) is induced by conditions representing physiological acidosis. *J Biol Chem* **273**, 12817-12826.
[16] Su Y, Chang PT (2001) Acidic pH promotes the formation of toxic fibrils from beta-amyloid peptide. *Brain Res* **893**, 287-291.
[17] Grasso G, Satriano C, Milardi D (2015) A neglected modulator of insulin-degrading enzyme activity and conformation: The pH. *Biophys Chem* **203-204**, 33-40.
[18] Bidani A, Wang CZ, Saggi SJ, Heming TA (1998) Evidence for pH sensitivity of tumor necrosis factor-alpha release by alveolar macrophages. *Lung* **176**, 111-121.
[19] Kraus M, Wolf B (1996) Implications of acidic tumor microenvironment for neoplastic growth and cancer treatment: A computer analysis. *Tumour Biol* **17**, 133-154.
[20] Helmlinger G, Yuan F, Dellian M, Jain RK (1997) Interstitial pH and pO2 gradients in solid tumors *in vivo*: High-resolution measurements reveal a lack of correlation. *Nat Med* **3**, 177-182.
[21] Yates CM, Butterworth J, Tennant MC, Gordon A (1990) Enzyme activities in relation to pH and lactate in postmortem brain in Alzheimer-type and other dementias. *J Neurochem* **55**, 1624-1630.
[22] Bowen DM, Davison AN (1986) Biochemical studies of nerve cells and energy metabolism in Alzheimer's disease. *Br Med Bull* **42**, 75-80.
[23] Pirchl M, Humpel C (2009) [Does acidosis in brain play a role in Alzheimer's disease?] *Neuropsychiatr* **23**, 187-192.
[24] von Bernhardi R, Eugenin J (2004) Microglial reactivity to beta-amyloid is modulated by astrocytes and proinflammatory factors. *Brain Res* **1025**, 186-193.
[25] Uribe-San Martin R, Herrera-Molina R, Olavarria L, Ramirez G, von Bernhardi R (2009) Reduction of beta-amyloid-induced neurotoxicity on hippocampal cell cultures by moderate acidosis is mediated by transforming growth factor beta. *Neuroscience* **158**, 1338-1347.
[26] Shi Y, Kim D, Caldwell M, Sun D (2013) The role of Na(+)/h (+) exchanger isoform 1 in inflammatory responses: Maintaining H(+) homeostasis of immune cells. *Adv Exp Med Biol* **961**, 411-418.
[27] Liu Y, Kintner DB, Chanana V, Algharabli J, Chen X, Gao Y, Chen J, Ferrazzano P, Olson JK, Sun D (2010) Activation of microglia depends on Na+/H+exchange-mediated H+ homeostasis. *J Neurosci* **30**, 15210-15220.
[28] Kettenmann H, Hanisch UK, Noda M, Verkhratsky A (2011) Physiology of microglia. *Physiol Rev* **91**, 461-553.
[29] Baird NR, Orlowski J, Szabó EZ, Zaun HC, Schultheis PJ, Menon AG, Shull GE (1999) Molecular cloning, genomic organization, and functional expression of Na+/H+exchanger isoform 5 (NHE5) from human brain. *J Biol Chem* **274**, 4377-4382.
[30] Brookes N (1997) Intracellullar pH as a regulatory signal in astrocyte metabolism. *Glia* **21**, 64-73.
[31] Hirata Y, Oku Y (2010) TRP channels are involved in mediating hypercapnic Ca2+ responses in rat glia-rich medullary cultures independent of extracellular pH. *Cell Calcium* **48**, 124-132.
[32] Gourine AV, Kasymov V, Marina N, Tang F, Figueiredo MF, Lane S, Teschemacher AG, Spyer KM, Deisseroth K, Kasparov S (2010) Astrocytes control breathing through pH-dependent release of ATP. *Science* **329**, 571-575.
[33] Wu LJ (2014) Voltage-gated proton channel HV1 in microglia. *Neuroscientist* **20**, 599-609.
[34] Hibino H, Fujita A, Iwai K, Yamada M, Kurachi Y (2004) Differential assembly of inwardly rectifying K+ channel subunits, Kir4.1 and Kir5.1, in brain astrocytes. *J Biol Chem* **279**, 44065-44073.
[35] Wenker IC, Kreneisz O, Nishiyama A, Mulkey DK (2010) Astrocytes in the retrotrapezoid nucleus sense H+ by inhibition of a Kir4.1-Kir5.1-like current and may contribute to chemoreception by a purinergic mechanism. *J Neurophysiol* **104**, 3042-3052.
[36] Meigh L, Greenhalgh SA, Rodgers JL, Cann MJ, Roper DI, Dale N (2013) CO2 directly modulates connexin 26 by formation of carbamate bridges between subunits. *eLIFE* **2**, e01213.
[37] von Bernhardi R (2007) Glial cell dysregulation: A new perspective on Alzheimer disease. *Neurotox Res* **12**, 215-232.
[38] Kim J, Basak JM, Holtzman DM (2009) The role of apolipoprotein E in Alzheimer's disease. *Neuron* **63**, 287-303.
[39] Ji C, Song C, Zuo P (2011) The mechanism of memory impairment induced by Abeta chronic administration involves imbalance between cytokines and neurotrophins in the rat hippocampus. *Curr Alzheimer Res* **8**, 410-420.

