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Inflammatory components in human Alzheimer's disease and after active amyloid-β_{42} immunization

Elina Zotova,[1] Viraj Bharambe,[1] Matthew Cheaveau,[1] William Morgan,[1] Clive Holmes,[1,2] Scott Harris,[3] James W Neal,[4] Seth Love,[5] James A. R. Nicoll[1,6] and Delphine Boche[1]

1 Clinical Neurosciences, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Mailpoint 806, Southampton General Hospital, Southampton SO16 6YD, UK
2 Moorgreen Hospital, M.A.R.C., Southern Health Foundation Trust, Southampton, UK
3 Public Health Sciences and Medical Statistics, Faculty of Medicine, University of Southampton, Southampton, UK
4 Department of Histopathology, University of Wales, Heath Park, Cardiff, UK
5 Department of Neuropathology, Institute of Clinical Neurosciences, School of Clinical Sciences, University of Bristol, Bristol, UK
6 Department of Cellular Pathology, University Hospital Southampton NHS Foundation Trust, Southampton, UK

Correspondence to: Delphine Boche,
Clinical Neurosciences,
Clinical and Experimental Sciences,
Faculty of Medicine,
University of Southampton,
Mailpoint 806,
Southampton General Hospital,
Southampton SO16 6YD
UK
E-mail: d.boche@soton.ac.uk

Inflammatory processes are important in the pathogenesis of Alzheimer's disease and in response to amyloid-β immunotherapy. We investigated the expression of multiple inflammatory markers in the brains of 28 non-immunized patients with Alzheimer's disease and 11 patients with Alzheimer's disease immunized against amyloid-β_{42} (AN1792): microglial ionized calcium-binding adaptor Iba-1, lysosome marker CD68, macrophage scavenger receptor A, Fcγ receptors I (CD64) and II (CD32); and also immunoglobulin IgG, complement C1q and the T lymphocyte marker CD3 using immunohistochemistry. The data were analysed with regard to amyloid-β and phospho-tau pathology, severity of cerebral amyloid angiopathy and cortical microhaemorrhages. In non-immunized Alzheimer's disease cases, amyloid-β_{42} correlated inversely with CD32 and Iba-1, whereas phospho-tau correlated directly with all microglial markers, IgG, C1q and the number of T cells. In immunized Alzheimer's disease cases, amyloid-β_{42} load correlated directly with macrophage scavenger receptor A-positive clusters and inversely with C1q. The severity of cerebral amyloid angiopathy and microhaemorrhages did not relate to any of the analysed markers. Overall, the levels of CD68, macrophage scavenger receptor A, CD64, CD32 and the number of macrophage scavenger receptor A-positive plaque-related clusters were significantly lower in immunized than non-immunized cases, although there was no significant difference in Iba-1 load, number of Iba-1-positive cells, IgG load, C1q load or number of T cells. Our findings indicate that different microglial populations co-exist in the Alzheimer's disease brain, and that the local inflammatory status within the grey matter is importantly linked with tau pathology. After amyloid-β immunization, the microglial functional state is altered in association with reduced amyloid-β and tau pathology. The results suggest that, in the long term, amyloid-β immunotherapy results in downregulation of microglial activation and potentially reduces the inflammation-mediated component of the neurodegeneration of Alzheimer's disease.

Keywords: Alzheimer's disease; microglia, immunotherapy; innate immunity; clinical trial

Abbreviation: MSR-A = macrophage scavenger receptor

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Introduction

Alzheimer's disease accounts for >60% of all dementia cases (Albanese *et al.*, 2007). The disease is characterized by the presence of cortical extracellular plaques of amyloid-β protein and intraneuronal phospho-tau protein aggregates (Lowe *et al.*, 2008). Both amyloid-β and tau have been hypothesized as initiators of the tissue damage in Alzheimer's disease (Gray *et al.*, 1987; Hardy and Higgins, 1992). Neuroinflammation, manifested by activation of microglia, is an important component of Alzheimer's disease pathology with evidence to suggest that it is both a reaction to the disease process and a contributor to the neuronal damage (Akiyama *et al.*, 2000; Perry *et al.*, 2010; Zotova *et al.*, 2010). Recent genetic studies provide further evidence that inflammatory processes are important in the pathogenesis of Alzheimer's disease (Harold *et al.*, 2009; Lambert *et al.*, 2009; Guerreiro *et al.*, 2013; Jonsson *et al.*, 2013).

Microglial cells play a key role in the innate immune system response within the CNS. In Alzheimer's disease, microglia have been implicated in the generation of chronic low-grade neurotoxic inflammation around amyloid-β plaques, while being unable to clear them effectively (McGeer *et al.*, 1987; Rogers *et al.*, 1988; Griffin *et al.*, 1989; Rozemuller *et al.*, 1989; Mattiace *et al.*, 1990; Paresce *et al.*, 1997; Overmyer *et al.*, 1999). *In vivo* imaging of microglia (by use of PK11195-PET) and fibrillar amyloid (Pittsburgh compound B-PET) show higher levels of both markers in the cortex in patients with Alzheimer's disease compared with non-demented control subjects (Cagnin *et al.*, 2001; Edison *et al.*, 2007, 2008). Increasing microglial activation correlates with progression of cognitive decline without further changes in the amyloid load (Edison *et al.*, 2008). Although the exact functional state of the microglia is not clear from these studies, they support a role for persistent neuroinflammation in disease progression.

Based on the amyloid cascade hypothesis, suggesting that amyloid-β accumulation is the trigger for Alzheimer's disease pathogenesis (Hardy and Higgins, 1992), active immunization against amyloid-β_{42} peptide was proposed as a treatment. This approach was shown to result in the removal of amyloid plaques from the brains of human amyloid-β precursor protein (APP) transgenic mice (Schenk *et al.*, 1999; Bard *et al.*, 2000; Janus *et al.*, 2000; Morgan *et al.*, 2000), and the plaque clearance was associated with behavioural improvements (Janus *et al.*, 2000; Morgan *et al.*, 2000; Dodart *et al.*, 2002). Microglial activity was implicated in this effect (Bard *et al.*, 2000; Wilcock *et al.*, 2001, 2003, 2004*a*, *b*). In the active amyloid-β_{42} immunization clinical trial that followed (Elan Pharmaceuticals, AN1792), most aspects of cognitive change did not differ significantly between immunized patients and control subjects (Hock *et al.*, 2003; Bayer *et al.*, 2005; Gilman *et al.*, 2005), with longer term clinical follow-up revealing continuing cognitive decline despite removal of plaques (Holmes *et al.*, 2008). In addition, the development of side-effects including 'meningoencephalitis' with infiltration by T lymphocytes, and 'amyloid-related imaging abnormalities', associated with cerebral amyloid angiopathy and cortical microhaemorrhages in a proportion of patients with Alzheimer's disease immunized against amyloid-β has been problematic (Nicoll *et al.*, 2003; Orgogozo *et al.*, 2003; Boche *et al.*, 2008; Sperling *et al.*, 2011).

In this study, we explored in detail the inflammatory processes in the brain in Alzheimer's disease and compared the results with those in patients with Alzheimer's disease following active amyloid-β_{42} immunization (Elan Pharmaceuticals, AN1792). We assessed the microglial load by quantifying immunostaining for ionized calcium-binding adaptor molecule 1 (Iba-1), which has been reported to be an effective marker of both resting and activated microglia (Imai *et al.*, 1996; Ahmed *et al.*, 2007; Streit *et al.*, 2009). In an attempt to define not only their activation level but also their functional status, we have investigated the expression of macrophage scavenger receptor-A (MSR-A) involved in microglial endocytosis of extracellular material, including fibrillar amyloid-β (El Khoury *et al.*, 1996; Chung *et al.*, 2001), the microglial lysosomal protein CD68 as an indicator of phagocytic activity (Rabinowitz and Gordon, 1989, 1991; Zotova *et al.*, 2011), and the microglial cell surface receptors that bind IgG and immune complexes (Fcγ receptors I and II) (Ravetch and Bolland, 2001; Nimmerjahn and Ravetch, 2006). The level of C1q was assessed to investigate the role of the complement system in relation to Alzheimer's disease pathology, microglial activation, and immunization status (Eikelenboom and Veerhuis, 1996). As we previously found that anti-amyloid-β IgG persisted in the blood of immunized patients with Alzheimer's disease for many years (Holmes *et al.*, 2008), we also investigated whether there were changes in the overall amount of IgG antibody in the brain. T lymphocytes were previously identified in some immunized patients with Alzheimer's disease (Nicoll *et al.*, 2003; Orgogozo *et al.*, 2003; Ferrer *et al.*, 2004) and were also analysed. We explored the relationships of these inflammatory markers to amyloid-β and phospho-tau load, the severity of cerebral amyloid angiopathy and cortical microhaemorrhages.

Materials and methods

Immunized Alzheimer's disease cases

We performed a follow-up study of patients who were enrolled in the Phase I Elan Pharmaceuticals Inc. active amyloid-β_{42} immunization (AN1792) clinical trial for Alzheimer's disease (Bayer *et al.*, 2005; Holmes *et al.*, 2008). As part of the study, participants were invited to consent to post-mortem neuropathology. The study received ethical approval from Southampton and South West Hampshire Local Research Ethics Committees (Reference No: LRC 075/03/w). In the study reported here, we investigated brain tissue from 11 patients who took part in the clinical trial and had confirmation of the diagnosis of Alzheimer's disease by post-mortem neuropathology (Table 1). Neurodegenerative pathology was assessed by standard methods including analysis of paraffin sections stained with haematoxylin and eosin, Luxol fast blue/Cresyl violet, and modified Bielschowsky silver impregnation, and immunostained for amyloid-β, tau, α-synuclein and TAR-DNA binding protein 43 (TDP43). All immunized Alzheimer's disease cases were Braak stage V/VI, indicating an advanced stage of Alzheimer's tau pathology (Table 1). Two of the 11 cases had concomitant Lewy body pathology (one neocortical and nigral, one nigral only). No TDP43 inclusions and no argyrophilic grains were identified. In terms of cerebrovascular disease, none had macroscopic infarcts; one had an old frontal cortical haemorrhage (2-cm diameter);

Table 1 Clinical characteristics of immunized Alzheimer's disease (iAD) and non-immunized (control) Alzheimer's disease (cAD) subjects

ID	Gender	Age	Braak stage	Dementia duration (years)	APOE genotype	Mean antibody response (ELISA units)	Survival time from first immunization dose (months)
iAD1	F	74	VI	6	3.4	1:119	20
iAD2	M	83	V	11	3.3	<1:100	4
iAD3	M	63	VI	6	3.3	<1:100	41
iAD4	F	71	VI	10	3.3	1:4072	44
iAD5	M	81	VI	7	3.4	1:1707	57
iAD6	M	82	VI	6	3.4	1:4374	60
iAD7	M	63	VI	10	3.4	1:6470	64
iAD8	M	81	VI	11	4.4	1:491	63
iAD9	F	88	VI	11	3.3	1:137	86
iAD10	M	88	VI	12	3.4	1:142	94
iAD11	F	89	VI	15	3.4	1:142	111
cAD ($n = 28$)	15F:13M	63–88	V/VI	3–17	21$\varepsilon4^+$: 6 $\varepsilon4^-$	–	–

all had varying degrees of arteriolosclerosis, most marked in the cerebral white matter where it was moderate or severe.

Non-immunized Alzheimer's disease controls

Post-mortem material from sufficient numbers of placebo patients in the immunotherapy trial was not available, therefore paraffin sections from 28 cases with Alzheimer's disease from the South-West Dementia Brain Bank (Frenchay Hospital, Bristol, UK) were used as non-immunized controls (Table 1) (Research ethics committee reference: 08/H0106/28). All control Alzheimer's disease cases had a clinical diagnosis of dementia made during life by an experienced clinician, a Mini-Mental State Examination score of <17 before death and satisfied post-mortem neuropathological Consensus Criteria for Alzheimer's disease (Hyman and Trojanowski, 1997). The immunized and control Alzheimer's disease cases were matched as closely as possible for age, gender, duration of dementia and *APOE* genotype.

The most common causes of death cited from all listed causes, in addition to dementia, were bronchopneumonia (immunized Alzheimer's disease, 27%; non-immunized controls, 46%), myocardial infarction (immunized Alzheimer's disease, 9%; non-immunized controls 7%) and cerebrovascular accident (non-immunized controls, 7%). Other causes of death included: immunized Alzheimer's disease—a ruptured aortic aneurysm, pulmonary embolism; non-immunized controls—pyelonephritis, sigmoid volvulus. One immunized Alzheimer's disease case and six non-immunized control cases have unknown cause of death.

Immunohistochemistry

For the purposes of this study, the following regions of cerebral cortex usually markedly affected by the Alzheimer's disease process were used: superior and middle temporal gyrus, medial frontal gyrus and inferior parietal lobule. Four micrometre-thick sections of formalin-fixed paraffin-embedded tissue from these areas were used for immunohistochemistry.

Primary antibodies

Sections were immunostained with the following antibodies: mouse anti-human specific for amyloid-β_{42} (clone 21F12) provided by Elan Pharmaceuticals Inc. (Johnson-Wood *et al.*, 1997); mouse anti-human phospho-tau (clone AT8, Autogen Bioclear); mouse anti-human CD68 (Dako); goat anti-human CD64 (Fcγ receptor I), goat anti-human CD32 (Fcγ receptor II), goat anti-human MSR-A (all from R&D Systems); rabbit anti-human Iba-1 (Wako Laboratories); rabbit anti-human IgG (Dako); rabbit anti-human C1q (Dako); mouse anti-human CD3 (Abcam). Table 2 specifies the functions associated with each of the microglial markers.

Immunohistochemistry

Sections from immunized Alzheimer's disease and non-immunized control cases were immunostained together in batches for each antibody to ensure comparability of labelling. Immunohistochemistry was performed using the appropriate antigen retrieval methods for each primary antibody. Biotinylated secondary antibodies (rabbit anti-mouse, swine anti-rabbit, and rabbit anti-goat) were from Dako, normal serum and avidin–biotin complex were from Vector Laboratories. Bound antibody was visualized using the avidin–biotin-peroxidase complex method (Vectastain Elite ABC) with 3,3′ diaminobenzidine as chromogen and 0.05% hydrogen peroxide as substrate to produce a brown reaction product. All sections were dehydrated before mounting in DePeX (BDH Laboratory Supplies). Sections incubated in the absence of the primary antibody were included as negative controls.

Quantification

All quantification was performed blind to the experimental group and identity of the cases. Images of the slides were taken from the same anatomical regions in every case. For a given antibody, a minimum of 20 fields of cortical grey matter at magnification ×10 (for amyloid-β_{42}, CD64, CD32, Iba-1, MSR-A, and IgG) or 30 fields of cortical grey matter at magnification ×20 (for AT8 and CD68) that covered a similar area, were analysed to ensure representative detection of immunostaining for image analysis. Images were acquired in a

Table 2 Details of microglial markers used

Antibody	Associated function or marker of
Iba-1 (ionized calcium-binding adaptor molecule 1)	Resting and activated microglia/macrophages, upregulated during activation (Imai *et al.*, 1996; Ahmed *et al.*, 2007; Streit *et al.*, 2009)
CD64 (Fcγ RI)	High affinity receptor for human IgG, main role in mounting an immune response (Ravetch and Bolland, 2001; Nimmerjahn and Ravetch, 2006)
CD32 (Fcγ RII)	Low affinity receptor for human IgG, phagocytosis of immune complexes (Ravetch and Bolland, 2001; Nimmerjahn and Ravetch, 2006)
MSR-A	Lipoprotein receptor involved in direct ligand recognition, including that of amyloid-β (El Khoury *et al.*, 1996)
CD68	Activated/phagocytic macrophages/microglia (Rabinowitz and Gordon, 1989, 1991; Zotova *et al.*, 2011)

zigzag sequence along the cortical ribbon to ensure that all cortical layers were represented in the quantification.

Images were analysed using ImageJ version 1.41 software (developed by Wayne Rasband NIH, USA). Each image was split into channels (red, green and blue). The eight-bit blue channel image was corrected for background illumination using the eight-bit blue channel of a brightfield image and applying a correction method adopted from Landini (2006–2010) to produce an evenly illuminated image. A threshold was then applied to the image to measure the total amount of specific immunostaining. The same threshold was maintained for all images (immunized Alzheimer's disease and non-immunized control cases) of slides stained together in the same batch. The percentage of the area examined for each antibody was used to indicate the corresponding protein load.

Counting of Iba-1-positive microglia and MSR-A-positive microglial clusters was performed in 30 fields of each of the three brain areas at magnification $\times 20$, aided by an ocular morphometric grid inserted into one of the eyepieces. The rationale for the additional quantification of Iba-1 and MSR-A was based on the reported detection of all microglia (resting and activated) with Iba-1 antibody (Streit *et al.*, 2009), and the marked clustering pattern of the MSR-A immunostaining we observed. The CD3-positive T lymphocytes were counted in the entire tissue section of each brain area analysed, at magnification $\times 10$, and expressed as the number of cells per 100 fields. Parenchymal and perivascular T lymphocytes were included in the quantification.

The grading of cerebral amyloid angiopathy was performed as described by Chalmers *et al.* (2003). Briefly, amyloid-β_{42}-immunostained sections were examined and assigned a grade ranging from zero (no amyloid-laden blood vessels) to four (blood vessels having a heavy deposit of amyloid that also extends into the surrounding parenchyma). Cortical and leptomeningeal cerebral amyloid angiopathy in the three brain areas was assessed separately and a median combined grade was calculated for each case.

Haematoxylin and eosin-stained sections of the three brain areas were examined for cortical microhaemorrhages (i.e. collections of haemosiderin-laden macrophages) and microvascular lesions (microscopic foci of parenchymal haemorrhage or infarction) which might influence the microglial status. Entire sections were assessed at magnification $\times 10$ and the sum of microhaemorrhages and microvascular lesions in the three brain areas was used for the subsequent analyses.

The immunostaining and quantification of amyloid-β_{42} and phospho-tau in the brains of 10 of the 11 immunized patients with Alzheimer's disease and the 28 non-immunized Alzheimer's disease control subjects were previously described (Boche *et al.*, 2010). The data set for this study was augmented by one extra immunized case and re-analysed using the same image analysis method as for the microglial markers.

Statistical analysis

The data on antigen load (% area immunostained), sum counts of Iba-1 or CD-3-positive cells, MSR-A-positive clusters, microhaemorrhages/microvascular lesions, and the cerebral amyloid angiopathy severity for the three areas were analysed. The continuous outcome variables were assessed for normality using one-sample Kolmogorov-Smirnoff tests and through examination of Q-Q plots. In view of the skewed distribution of the data, the non-parametric Mann-Whitney U-test was used for comparisons between the two groups (non-immunized control versus immunized Alzheimer's disease cases), with median values reported. Similarly, Spearman's Rank correlation was used to assess measurements of inflammatory markers in relation to amyloid-β_{42} and tau load. The data of the three areas combined were analysed using SPSS 19 software (SPSS, Inc). *P*-values <0.05 were considered statistically significant.

Results

Microglial markers

Iba-1

Immunostaining with Iba-1, which has been reported to label all microglia, active and resting (Streit *et al.*, 2009), revealed microglial cells with both ramified and amoeboid morphology (Fig. 1A and B), although most cells displayed ramified morphology regardless of the Alzheimer's disease group (non-immunized controls or immunized Alzheimer's disease patients). Quantification showed no significant difference in the Iba-1 load between the immunized Alzheimer's disease and non-immunized control cases, although there was a trend towards less Iba-1 in the immunized group (Fig. 1C). Similarly, the total number of Iba-1-positive microglial cells in 90 fields was not significantly different between immunized Alzheimer's disease and non-immunized control groups (Fig. 1D).

Macrophage scavenger receptor A

Immunostaining for MSR-A demonstrated microglia, including their processes, in a pattern consistent with cell surface membrane expression of this receptor (Fig. 2A and B). A striking feature, not observed so markedly with the other microglial antibodies, was clustering of labelled cells, often associated with amyloid plaques, which prompted us to undertake manual counting of clusters in addition to the quantification of the total antigen load. The total

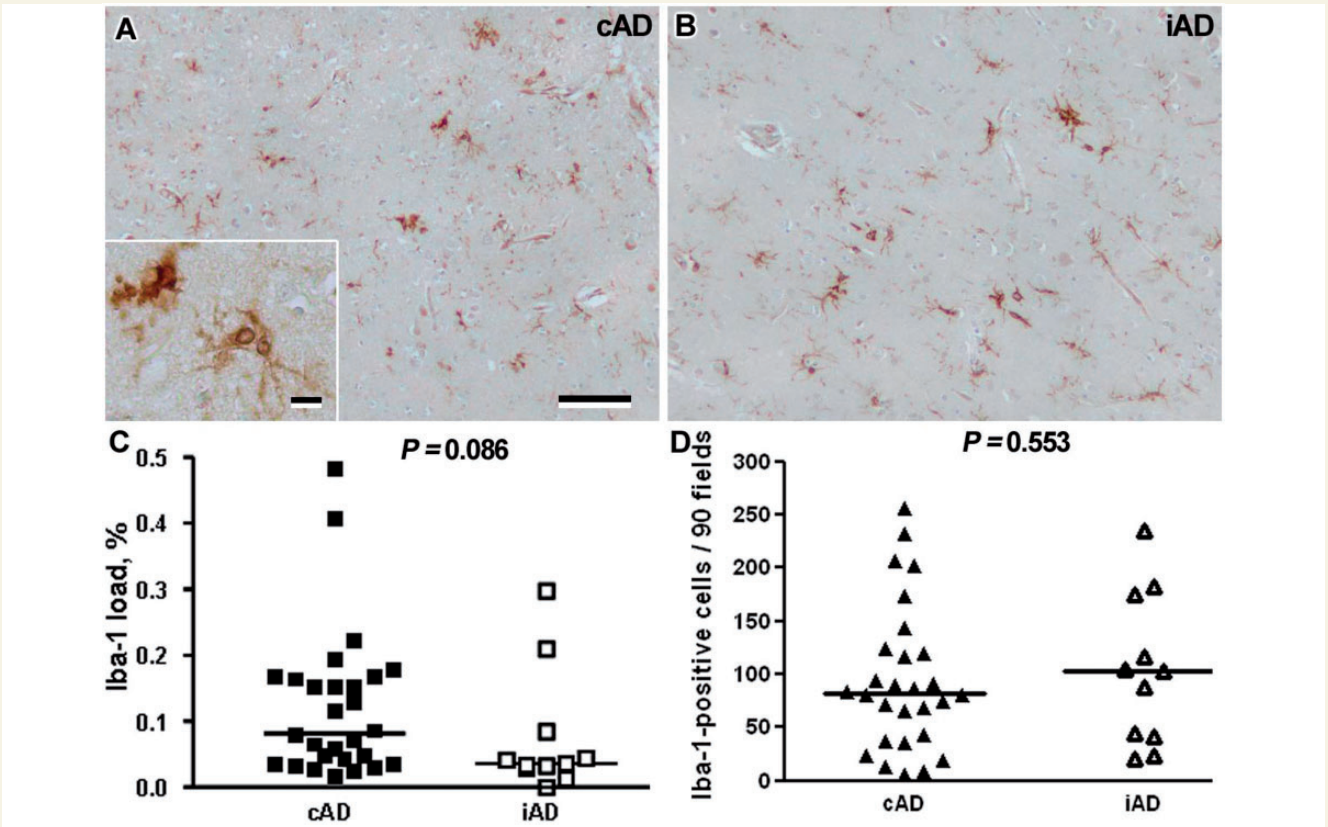

Figure 1 Illustrations of immunostaining for microglial Iba-1 in non-immunized control (cAD) and immunized Alzheimer's disease (iAD) cases (**A** and **B**), with inset of higher magnification. Quantification results of Iba-1 protein load (**C**) and the number of Iba-1-positive cells (**D**) expressed as scatter plots with lines indicating median values, and Mann-Whitney *P*-values included. Scale bars = 100 μm; insets = 10 μm.