[40] Lee YB, Nagai A, Kim SU (2002) Cytokines, chemokines, and cytokine receptors in human microglia. *J Neurosci Res* **69**, 94-103.

[41] Lue LF, Rydel R, Brigham EF, Yang LB, Hampel H, Murphy GM, Jr., Brachova L, Yan SD, Walker DG, Shen Y, Rogers J (2001) Inflammatory repertoire of Alzheimer's disease and nondemented elderly microglia *in vitro*. *Glia* **35**, 72-79.

[42] Smits HA, Rijsmus A, van Loon JH, Wat JW, Verhoef J, Boven LA, Nottet HS (2002) Amyloid-beta-induced chemokine production in primary human macrophages and astrocytes. *J Neuroimmunol* **127**, 160-168.

[43] Ito K, Hirao A, Arai F, Takubo K, Matsuoka S, Miyamoto K, Ohmura M, Naka K, Hosokawa K, Ikeda Y, Suda T (2006) Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. *Nat Med* **12**, 446-451.

[44] von Bernhardi R, Eugenin-von Bernhardi L, Eugenin J (2015) Microglial cell dysregulation in brain aging and neurodegeneration. *Front Aging Neurosci* **7**, 124.

[45] Ramirez G, Toro R, Dobeli H, von Bernhardi R (2005) Protection of rat primary hippocampal cultures from A beta cytotoxicity by pro-inflammatory molecules is mediated by astrocytes. *Neurobiol Dis* **19**, 243-254.

[46] Town T, Nikolic V, Tan J (2005) The microglial "activation" continuum: From innate to adaptive responses. *J Neuroinflammation* **2**, 24.

[47] Hickman SE, Allison EK, El Khoury J (2008) Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer's disease mice. *J Neurosci* **28**, 8354-8360.

[48] Schwab C, McGeer PL (2008) Inflammatory aspects of Alzheimer disease and other neurodegenerative disorders. *J Alzheimers Dis* **13**, 359-369.

[49] Colton CA, Vitek MP, Wink DA, Xu Q, Cantillana V, Previti ML, Van Nostrand WE, Weinberg JB, Dawson H (2006) NO synthase 2 (NOS2) deletion promotes multiple pathologies in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A* **103**, 12867-12872.

[50] Alarcon R, Fuenzalida C, Santibanez M, von Bernhardi R (2005) Expression of scavenger receptors in glial cells. Comparing the adhesion of astrocytes and microglia from neonatal rats to surface-bound beta-amyloid. *J Biol Chem* **280**, 30406-30415.

[51] von Bernhardi R, Ramírez G (2001) Microglia-astrocyte interaction in Alzheimer's disease: Friends or foes for the nervous system? *Biol Res* **34**, 123-128.