MSR-A load was significantly lower in the immunized Alzheimer's disease (0.01%) than the non-immunized control group (0.10%, $P < 0.001$, Fig. 2C). Similarly, the sum number of MSR-A-positive clusters was also significantly lower (immunized Alzheimer's disease = 22 compared with non-immunized controls = 70, $P < 0.001$, Fig. 2D).

Fcγ receptor I (CD64)

Antibody to CD64 strongly labelled microglial cells, including their processes, consistent with the expected pattern for a cell surface membrane receptor (Fig. 3A and B). The CD64 load was significantly lower in the immunized Alzheimer's disease (0.36%) than the non-immunized control group (0.82%, $P = 0.001$, Fig. 3C). The CD64 load was several-fold higher than any of the other microglial antigens. In both immunized and non-immunized Alzheimer's disease cases the CD64 load was ~10 times that obtained for Iba-1, which has been reported to be expressed by all microglia.

Fcγ receptor II (CD32)

Immunostaining for CD32 demonstrated microglial cells, although the cell processes were less strongly defined than with CD64 antibody (Fig. 3D and E). The CD32 load was also significantly lower in the immunized Alzheimer's disease (0.02%) than the non-immunized control group (0.04%, $P = 0.002$, Fig. 3F).

CD68

CD68 was distributed in an intracytoplasmic dot-like pattern consistent with the labelling of lysosomes within microglia, as previously shown (Zotova *et al.*, 2011) (Fig. 3G and H). The CD68 load was lower in the immunized (0.02%) than the non-immunized group (0.05%, $P = 0.018$, Fig. 3I).

Amyloid-β_{42} and phospho-tau load

As previously described (Boche *et al.*, 2010), the amyloid-β_{42} load was 82% lower in the immunized (0.72%) than the non-immunized Alzheimer's disease cases (4.06%; $P < 0.001$, Fig. 4), reflecting the removal of amyloid-β as a consequence of amyloid-β immunization. The phospho-tau load was 40% lower in the immunized (0.46%) than the non-immunized group (0.77%, $P = 0.034$, Fig. 4), reflecting a reduction of phospho-tau mainly in neuronal processes (Boche *et al.*, 2010).

Correlations between microglial markers and amyloid-β_{42} and phospho-tau pathology

Correlations between the microglial antigen load and amyloid-β_{42} and phospho-tau load were examined in the non-immunized control and immunized Alzheimer's disease groups (Tables 3 and 4).

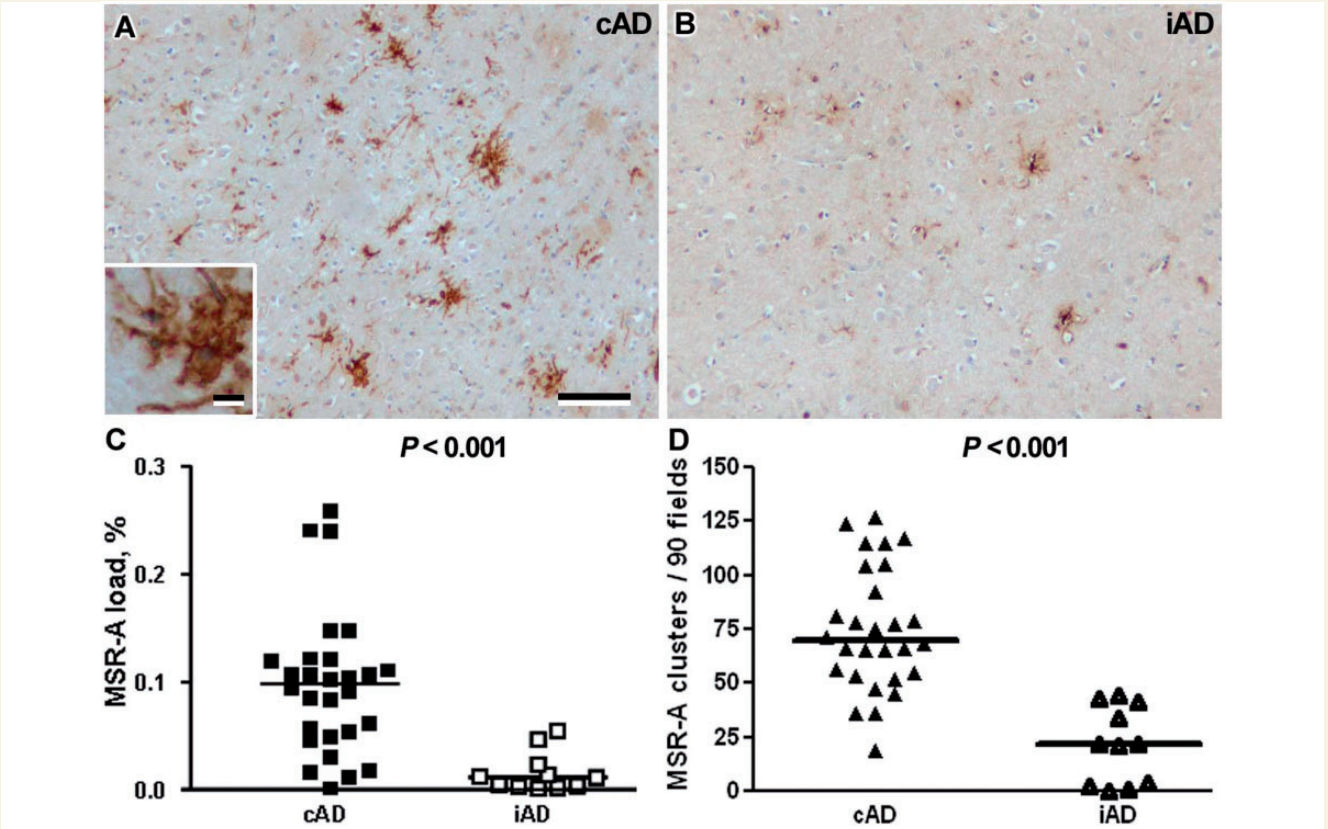

Figure 2 Illustrations of immunostaining for microglial MSR-A in non-immunized control (cAD) and immunized Alzheimer's disease (iAD) cases (**A** and **B**), with inset of higher magnification. Quantification results of MSR-A protein load (**C**) and the number of MSR-A-positive clusters (**D**) expressed as scatter plots with lines indicating median values, and Mann-Whitney *P*-values included. Scale bars = 100 μm; inset = 10 μm.

Correlations with amyloid-β_{42}

Inverse correlations were observed between the amyloid-β_{42} load and two microglial markers in the non-immunized control group: Iba-1, both the load (Spearman's $\rho = -0.6$, $P = 0.001$, Fig. 5A) and the number of cells (Spearman's $\rho = -0.4$, $P = 0.026$, Fig. 5B), and the CD32 load (Fcγ receptor II, Spearman's $\rho = -0.6$, $P < 0.001$, Fig. 5C). Within the immunized Alzheimer's disease group, a positive correlation was noted between the amyloid-β_{42} load and the number of MSR-A-positive clusters (Spearman's $\rho = 0.7$, $P = 0.024$, Fig. 5D). No other correlations between microglial markers and amyloid-β_{42} load were observed within the immunized Alzheimer's disease and non-immunized control groups (Tables 3 and 4).

Correlations with tau

Correlations were observed between the phospho-tau load and all of the microglial markers in the non-immunized control group (Fig. 6 and Table 3): Iba-1 load (Spearman's $\rho = 0.4$, $P = 0.046$, Fig. 6A), Fcγ receptor II load (Spearman's $\rho = 0.4$, $P = 0.032$, Fig. 6B), MSR-A load (Spearman's $\rho = 0.4$, $P = 0.045$, Fig. 6C), Fcγ receptor I load (Spearman's $\rho = 0.5$, $P = 0.010$, Fig. 6D), and CD68 load (Spearman's $\rho = 0.4$, $P = 0.036$, Fig. 6E). No correlations between microglial markers and phospho-tau load were observed within the immunized group (Table 4). The number of Iba-1-positive cells and MSR-A-positive clusters did not correlate with the phospho-tau load in either of the Alzheimer's disease groups (Tables 3 and 4).

Immunoglobulin G

Human IgG was detected in amyloid-β plaques, on microglia surrounding plaques, and within neurons (often adjacent to plaques), in both immunized Alzheimer's disease and non-immunized control cases (Fig. 7A–F). It should be noted, however, that extensive areas of cortex did not contain immunodetectable IgG, even where abundant plaques were present. Some cortical and meningeal blood vessels immunopositive for IgG were also noted, particularly those with amyloid-β in their walls (i.e. with cerebral amyloid angiopathy; Fig. 7D and E). Quantification of the IgG load showed no significant difference between the immunized Alzheimer's disease and non-immunized control groups (Fig. 7G). A correlation between the IgG load and the phospho-tau load was noted within the non-immunized controls (Spearman's $\rho = 0.4$, $P = 0.030$, Fig. 7H, Table 3), but not the immunized Alzheimer's disease group (Table 4). In addition, within the non-immunized control group the IgG load correlated with the load of all microglial antigens examined (Table 3): Iba-1 (Spearman's $\rho = 0.4$, $P = 0.036$, Fig. 8A), Fcγ receptor II (CD32) load (Spearman's

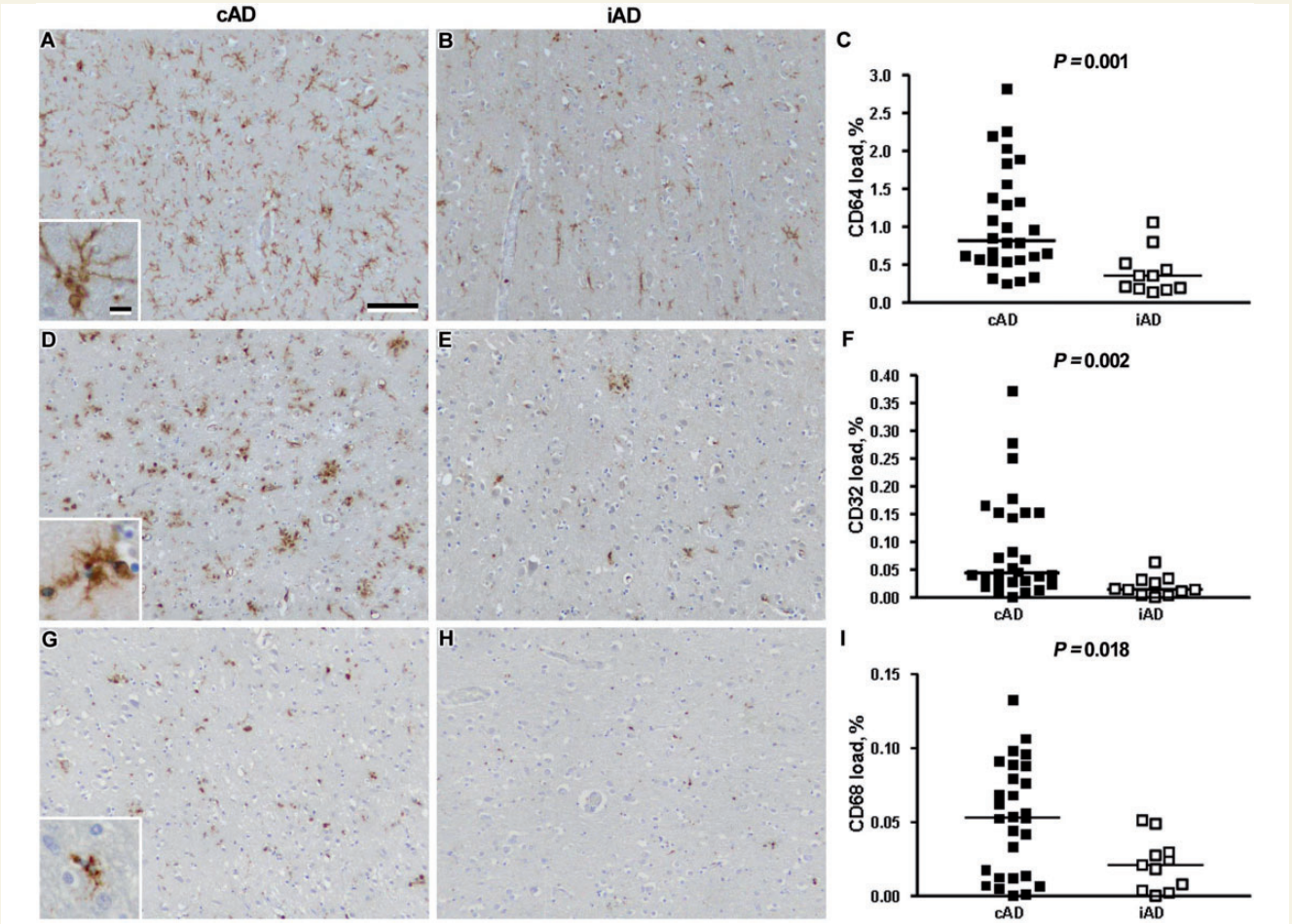

Figure 3 Illustrations of immunostaining for microglial markers in non-immunized control (cAD) and immunized Alzheimer's disease (iAD) cases using antibody against: CD64 (Fcγ RI) (**A** and **B**), CD32 (Fcγ RII) (**D** and **E**), and CD68 (**G** and **H**) with inset of higher magnification. Quantification results of protein load for each microglial marker is expressed as scatter plots with lines indicating median values, and Mann-Whitney *P*-values included (**C**, **F** and **I**). Scale bars = 100 μm; insets = 10 μm.

$\rho = 0.5$, $P = 0.003$, Fig. 8B), MSR-A load (Spearman's $\rho = 0.6$, $P = 0.001$, Fig. 8C), Fcγ receptor I (CD64) load (Spearman's $\rho = 0.7$, $P < 0.001$, Fig. 8D), and CD68 load (Spearman's $\rho = 0.7$, $P = 0.001$, Fig. 8E). Within the immunized Alzheimer's disease group, similar trends between the IgG load and microglial markers MSR-A, CD32, and CD64 were observed, but not with Iba-1 or CD68 (Table 4). No correlation was observed between the IgG load and the amyloid-β_{42} load in either of the Alzheimer's disease groups (Tables 3 and 4).

Complement C1q

Antibody to the complement element C1q labelled neurons, amyloid plaques, glia, and occasional blood vessels (Fig. 9A–D), similarly to anti-IgG antibody. Quantification of C1q also showed no difference between the immunized Alzheimer's disease and non-immunized control groups (Fig. 9E). Within the non-immunized control group, the C1q load correlated only with the phospho-tau load (Spearman's $\rho = 0.6$, $P = 0.003$, Fig. 9F and Table 3), whereas in the immunized group, only an inverse correlation between the C1q load and the amyloid-β_{42} load was noted (Spearman's $\rho = -0.6$, $P = 0.047$, Fig. 9G and Table 4). As for IgG, the C1q load correlated with the load of all microglial antigens examined within the non-immunized control group (Table 3): Iba-1 (Spearman's $\rho = 0.5$, $P = 0.017$, Fig. 10A), CD68 (Spearman's $\rho = 0.5$, $P = 0.012$, Fig. 10B), CD64 (FcγRI, Spearman's $\rho = 0.6$, $P = 0.001$, Fig. 10C), CD32 (FcγRII, Spearman's $\rho = 0.7$, $P < 0.001$, Fig. 10D), and MSR-A (Spearman's $\rho = 0.4$, $P = 0.036$, Fig. 10E). No such correlations were observed in the immunized Alzheimer's disease group (Table 4). Correlations between the C1q load and the IgG load were found in both the non-immunized control group (Spearman's $\rho = 0.7$, $P < 0.001$, Table 3) and the immunized Alzheimer's disease group (Spearman's $\rho = 0.6$, $P = 0.035$, Table 4).

CD3-positive T lymphocytes

The number of CD3-positive T lymphocytes did not differ significantly between the immunized and non-immunized groups (Fig. 11A). Parenchymal (Fig. 11E) and perivascular (Fig. 11F) T lymphocytes were included in the quantification. A correlation between the number of CD3-positive cells and the IgG load was

observed in both the non-immunized controls (Spearman's $\rho = 0.4$, $P = 0.030$, Fig. 11C and Table 3) and immunized Alzheimer's disease groups (Spearman's $\rho = 0.8$, $P = 0.007$, Fig. 11D and Table 4). The number of CD3-positive T cells correlated with the phospho-tau load within the non-immunized controls (Spearman's $\rho = 0.4$, $P = 0.026$, Fig. 11B and Table 3) but not the immunized Alzheimer's disease group (Table 4). No relationships between the CD3 data and any of the microglial markers, amyloid-β_{42}, or complement C1q were noted in either of the Alzheimer's disease groups (Tables 3 and 4).

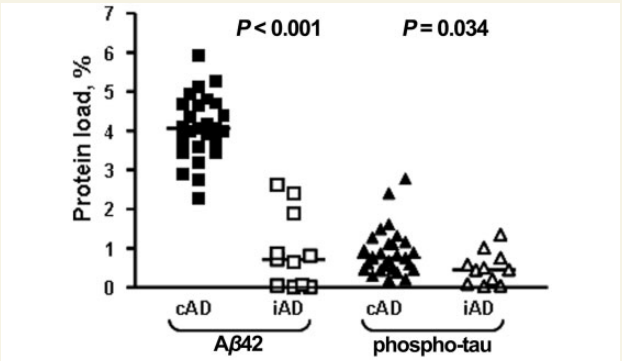

Figure 4 Results of the amyloid-β_{42} and phospho-tau quantification summarized in a scatter plot with lines indicating median values and Mann-Whitney *P*-values included. cAD = non-immunized Alzheimer's disease controls; iAD = immunized Alzheimer's disease cases.

Cerebral amyloid angiopathy

The assessment of cerebral amyloid angiopathy in the cerebral cortex and leptomeninges demonstrated more severe cerebral amyloid angiopathy in the immunized Alzheimer's disease (median score = 2.0) than the non-immunized control group (median score = 1.0, $P = 0.044$), in accordance with previously reported findings (Boche *et al.*, 2008). However, no relationships were observed between the cerebral amyloid angiopathy severity and the microglial markers, amyloid-β_{42} load, phospho-tau load or any other parameters investigated in this study.

Microhaemorrhages and microvascular lesions

The assessment of the haematoxylin and eosin-stained sections for the number of microhaemorrhages and microvascular lesions did not reveal any significant differences between the immunized, and the non-immunized group. No relationships were noted between the number of microhaemorrhages and microvascular lesions and the level of microglial markers, amyloid-β_{42} load, phospho-tau load or any other parameters assessed in the two groups.

Discussion

Microglial activity is closely linked to the development of Alzheimer's disease, but the precise role of microglia in disease pathogenesis and the response to amyloid-β immunization is

Table 3 Results of correlation analyses within non-immunized Alzheimer's disease control group – Spearman's ρ and *P*-values

cAD	Phospho-tau load	CD68 load	CD64 load	CD32 load	MSR load	MSR clusters	Iba-1 load	Iba-1 + cells	IgG load	C1q load	CD3 + cells
Amyloid-β_{42} load	$\rho = -0.2$ $P = 0.428$	$\rho = -0.3$ $P = 0.091$	$\rho = -0.1$ $P = 0.477$	**$\rho = -0.6$ $P < 0.001$**	$\rho = -0.2$ $P = 0.438$	$\rho = 0.1$ $P = 0.660$	**$\rho = -0.6$ $P = 0.001$**	**$\rho = -0.4$ $P = 0.026$**	$\rho = -0.2$ $P = 0.370$	$\rho = -0.2$ $P = 0.258$	$\rho = -0.1$ $P = 0.553$
Phospho-tau load		**$\rho = 0.4$ $P = 0.036$**	**$\rho = 0.5$ $P = 0.010$**	**$\rho = 0.4$ $P = 0.032$**	**$\rho = 0.4$ $P = 0.045$**	$\rho < 0.1$ $P = 0.936$	**$\rho = 0.4$ $P = 0.046$**	$\rho = 0.3$ $P = 0.151$	**$\rho = 0.4$ $P = 0.030$**	**$\rho = 0.6$ $P = 0.003$**	**$\rho = 0.4$ $P = 0.026$**
CD68 load			**$\rho = 0.7$ $P < 0.001$**	**$\rho = 0.7$ $P < 0.001$**	**$\rho = 0.6$ $P < 0.001$**	$\rho = 0.2$ $P = 0.441$	**$\rho = 0.5$ $P = 0.004$**	$\rho = 0.1$ $P = 0.586$	**$\rho = 0.7$ $P < 0.001$**	**$\rho = 0.5$ $P = 0.012$**	$\rho = 0.2$ $P = 0.244$
CD64 load				**$\rho = 0.6$ $P = 0.001$**	**$\rho = 0.5$ $P = 0.003$**	$\rho = 0.2$ $P = 0.387$	**$\rho = 0.5$ $P = 0.007$**	$\rho = 0.2$ $P = 0.444$	**$\rho = 0.7$ $P < 0.001$**	**$\rho = 0.6$ $P = 0.001$**	$\rho = 0.3$ $P = 0.095$
CD32 load					$\rho = 0.4$ $P = 0.052$	$\rho < 0.1$ $P = 0.912$	**$\rho = 0.7$ $P < 0.001$**	**$\rho = 0.5$ $P = 0.011$**	**$\rho = 0.5$ $P = 0.003$**	**$\rho = 0.7$ $P < 0.001$**	$\rho = 0.1$ $P = 0.446$
MSR load						**$\rho = 0.5$ $P = 0.002$**	$\rho = 0.4$ $P = 0.054$	$\rho = -0.1$ $P = 0.725$	**$\rho = 0.6$ $P = 0.001$**	**$\rho = 0.4$ $P = 0.036$**	$\rho < 0.1$ $P = 0.899$
MSR clusters							$\rho = 0.1$ $P = 0.750$	$\rho = -0.1$ $P = 0.524$	$\rho = 0.1$ $P = 0.459$	$\rho < 0.1$ $P = 0.801$	$\rho = -0.2$ $P = 0.208$
Iba-1 load								**$\rho = 0.7$ $P < 0.001$**	**$\rho = 0.4$ $P = 0.036$**	**$\rho = 0.5$ $P < 0.017$**	$\rho < 0.1$ $P = 0.818$
Iba-1 + cells									$\rho = -0.1$ $P = 0.701$	$\rho = 0.3$ $P = 0.165$	$\rho = 0.1$ $P = 0.750$
IgG load										**$\rho = 0.7$ $P < 0.001$**	**$\rho = 0.4$ $P = 0.030$**
C1q load											$\rho = 0.3$ $P = 0.141$

cAD = non-immunized Alzheimer's disease control.
Significant correlations are highlighted in bold.