[52] Tichauer JE, Flores B, Soler B, Eugenin-von Bernhardi L, Ramirez G, von Bernhardi R (2014) Age-dependent changes on TGFbeta1 Smad3 pathway modify the pattern of microglial cell activation. *Brain Behav Immun* **37**, 187-196.

[53] von Bernhardi R, Tichauer J, Eugenin-von Bernhardi L (2011) Proliferating culture of aged microglia for the study of neurodegenerative diseases. *J Neurosci Methods* **202**, 65-69.

[54] Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper NR, Eikelenboom P, Emmerling M, Fiebich BL, Finch CE, Frautschy S, Griffin WS, Hampel H, Hull M, Landreth G, Lue L, Mrak R, Mackenzie IR, McGeer PL, O'Banion MK, Pachter J, Pasinetti G, Plata-Salaman C, Rogers J, Rydel R, Shen Y, Streit W, Strohmeyer R, Tooyoma I, Van Muiswinkel FL, Veerhuis R, Walker D, Webster S, Wegrzyniak B, Wenk G, Wyss-Coray T (2000) Inflammation and Alzheimer's disease. *Neurobiol Aging* **21**, 383-421.

[55] Wyss-Coray T (2006) Inflammation in Alzheimer disease: Driving force, bystander or beneficial response? *Nat Med* **12**, 1005-1015.

[56] von Bernhardi R, Tichauer JE, Eugenin J (2010) Aging-dependent changes of microglial cells and their relevance for neurodegenerative disorders. *J Neurochem* **112**, 1099-1114.

[57] Ramírez G, Rey S, von Bernhardi R (2008) Proinflammatory stimuli are needed for induction of microglial cell-mediated AbetaPP_244-C and Abeta-neurotoxicity in hippocampal cultures. *J Alzheimers Dis* **15**, 45-59.

[58] Cornejo F, von Bernhardi R (2013) Role of scavenger receptors in glia-mediated neuroinflammatory response associated with Alzheimer's disease. *Mediators Inflamm* **2013**, 895651.

[59] Murgas P, Godoy B, von Bernhardi R (2012) Abeta potentiates inflammatory activation of glial cells induced by scavenger receptor ligands and inflammatory mediators in culture. *Neurotox Res* **22**, 69-78.

[60] Krieger M, Acton S, Ashkenas J, Pearson A, Penman M, Resnick D (1993) Molecular flypaper, host defense, and atherosclerosis. Structure, binding properties, and functions of macrophage scavenger receptors. *J Biol Chem* **268**, 4569-4572.

[61] Elomaa O, Kangas M, Sahlberg C, Tuukkanen J, Sormunen R, Liakka A, Thesleff I, Kraal G, Tryggvason K (1995) Cloning of a novel bacteria-binding receptor structurally related to scavenger receptors and expressed in a subset of macrophages. *Cell* **80**, 603-609.

[62] Husemann J, Loike JD, Anankov R, Febbraio M, Silverstein SC (2002) Scavenger receptors in neurobiology and neuropathology: Their role on microglia and other cells of the nervous system. *Glia* **40**, 195-205.

[63] Coraci IS, Husemann J, Berman JW, Hulette C, Dufour JH, Campanella GK, Luster AD, Silverstein SC, El-Khoury JB (2002) CD36, a class B scavenger receptor, is expressed on microglia in Alzheimer's disease brains and can mediate production of reactive oxygen species in response to beta-amyloid fibrils. *Am J Pathol* **160**, 101-112.

[64] Yan SD, Chen X, Fu J, Chen M, Zhu H, Roher A, Slattery T, Zhao L, Nagashima M, Morser J, Migheli A, Nawroth P, Stern D, Schmidt AM (1996) RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. *Nature* **382**, 685-691.

[65] Marzolo MP, von Bernhardi R, Bu G, Inestrosa NC (2000) Expression of alpha(2)-macroglobulin receptor/low density lipoprotein receptor-related protein (LRP) in rat microglial cells. *J Neurosci Res* **60**, 401-411.

[66] Marzolo MP, von Bernhardi R, Inestrosa NC (1999) Mannose receptor is present in a functional state in rat microglial cells. *J Neurosci Res* **58**, 387-395.