Table 4 Results of correlation analyses within the immunized Alzheimer's disease group – Spearman's ρ and *P*-values

iAD	Phospho-tau load	CD68 load	CD64 load	CD32 load	MSR load	MSR clusters	Iba-1 load	Iba-1 + cells	IgG load	C1q load	CD3 + cells
Amyloid-β_{42} load	$\rho = 0.4$ $P = 0.212$	$\rho = 0.2$ $P = 0.484$	$\rho = 0.5$ $P = 0.151$	$\rho = 0.3$ $P = 0.326$	$\rho = 0.4$ $P = 0.272$	**$\rho = 0.7$** **$P = 0.024$**	$\rho = 0.1$ $P = 0.689$	$\rho = 0.2$ $P = 0.650$	$\rho = -0.2$ $P = 0.537$	**$\rho = -0.6$** **$P = 0.047$**	$\rho = -0.2$ $P = 0.555$
Phospho-tau load		$\rho = 0.2$ $P = 0.484$	$\rho = 0.2$ $P = 0.574$	$\rho = 0.5$ $P = 0.110$	$\rho = 0.4$ $P = 0.201$	$\rho = 0.5$ $P = 0.098$	$\rho = 0.2$ $P = 0.612$	$\rho < -0.1$ $P = 0.915$	$\rho = -0.1$ $P = 0.811$	$\rho = -0.2$ $P = 0.502$	$\rho = -0.3$ $P = 0.433$
CD68 load			**$\rho = 0.8$** **$P = 0.004$**	$\rho = 0.4$ $P = 0.180$	$\rho = 0.6$ $P = 0.060$	$\rho = 0.3$ $P = 0.377$	$\rho = -0.1$ $P = 0.770$	$\rho = -0.6$ $P = 0.051$	$\rho = 0.5$ $P = 0.177$	$\rho = 0.3$ $P = 0.450$	$\rho = 0.5$ $P = 0.125$
CD64 load				$\rho = 0.5$ $P = 0.102$	**$\rho = 0.7$** **$P = 0.016$**	$\rho = 0.4$ $P = 0.174$	$\rho = 0.2$ $P = 0.593$	$\rho = -0.2$ $P = 0.593$	$\rho = 0.6$ $P = 0.077$	$\rho = 0.3$ $P = 0.417$	$\rho = 0.5$ $P = 0.170$
CD32 load					$\rho = 0.6$ $P = 0.071$	$\rho = 0.5$ $P = 0.141$	$\rho = 0.5$ $P = 0.096$	$\rho < 0.1$ $P = 0.979$	$\rho = 0.6$ $P = 0.066$	$\rho = 0.1$ $P = 0.770$	$\rho = 0.5$ $P = 0.110$
MSR load						$\rho = 0.4$ $P = 0.264$	$\rho = 0.6$ $P = 0.051$	$\rho < -0.1$ $P = 0.915$	$\rho = 0.6$ $P = 0.066$	$\rho = 0.3$ $P = 0.417$	$\rho = 0.4$ $P = 0.201$
MSR clusters							$\rho = 0.4$ $P = 0.194$	$\rho = 0.2$ $P = 0.492$	$\rho = -0.1$ $P = 0.709$	$\rho = -0.1$ $P = 0.852$	$\rho = -0.1$ $P = 0.884$
Iba-1 load								$\rho = 0.6$ $P = 0.056$	$\rho = 0.4$ $P = 0.285$	$\rho = 0.3$ $P = 0.340$	$\rho = 0.3$ $P = 0.340$
Iba-1 + cells									$\rho = -0.3$ $P = 0.450$	$\rho = -0.1$ $P = 0.750$	$\rho = -0.3$ $P = 0.370$
IgG load										**$\rho = 0.6$** **$P = 0.035$**	**$\rho = 0.8$** **$P = 0.007$**
C1q load											$\rho = 0.6$ $P = 0.077$

iAD = immunized Alzheimer's disease.
Significant correlations are highlighted in bold.

unclear. The poor cognitive outcome of patients in the AN1792 clinical trial suggested that clearance of amyloid-β plaques alone is not sufficient to halt the progression of dementia (Holmes *et al.*, 2008), raising the possibility that persistent microglial activation results in continuing neurodegeneration. In the current study, we have investigated microglial receptors involved in direct and antibody-mediated ligand recognition and clearance in human post-mortem Alzheimer's disease tissue, their relation to amyloid-β and phospho-tau pathology and other inflammatory processes, and how these relationships are altered after active amyloid-β42 immunization.

Microglial markers and pathological features of Alzheimer's disease, amyloid-β_{42} and tau

Consistent with previously reported findings, we observed significantly lower amyloid-β_{42} and phospho-tau loads after amyloid-β immunization (Boche *et al.*, 2010). In conjunction with these alterations in the pathology of the disease, we observed significant changes in microglial receptors and markers of the CNS innate immune response.

Correlations between amyloid-β_{42} and microglial markers

With respect to amyloid-β_{42}, inverse correlations were observed with Iba-1 and Fcγ receptor II in non-immunized Alzheimer's disease only. Iba-1 is expressed constitutively by microglia in the brain, with increased expression upon activation (Imai *et al.*, 1996; Imai and Kohsaka, 2002). It was proposed that Iba-1 is involved in cytoskeletal reorganization, membrane ruffling and actin cross-linking in microglia, necessary for phagocytosis (Ohsawa *et al.*, 2000; Sasaki *et al.*, 2001; Kanazawa *et al.*, 2002). The Fcγ receptors expressed by microglia are important players in phagocytosis, transport and clearance of antibodies and immune complexes from the brain, and antibody-dependent cell-mediated cytotoxicity (Ulvestad *et al.*, 1994*a*; Deane *et al.*, 2009). The ability of anti-amyloid-β antibodies to cross the blood–brain barrier has been reported in animal models (Bard *et al.*, 2000; Deane *et al.*, 2005). Anti-amyloid-β autoantibodies were detected in the plasma of patients with Alzheimer's disease (Hyman *et al.*, 2001), consistent with the detection of Fcγ receptors in the non-immunized Alzheimer's disease brains. One explanation for the inverse correlations between amyloid-β and the microglial Iba-1 and Fcγ receptor II may be that in Alzheimer's disease, the expression of these receptors limits the accumulation of amyloid. Alternatively, the inverse relationship could reflect a mechanism whereby amyloid-β aggregation and, presumably, amyloid-β immune complexes, downregulate microglia as a means of minimizing neuronal damage in the context of a prolonged inflammatory reaction. It was suggested that activation of Fcγ receptors leads to increased levels of intracellular Ca^{2+} (Ravetch and Kinet, 1991), possibly indicating a link with the calcium adapter protein Iba-1.

Immunization alters the relationship between amyloid-β_{42} and these microglial receptors. This may contribute to a change in microglial function that facilitates amyloid-β clearance, as suggested by the correlation between the cell surface scavenger receptor MSR-A-positive clusters and amyloid-β_{42} in immunized Alzheimer's disease cases. MSR-A was reported to be involved in the activation of microglia for uptake of extracellular matter, including amyloid (Christie *et al.*, 1996). We observed no relationship between microglia and vascular amyloid-β (cerebral amyloid

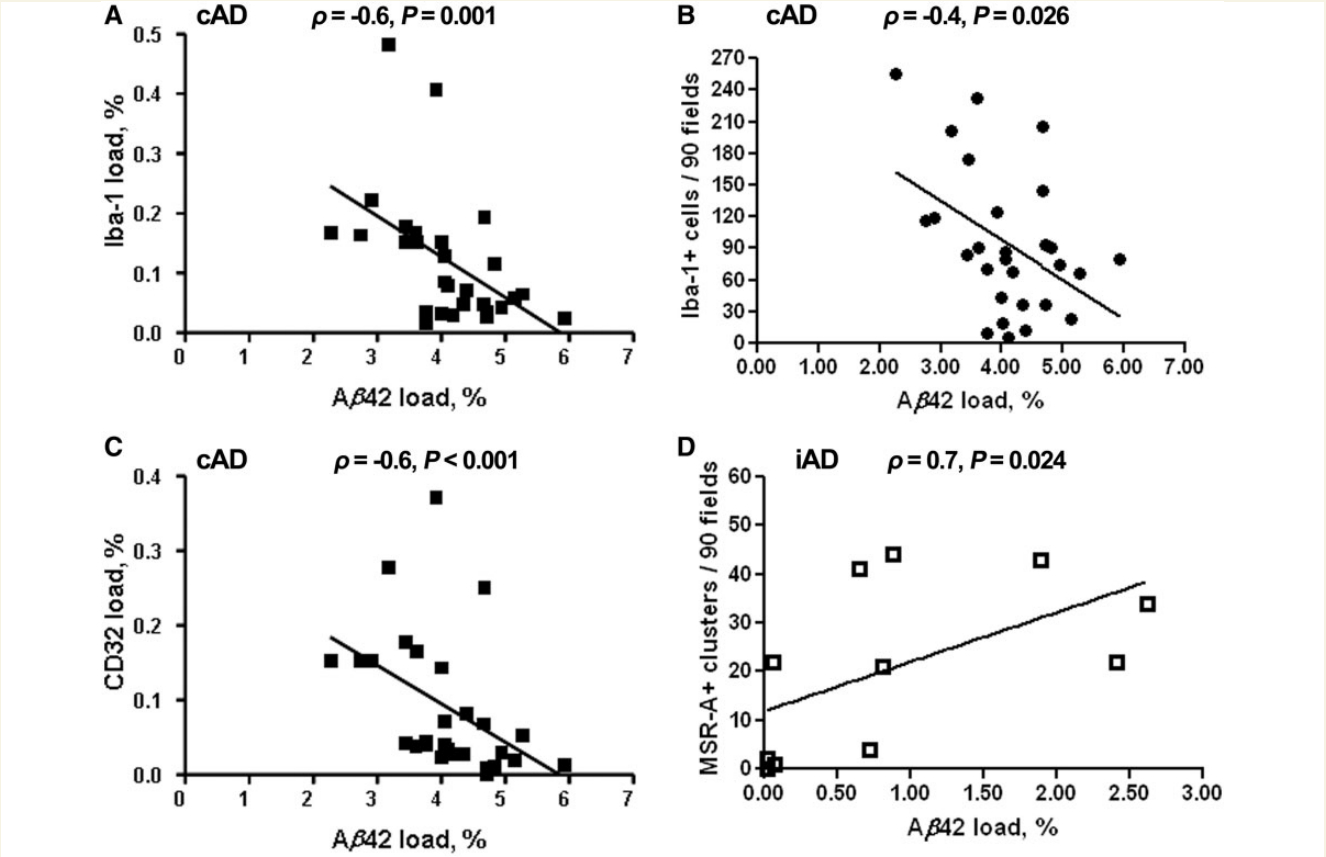

Figure 5 Correlation plots between microglial and amyloid-β_{42} (21F12) within non-immunized control (cAD) (**A–C**) and immunized Alzheimer's disease (iAD) groups (**D**) with linear regression lines and Spearman's ρ and *P*-values included. Within the non-immunized control group, the amyloid-β_{42} load inversely correlated with Iba-1 load (**A**), the number of Iba-1-positive cells (**B**), and CD32 (Fcγ RII) load (**C**). Within the immunized Alzheimer's disease group, the amyloid-β_{42} load correlated with the number of MSR-A-positive microglial clusters (**D**).

angiopathy) in Alzheimer's disease brains. Even after the immunization, which results in a transient increase in cerebral amyloid angiopathy (Boche *et al.*, 2008), no association between microglial markers and the severity of cerebral amyloid angiopathy was noted. Similarly, microglial activity was not related to the number of microhaemorrhages and microvascular lesions, although the lack of apparent relationship may simply reflect the relatively infrequent occurrence of these lesions in the present cohort.

Correlation between tau and microglial markers

In the non-immunized Alzheimer's disease cases, phospho-tau load, but not amyloid-β, correlated with the levels of all of the microglial markers assessed. There is evidence for a connection between microglial activation and tau pathology in Alzheimer's disease and other tauopathies (DiPatre and Gelman, 1997; Ishizawa and Dickson, 2001; Streit *et al.*, 2009; Serrano-Pozo *et al.*, 2011). Chronic microglial activation resulting in the release of pro-inflammatory cytokines (e.g. IL-1α, IL-1β and TNFα) and other molecules (e.g. quinolinic acid) may cause tau hyperphosphorylation (Iqbal and Grundke-Iqbal, 2002; Li *et al.*, 2003), suggesting that microglia may contribute to the ongoing neurodegeneration (Serrano-Pozo *et al.*, 2011). Conversely, microglial activity may be a response to the synaptic loss associated with tau pathology (Iqbal and Grundke-Iqbal, 2002), supporting the concept that microglia are losing their protective capacities with ageing (Streit *et al.*, 2009).

Our observations of correlations between microglia and tau, and between microglia and amyloid-β, but not between tau and amyloid-β in Alzheimer's disease, suggest that microglia may play a pivotal role in Alzheimer's disease. We did not observe any relation between the tau pathology and microglial markers in the immunized Alzheimer's disease group, in keeping with a change in the microglial profile after immunization focusing on amyloid-β clearance (Zotova *et al.*, 2011). The exact pathway and sequence of events that involve microglia, amyloid-β and phospho-tau, in the pathogenesis of Alzheimer's disease are still not deciphered. Microglial activation may represent a response that is aimed at limiting amyloid-β deposition (inverse correlation between some microglial markers and amyloid-β_{42}), as observed by *in vivo* imaging of transgenic mice (Bolmont *et al.*, 2008) but that causes secondary neuronal damage (direct correlation between all microglial markers and phospho-tau). Alternatively, microglial activation may be triggered by the neuronal damage in Alzheimer's disease,

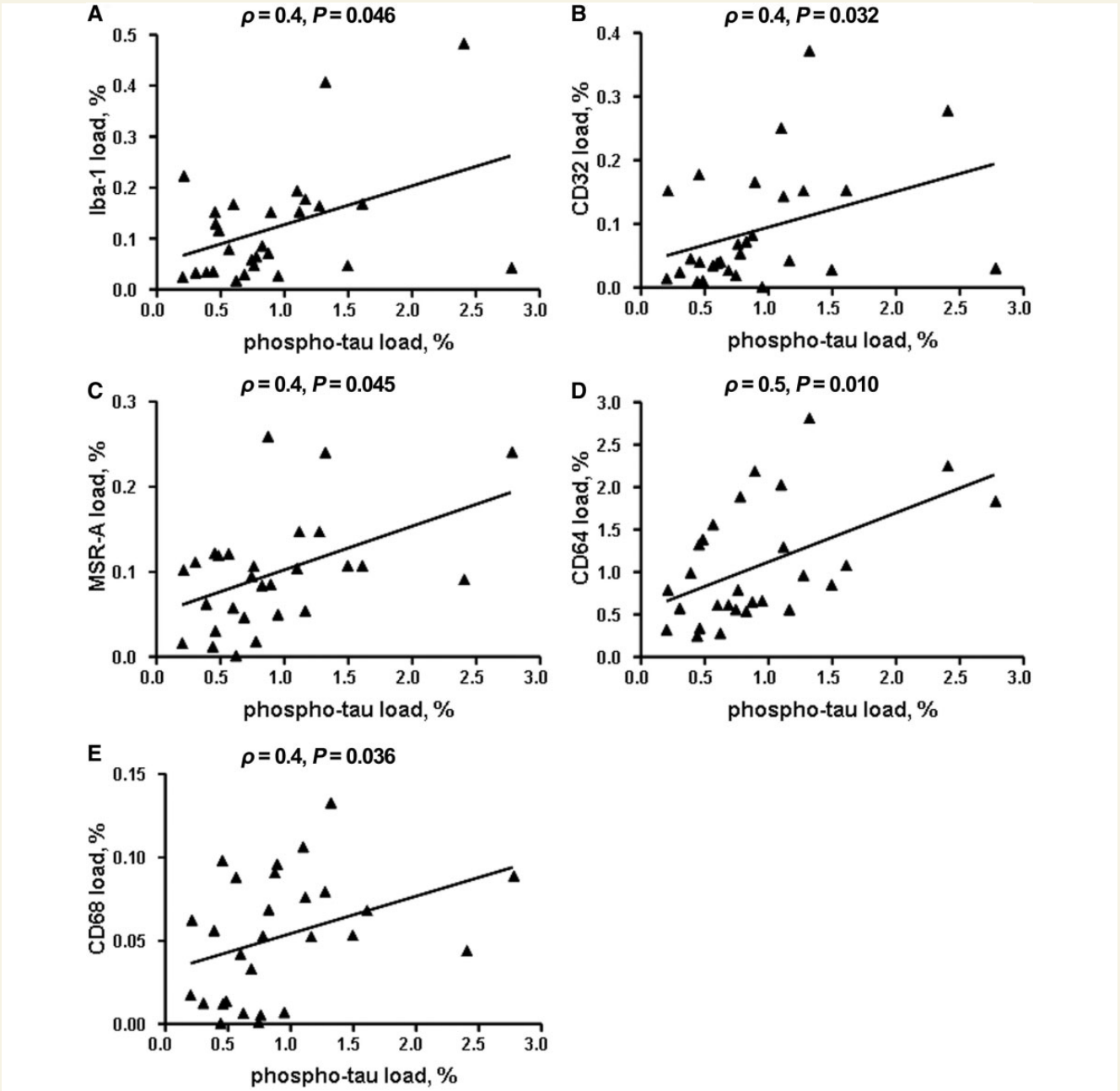

Figure 6 Correlation plots between microglial and phospho-tau (AT8) within the non-immunized control group with linear regression lines and Spearman's ρ and *P*-values included. Phospho-tau load correlated with Iba-1 load (**A**), CD32 (Fcγ RII) load (**B**), MSR-A load (**C**), CD64 (Fcγ RI) load (**D**), and CD68 load (**E**).

with amyloid-β deposition serving as an attempt to control the inflammation (Soscia *et al.*, 2010). The possibility that plaque-associated amyloid-β is not directly involved in neurodegeneration in sporadic Alzheimer's disease is consistent with our observations of continued decline in cognitive function, despite the removal of amyloid-β plaques by immunotherapy (Holmes *et al.*, 2008).

Immunoglobulin G

We quantified IgG to assess the extent of opsonization of amyloid-β plaques before and after immunization, but found no significant difference in total load of IgG within the cortex. This is despite evidence that anti-amyloid-β antibodies persist for many years in the blood of patients immunized with AN1972 (Holmes *et al.*, 2008). It should be noted that the immunohistochemistry did not differentiate between host-generated antibodies of different specificities (e.g. anti-amyloid-β) that might be present in the brain. It is possible that an increase in amyloid-β-specific antibody level and immune complex formation within the brain takes place in Alzheimer's disease brain immediately after the treatment, as observed in animal studies using passive immunization (Bard *et al.*, 2000). If so, our observations of lower levels of Fcγ receptors I

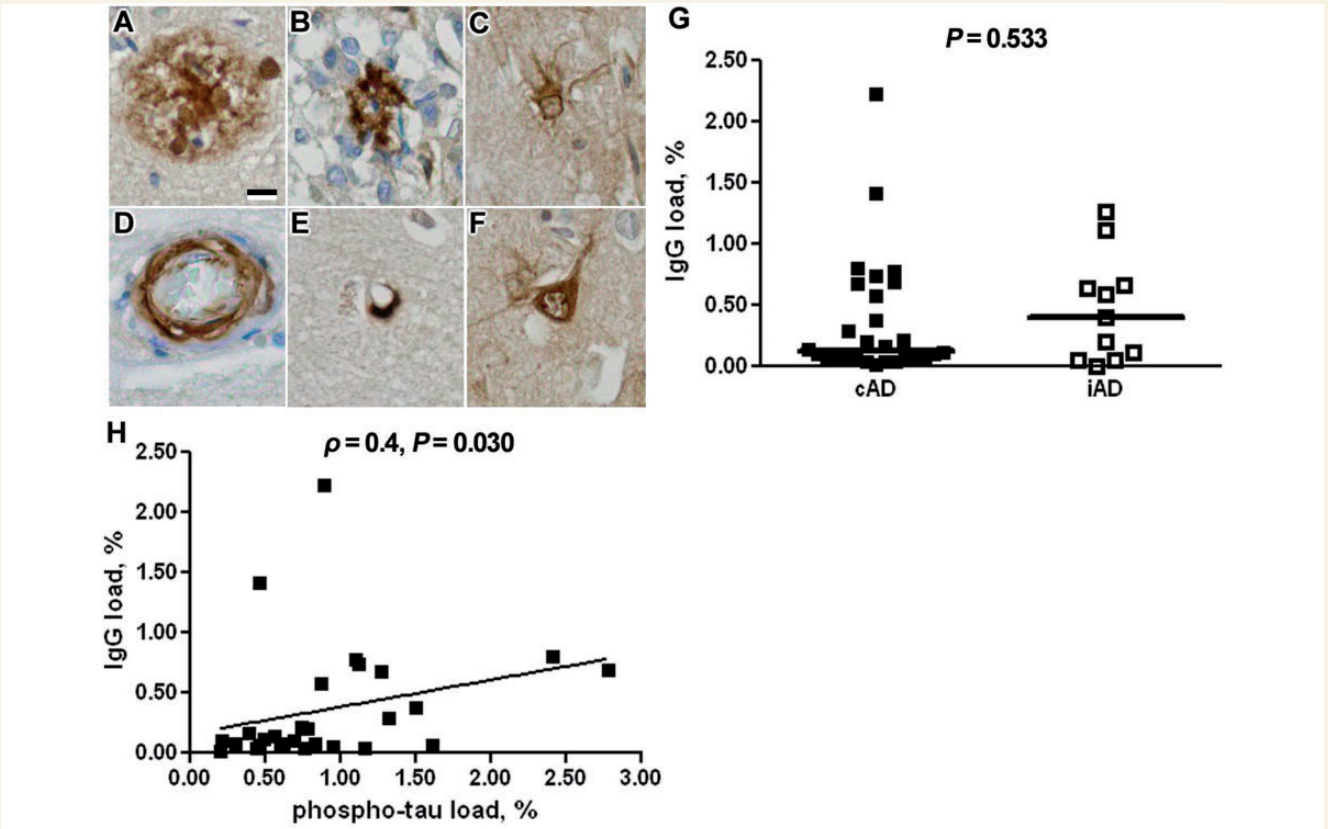

Figure 7 Illustrations of IgG immunostaining: amyloid-β plaque (**A**); amyloid-β core (**B**); glial cell (**C**); IgG within blood vessel wall (**D**) capillary angiopathy (**E**); and neuron (**F**). Scale bar = 10 μm. (**G**) Quantification results of IgG load in non-immunized controls and immunized Alzheimer's disease cases expressed as scatter plot with lines indicating median values, and Mann-Whitney *P*-values included. (**H**) Correlation plot between the IgG load and phospho-tau (AT8) load within the non-immunized control group with linear regression line and Spearman's ρ and *P*-values included. cAD = non-immunized Alzheimer's disease controls; iAD = immunized Alzheimer's disease cases.

and II in immunized cases by the time of autopsy suggest that the deployment of these receptors for the clearance of amyloid-β is followed by their downregulation.

There was a correlation between IgG and phospho-tau within the non-immunized but not the immunized Alzheimer's disease group. A link has been proposed between changes in brain–blood barrier permeability in Alzheimer's disease and the influx and localization of IgG within neurons, leading to tau-mediated reorganization of neuronal microtubules (Bouras *et al.*, 2005). Our findings would be consistent with the presence of IgG in the brain influencing the extent of the tau pathology. This is in keeping with the strong correlations between the amount of IgG and the levels of all microglial receptors, and between these receptors and phospho-tau, as discussed above. Similarly, T cell numbers correlated with the IgG load and phospho-tau pathology in the non-immunized Alzheimer's disease group, supporting the involvement of humoral immunity, in addition to cellular immunity in the pathogenesis of Alzheimer's disease (Bouras *et al.*, 2005). Immunization against amyloid-β_{42} seems to alter these relationships, perhaps reflecting alteration in the type of IgG present in the brain following the treatment. However, the relationship between T cells and IgG was still present in the immunized cases and at a stronger level. Consistent with this observation, the ability of microglia-mediated antibody-dependent phagocytosis involving Fcγ receptors to stimulate T cells has been demonstrated *in vitro* (Ulvestad *et al.*, 1994*b*).