[67] Zlokovic BV (2005) Neurovascular mechanisms of Alzheimer's neurodegeneration. *Trends Neurosci* **28**, 202-208.

[68] Benarroch EE (2007) Neurovascular unit dysfunction: A vascular component of Alzheimer disease? *Neurology* **68**, 1730-1732.

[69] Chang ZL (2010) Important aspects of Toll-like receptors, ligands and their signaling pathways. *Inflamm Res* **59**, 791-808.

[70] Murgas P, Cornejo FA, Merino G, von Bernhardi R (2014) SR-A regulates the inflammatory activation of astrocytes. *Neurotox Res* **25**, 68-80.

[71] Hayes GM, Woodroofe MN, Cuzner ML (1988) Characterisation of microglia isolated from adult human and rat brain. *J Neuroimmunol* **19**, 177-189.
[72] Querfurth HW, LaFerla FM (2010) Alzheimer's disease. *N Engl J Med* **362**, 329-344.
[73] Webster SD, Galvan MD, Ferran E, Garzon-Rodriguez W, Glabe CG, Tenner AJ (2001) Antibody-mediated phagocytosis of the amyloid beta-peptide in microglia is differentially modulated by C1q. *J Immunol* **166**, 7496-7503.
[74] Herrera-Molina R, von Bernhardi R (2005) Transforming growth factor-beta 1 produced by hippocampal cells modulates microglial reactivity in culture. *Neurobiol Dis* **19**, 229-236.
[75] Qin L, Liu Y, Cooper C, Liu B, Wilson B, Hong JS (2002) Microglia enhance beta-amyloid peptide-induced toxicity in cortical and mesencephalic neurons by producing reactive oxygen species. *J Neurochem* **83**, 973-983.
[76] Ii M, Sunamoto M, Ohnishi K, Ichimori Y (1996) beta-Amyloid protein-dependent nitric oxide production from microglial cells and neurotoxicity. *Brain Res* **720**, 93-100.
[77] Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH (2010) Mechanisms underlying inflammation in neurodegeneration. *Cell* **140**, 918-934.
[78] Pignataro G, Simon RP, Xiong ZG (2007) Prolonged activation of ASIC1a and the time window for neuroprotection in cerebral ischaemia. *Brain* **130**, 151-158.
[79] Vagelatos NT, Eslick GD (2013) Type 2 diabetes as a risk factor for Alzheimer's disease: The confounders, interactions, and neuropathology associated with this relationship. *Epidemiol Rev* **35**, 152-160.
[80] Guglielmotto M, Giliberto L, Tamagno E, Tabaton M (2010) Oxidative stress mediates the pathogenic effect of different Alzheimer's disease risk factors. *Front Aging Neurosci* **2**, 3.
[81] Bozluolcay M, Andican G, Firtina S, Erkol G, Konukoglu D (2015) Inflammatory hypothesis as a link between Alzheimer's disease and diabetes mellitus. *Geriatr Gerontol Int*, doi: 10.1111/ggi.12602.
[82] Gupta A, Iadecola C (2015) Impaired Abeta clearance: A potential link between atherosclerosis and Alzheimer's disease. *Front Aging Neurosci* **7**, 115.
[83] Biessels GJ, Reagan LP (2015) Hippocampal insulin resistance and cognitive dysfunction. *Nat Rev Neurosci* **16**, 660-671.
[84] Lo W, O'Donnell M, Tancredi D, Orgain M, Glaser N (2016) Diabetic ketoacidosis in juvenile rats is associated with reactive gliosis and activation of microglia in the hippocampus. *Pediatr Diabetes* **17**, 127-139.
[85] Marunaka Y, Yoshimoto K, Aoi W, Hosogi S, Ikegaya H (2014) Low pH of interstitial fluid around hippocampus of the brain in diabetic OLETF rats. *Mol Cell Ther* **2**, 6.
[86] Xiong ZG, Pignataro G, Li M, Chang SY, Simon RP (2008) Acid-sensing ion channels (ASICs) as pharmacological targets for neurodegenerative diseases. *Curr Opin Pharmacol* **8**, 25-32.
[87] Yamamoto M, Ujike H, Wada K, Tsuji T (1997) Cerebrospinal fluid lactate and pyruvate concentrations in patients with Parkinson's disease and mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS). *J Neurol Neurosurg Psychiatry* **62**, 290.
[88] Rehncrona S, Rosen I, Smith ML (1985) Effect of different degrees of brain ischemia and tissue lactic acidosis on the short-term recovery of neurophysiologic and metabolic variables. *Exp Neurol* **87**, 458-473.
[89] Basurto-Islas G, Grundke-Iqbal I, Tung YC, Liu F, Iqbal K (2013) Activation of asparaginyl endopeptidase leads to Tau hyperphosphorylation in Alzheimer disease. *J Biol Chem* **288**, 17495-17507.
[90] Marksteiner J, Humpel C (2008) Beta-amyloid expression, release and extracellular deposition in aged rat brain slices. *Mol Psychiatry* **13**, 939-952.
[91] Li PA, Siesjo BK (1997) Role of hyperglycaemia-related acidosis in ischaemic brain damage. *Acta Physiol Scand* **161**, 567-580.
[92] Mohajeri MH, Wollmer MA, Nitsch RM (2002) Abeta 42-induced increase in neprilysin is associated with prevention of amyloid plaque formation *in vivo*. *J Biol Chem* **277**, 35460-35465.
[93] Bian L, Yang JD, Guo TW, Sun Y, Duan SW, Chen WY, Pan YX, Feng GY, He L (2004) Insulin-degrading enzyme and Alzheimer disease: A genetic association study in the Han Chinese. *Neurology* **63**, 241-245.
[94] Liu W, Tang Y, Feng J (2011) Cross talk between activation of microglia and astrocytes in pathological conditions in the central nervous system. *Life Sci* **89**, 141-146.
[95] Farina C, Aloisi F, Meinl E (2007) Astrocytes are active players in cerebral innate immunity. *Trends Immunol* **28**, 138-145.
[96] Godoy B, Murgas P, Tichauer J, Von Bernhardi R (2012) Scavenger receptor class A ligands induce secretion of IL1beta and exert a modulatory effect on the inflammatory activation of astrocytes in culture. *J Neuroimmunol* **251**, 6-13.
[97] Williams MR, Cauvi DM, Rivera I, Hawisher D, De Maio A (2016) Changes in macrophage function modulated by the lipid environment. *Innate Immun* **22**, 141-151.
[98] Suzuki H, Kurihara Y, Takeya M, Kamada N, Kataoka M, Jishage K, Ueda O, Sakaguchi H, Higashi T, Suzuki T, Takashima Y, Kawabe Y, Cynshi O, Wada Y, Honda M, Kurihara H, Aburatani H, Doi T, Matsumoto A, Azuma S, Noda T, Toyoda Y, Itakura H, Yazaki Y, Kodama T, et al. (1997) A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection. *Nature* **386**, 292-296.
[99] Li F, Faustino J, Woo MS, Derugin N, Vexler ZS (2015) Lack of the scavenger receptor CD36 alters microglial phenotypes after neonatal stroke. *J Neurochem* **135**, 445-452.
[100] Baranova IN, Souza AC, Bocharov AV, Vishnyakova TG, Hu X, Vaisman BL, Amar MJ, Chen Z, Kost Y, Remaley AT, Patterson AP, Yuen PS, Star RA, Eggerman TL (2016) Human SR-BI and SR-BII potentiate lipopolysaccharide-induced inflammation and acute liver and kidney injury in mice. *J Immunol* **196**, 3135-3147.
[101] Peiser L, Gordon S (2001) The function of scavenger receptors expressed by macrophages and their role in the regulation of inflammation. *Microbes Infect* **3**, 149-159.
[102] Becker M, Cotena A, Gordon S, Platt N (2006) Expression of the class A macrophage scavenger receptor on specific subpopulations of murine dendritic cells limits their endotoxin response. *Eur J Immunol* **36**, 950-960.
[103] Ozeki Y, Tsutsui H, Kawada N, Suzuki H, Kataoka M, Kodama T, Yano I, Kaneda K, Kobayashi K (2006) Macrophage scavenger receptor down-regulates mycobacterial cord factor-induced proinflammatory cytokine production by alveolar and hepatic macrophages. *Microb Pathog* **40**, 171-176.