C1q

Although no changes were observed in the total C1q load between both groups, the differences in correlations between C1q and microglial markers, and C1q and amyloid-β_{42}/tau support a role for C1q in Alzheimer's disease pathogenesis and immunization-related changes. Consistent with the recent identification of complement gene variation as risk factor for Alzheimer's disease (Lambert *et al.*, 2009), and the suggested ability of C1q to activate microglial cells (Veerhuis *et al.*, 2003), C1q correlated with all microglial markers in the non-immunized Alzheimer's disease group. The findings are also in accordance with the *in vitro* demonstrations of microglial C1q-mediated Fc receptor-dependent phagocytosis of immune complexes (Webster *et al.*, 2000, 2001). In the non-immunized group there was also a relationship between C1q and phospho-tau, supporting a view that C1q expression is related to the degeneration of neurons in Alzheimer's disease (Fonseca *et al.*, 2004; D'Andrea, 2005). The ability of tau to activate complement through C1q was previously demonstrated *in vitro* and *in situ* (Shen *et al.*, 2001). In the immunized group, this relationship was no longer present. Instead, an inverse

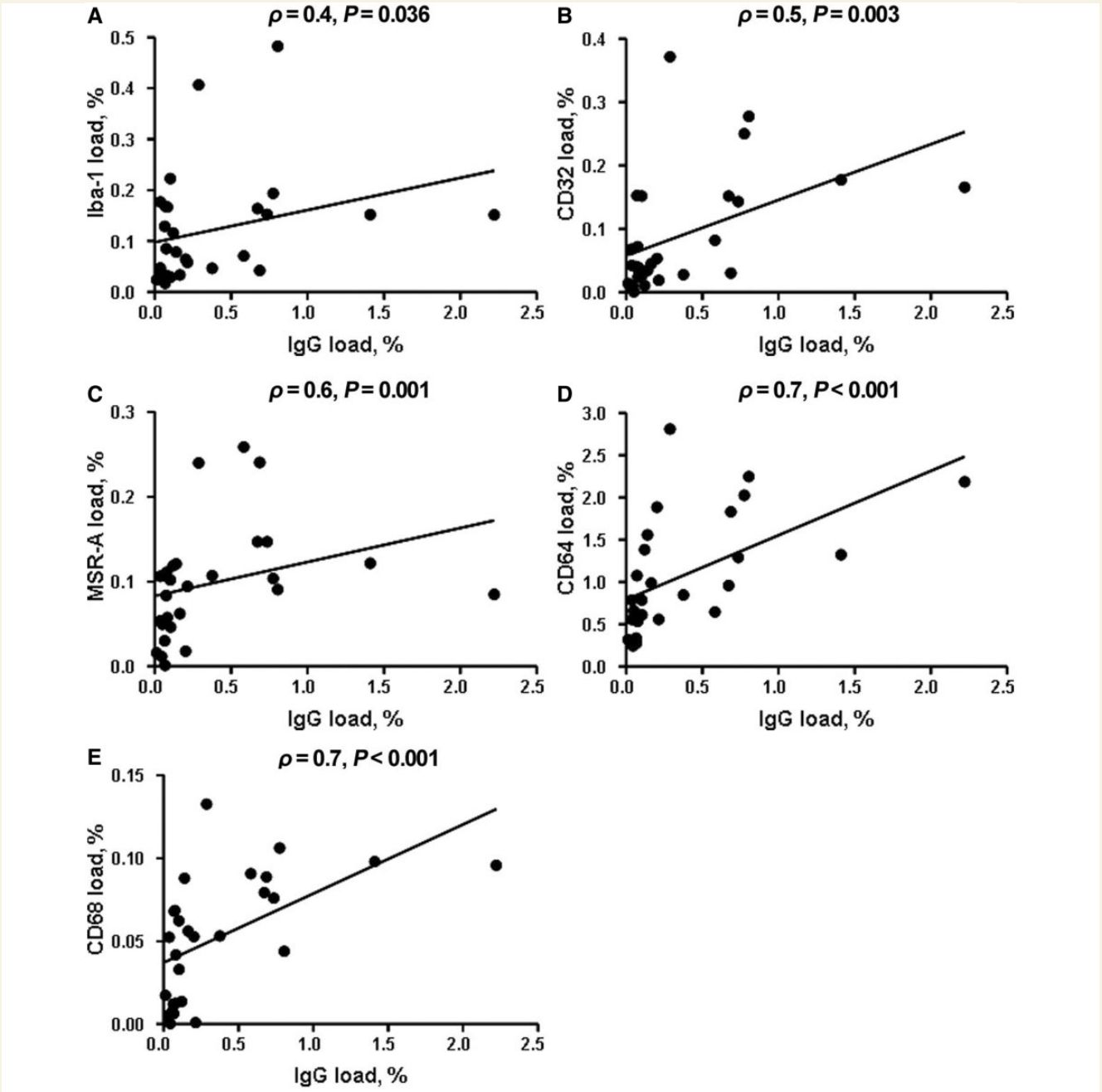

Figure 8 Correlation between microglial markers and IgG load in non-immunized controls with linear regression lines and Spearman's ρ and *P*-values included. Correlations were observed between the IgG load and Iba-1 load (**A**), CD32 (Fcγ RII) load (**B**), MSR-A load (**C**), CD64 (Fcγ RI) load (**D**), and CD68 load (**E**).

relationship between amyloid-β_{42} and C1q was observed, suggesting a different role for C1q after immunization, perhaps related to the clearance of amyloid-β, as increased uptake of C1q-coated fibrillar amyloid-β particles and immune complexes by rodent microglia *in vitro* has been reported (Brazil *et al.*, 2000; Webster *et al.*, 2001). Overall, our observations indicate that C1q is likely to be a mediator in the microglia-amyloid-β-tau network in Alzheimer's disease and that its role in this network is altered by immunization.

Effect of immunization on microglial receptors, cell number and distribution

Human data on the detailed microglial profile in chronic neurodegeneration and in response to stimulation of the immune system are scarce. Animal studies suggest that induction of an immune response in the brain leads to acutely elevated levels of microglial receptors (Herber *et al.*, 2006, 2007, Malm *et al.*, 2008, Ryu *et al.*, 2009), and alterations in an inflammatory gene profile

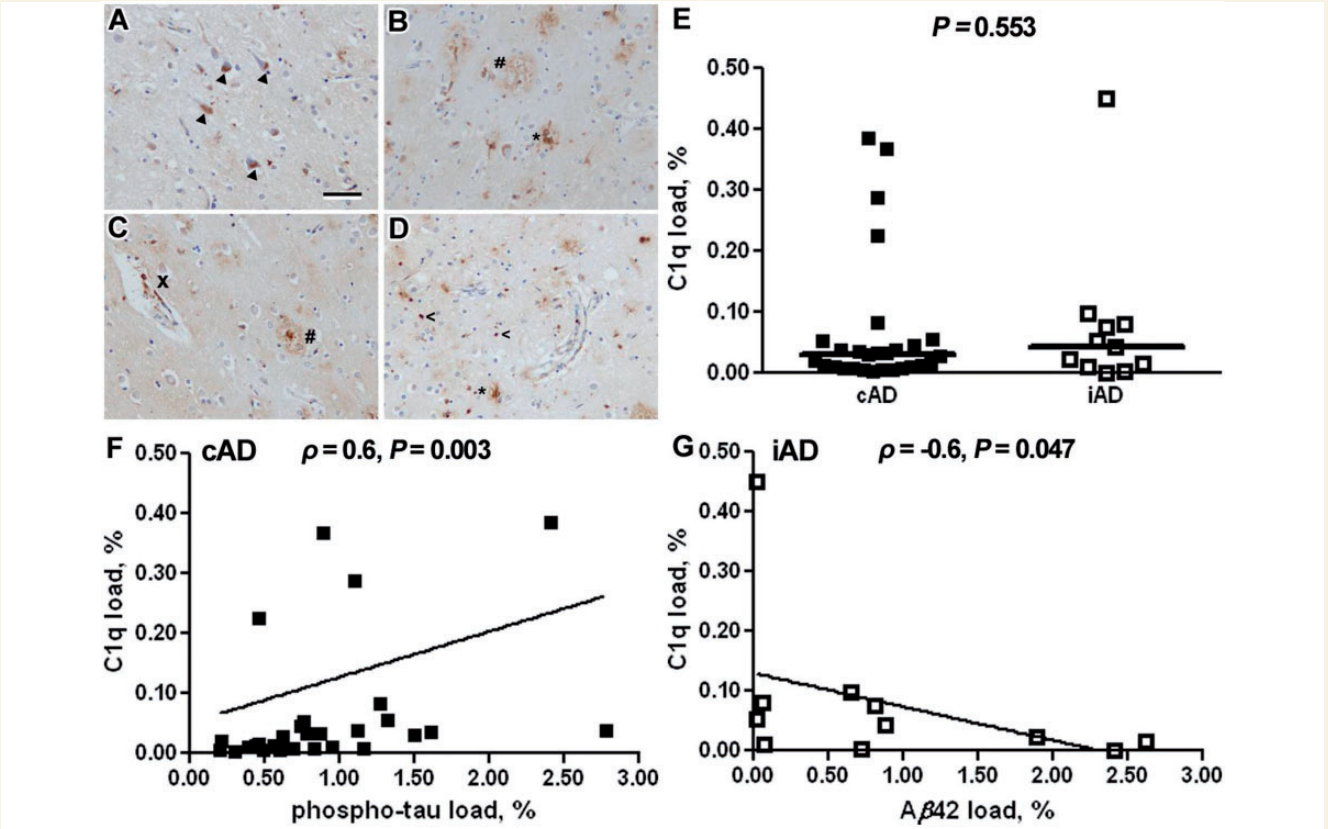

Figure 9 Illustrations of C1q immunostaining demonstrating positively stained neurons (filled triangle, **A**), amyloid-β plaques (hash symbol, **B** and **C**); glial cell (asterisk, **B** and **D**), blood vessels (×, **C**), and other cells (possibly astrocytes, <, **D**). Scale bar = 50 μm. (**E**) Quantification results of C1q load in non-immunized controls and immunized Alzheimer's disease cases expressed as scatter plot with lines indicating median values, and Mann-Whitney *P*-values included. (**F**) Correlation plot between the C1q load and phospho-tau (AT8) load within the non-immunized control group with linear regression line and Spearman's ρ and *P*-values included. (**G**) Correlation plot between the C1q load and amyloid-β_{42} load within the immunized Alzheimer's disease group with linear regression line and Spearman's ρ and *P*-values included.

towards classical activation associated with reduced brain amyloid-β levels (Wilcock *et al.*, 2011).

Our quantitative analysis of the microglial markers MSR-A, CD64, CD32 and CD68 revealed significantly lower levels of these receptors in immunized Alzheimer's disease cases, perhaps surprisingly suggesting downregulation of microglial activity after immunization, at least by the time of post-mortem examination, which in almost all cases was several years after treatment. In the immunized group, we observed an 89% lower load of the scavenger receptor MSR-A and a 68% lower number of MSR-A-positive clusters, consistent with the degree of plaque removal seen in these cases. The Fcγ receptors I and II, expected to be involved in the CNS innate immune response to immunotherapy, were 56% and 66% lower after immunization. The marker of intracellular microglial lysosomes, CD68, was also 60% lower in the immunized group. Although in our previous study we reported a higher load of CD68 in immunized cases (Zotova *et al.*, 2011), the current non-immunized Alzheimer's disease cohort is larger and better matched for age, gender, duration of dementia and *APOE* genotype to the immunized group, which includes some additional cases with longer post-immunization times. Our current finding of an overall lower CD68 load in the immunized cases is in accordance with our findings for all other microglial markers, and highlights the necessity of using large and well-matched control cohorts for studies of human tissue (Boche *et al.*, 2013). It should be noted that the findings on analysis of post-mortem tissue several years following the immunization may not necessarily reflect those immediately after receiving the treatment, and instead represent the late effects after plaque removal has occurred. Although all of the markers of microglial activation were lower after immunization, we detected no difference in Iba-1 load or the number of Iba-1-positive cells between the groups. Iba-1 antibody is reported to label all microglia, whether resting or active (Streit *et al.*, 2009). In accordance with this we expected, but did not observe, a higher Iba-1 load than that of the other microglial markers. Simard *et al.* (2006) showed that blood-derived monocytes can be recruited into the brain of transgenic mice and differentiate into microglia-like cells, which are more efficient at clearing amyloid-β plaques. The lack of increase in total microglia number after the immunization provides no evidence for immunization-induced recruitment of monocytes from the periphery or *in situ* proliferation of microglia.