[104] Arredouani MS, Yang Z, Imrich A, Ning Y, Qin G, Kobzik L (2006) The macrophage scavenger receptor SR-AI/II and lung defense against pneumococci and particles. *Am J Respir Cell Mol Biol* **35**, 474-478.

[105] Hollifield M, Bou Ghanem E, de Villiers WJ, Garvy BA (2007) Scavenger receptor A dampens induction of inflammation in response to the fungal pathogen Pneumocystis carinii. *Infect Immun* **75**, 3999-4005.

[106] Hsu HY, Chiu SL, Wen MH, Chen KY, Hua KF (2001) Ligands of macrophage scavenger receptor induce cytokine expression via differential modulation of protein kinase signaling pathways. *J Biol Chem* **276**, 28719-28730.

[107] Campa VM, Iglesias JM, Carcedo MT, Rodriguez R, Riera J, Ramos S, Lazo PS (2005) Polyinosinic acid induces TNF and NO production as well as NF-kappaB and AP-1 transcriptional activation in the monocytemacrophage cell line RAW 264.7. *Inflamm Res* **54**, 328-337.

[108] Kwon KH, Ohigashi H, Murakami A (2007) Dextran sulfate sodium enhances interleukin-1 beta release via activation of p38 MAPK and ERK1/2 pathways in murine peritoneal macrophages. *Life Sci* **81**, 362-371.

[109] Song ES, Juliano MA, Juliano L, Hersh LB (2003) Substrate activation of insulin-degrading enzyme (insulysin). A potential target for drug development. *J Biol Chem* **278**, 49789-49794.

[110] Astrup J, Symon L, Branston NM, Lassen NA (1977) Cortical evoked potential and extracellular K+ and H+ at critical levels of brain ischemia. *Stroke* **8**, 51-57.

[111] Siesjo BK (1992) Pathophysiology and treatment of focal cerebral ischemia. Part II: Mechanisms of damage and treatment. *J Neurosurg* **77**, 337-354.

[112] Cho S, Park EM, Febbraio M, Anrather J, Park L, Racchumi G, Silverstein RL, Iadecola C (2005) The class B scavenger receptor CD36 mediates free radical production and tissue injury in cerebral ischemia. *J Neurosci* **25**, 2504-2512.

[113] El Khoury J, Hickman SE, Thomas CA, Cao L, Silverstein SC, Loike JD (1996) Scavenger receptor-mediated adhesion of microglia to beta-amyloid fibrils. *Nature* **382**, 716-719.

[114] Husemann J, Loike JD, Kodama T, Silverstein SC (2001) Scavenger receptor class B type I (SR-BI) mediates adhesion of neonatal murine microglia to fibrillar beta-amyloid. *J Neuroimmunol* **114**, 142-150.

[115] Granucci F, Petralia F, Urbano M, Citterio S, Di Tota F, Santambrogio L, Ricciardi-Castagnoli P (2003) The scavenger receptor MARCO mediates cytoskeleton rearrangements in dendritic cells and microglia. *Blood* **102**, 2940-2947.

[116] Pikkarainen T, Brannstrom A, Tryggvason K (1999) Expression of macrophage MARCO receptor induces formation of dendritic plasma membrane processes. *J Biol Chem* **274**, 10975-10982.

[117] El Khoury J, Hickman SE, Thomas CA, Loike JD, Silverstein SC (1998) Microglia, scavenger receptors, and the pathogenesis of Alzheimer's disease. *Neurobiol Aging* **19**, S81-S84.

[118] Grewal RP, Yoshida T, Finch CE, Morgan TE (1997) Scavenger receptor mRNAs in rat brain microglia are induced by kainic acid lesioning and by cytokines. *Neuroreport* **8**, 1077-1081.