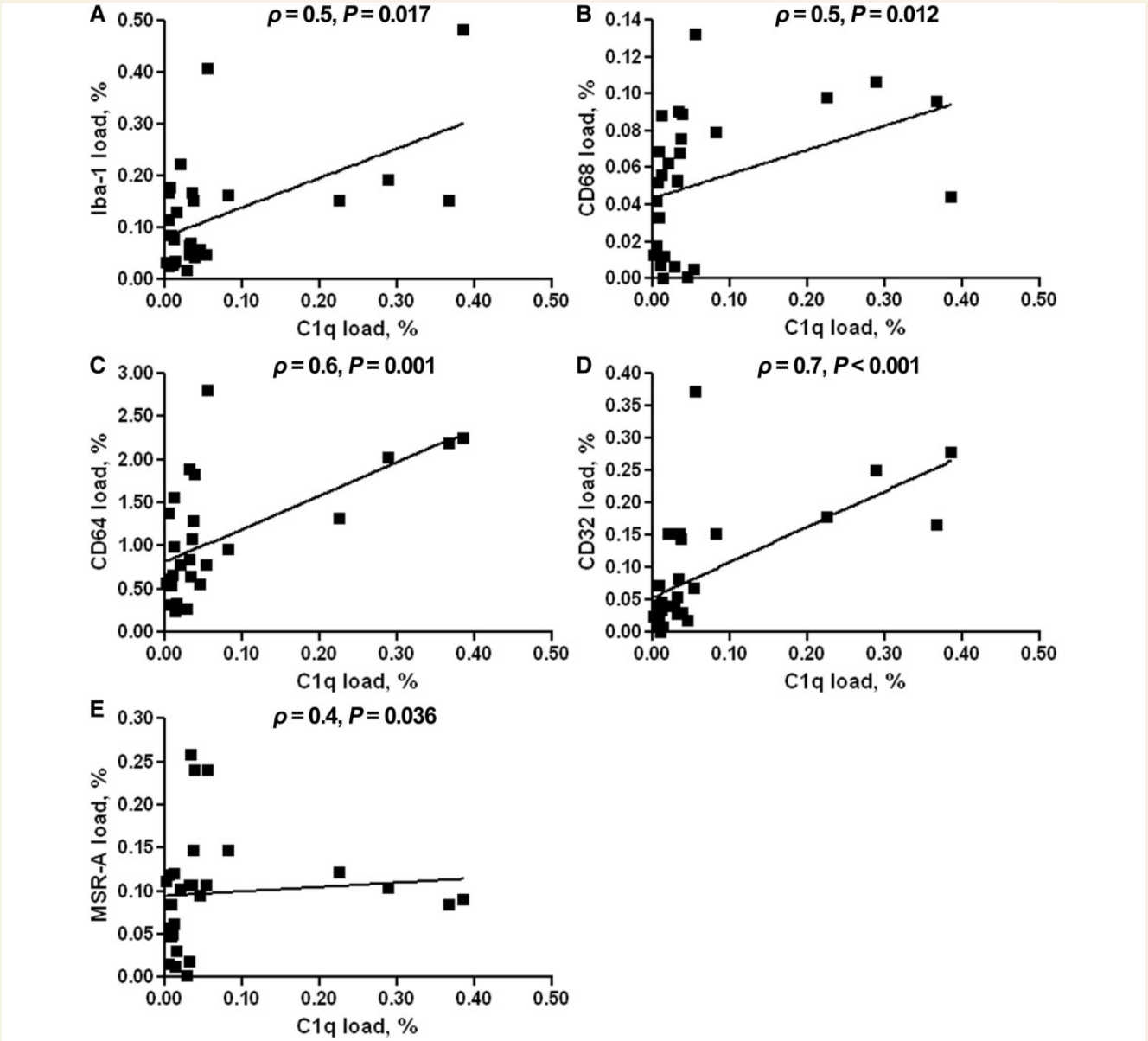

Figure 10 Correlation between microglial markers and C1q load in non-immunized controls with linear regression lines and Spearman's ρ and *P*-values included. Correlations were observed between the C1q load and Iba-1 load (**A**), CD68 load (**B**), CD64 (Fcγ RI) load (**C**), CD32 (Fcγ RII) load (**D**), and MSR-A load (**E**).

Our observation of a strong clustering pattern of MSR-A-positive microglia is in accordance with other studies that looked at expression of macrophage scavenger receptors in Alzheimer's disease and non-demented brains (Christie *et al.*, 1996; Honda *et al.*, 1998) suggesting that these clusters are related to amyloid-β plaques and consistent with the function of MSR-A (Chung *et al.*, 2001). We previously reported clustering and co-localization of microglial markers HLA-DR and CD68 around amyloid-β plaques (Zotova *et al.*, 2011). We did not observe any obvious amyloid-β-related clustering of cells expressing Fcγ receptors, in keeping with a previous report that these cells are distributed throughout the cortex of normal and Alzheimer's disease brains (Peress *et al.*, 1993). The variability in the distribution of microglial markers that we observed in human disease suggest that microglia may be present in different functional states in the same brain in Alzheimer's disease, with further alterations in microglial activation and function following amyloid-β immunization. The distinct distribution of MSR-A immunostaining may be explained by the selective immobilization of the MSR-A-positive cells when they encounter plaques. Indeed, *in vitro* studies using murine microglial cells showed that the murine homologue of MSR-A was the first receptor to engage in phagocytosis of amyloid-β fibrils (El Khoury *et al.*, 1996; Paresce *et al.*, 1996), resulting in immobilization of microglia and release of neurotoxic cytokines and reactive oxygen species (El Khoury *et al.*, 1996; Husemann *et al.*, 2002). Similar observations were made on cultured human microglia from

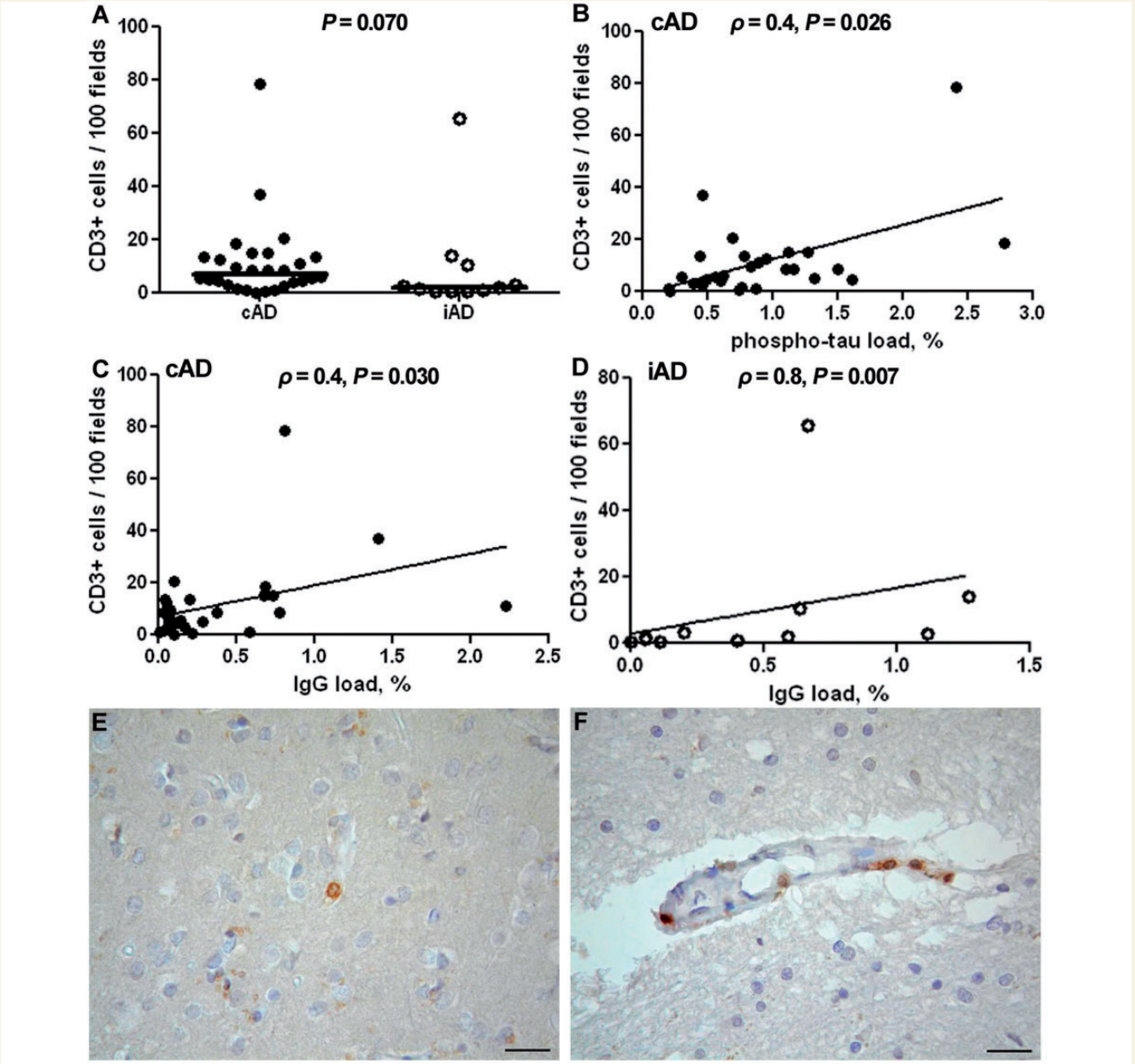

Figure 11 Quantification results of CD3-positive T cells in non-immunized controls and immunized Alzheimer's disease cases expressed as scatter plot with lines indicating median values, and Mann-Whitney *P*-values included (**A**). Correlation plots between the number of CD3-positive T cells and the phospho-tau (AT8) load within the non-immunized control group with linear regression line and Spearman's ρ and *P*-values included (**B**). Correlation plots between the number of CD3-positive T cells and the IgG load within the non-immunized control group (**C**) and the immunized Alzheimer's disease group (**D**) with linear regression line and Spearman's ρ and *P*-values included. Examples of CD3 immunostaining in parenchyma (**E**) and perivascular (**F**). Scale bar = 30 μm.

Alzheimer's disease and non-demented cases (Lue and Walker, 2002).

Together, our data on the levels of microglial receptors involved in antigen recognition and uptake suggest that, in the long term, immunotherapy has a downregulating effect on the expression of these receptors, while keeping the overall number of microglia unaltered. These findings are counter to the observations in animal models, challenging the fidelity of animal models as mimics of the complexity of the human disease. Importantly, the time-course also differed: in the experimental models the response was typically studied after a matter of days or few months, whereas we are investigating the brains of patients with Alzheimer's disease almost all of whom were studied several years following immunization. However, individual data on microglial markers from the immunized Alzheimer's disease cases examined shortly after the immunization (e.g. Patient iAD2, 4 months following the treatment), was similar to the rest of the cases within the immunized Alzheimer's disease group, despite signs of

active clearance of amyloid-β plaques (Nicoll *et al.*, 2006; Zotova *et al.*, 2011). In addition, no correlations were observed between the inflammatory markers and survival time post-immunization.

The mechanisms of amyloid-β removal may vary according to the different treatment protocols in clinical trials, of which there are currently >40 in progress worldwide (Menendez-Gonzalez *et al.*, 2011). We previously found elevated microglial phagocytosis in patients with Alzheimer's disease (Zotova *et al.*, 2011), with evidence of amyloid-β within microglia (Nicoll *et al.*, 2006), but also elevated soluble amyloid-β (Maarouf *et al.*, 2010) and increased severity of cerebral amyloid angiopathy, interpreted as a reflection of enhanced exit of soluble amyloid-β from the brain by the perivascular pathway (Boche *et al.*, 2008). Although based on few cases, the data suggested that, once the brain was cleared of amyloid-β plaques, all these features fell below the level seen in non-immunized Alzheimer's disease brain. Our current findings further confirm that microglial markers of activation and other inflammatory processes are all reduced following the immunization.

Conclusion

Overall, our data reveal antigenically and probably functionally different populations of microglia in the Alzheimer's disease brain: some are predominantly located in and around plaques (e.g. MSR-A-positive microglia) and therefore presumably responding in a specific way to plaque components, whereas others are diffusely distributed through the cortex, perhaps responding or contributing to neuronal damage. This reinforces the concept of specific phenotypes of microglia, with different functions as defined in animal studies (Perry *et al.*, 2010), and confirms its relevance to human neurodegenerative disease. Interestingly, for the first time in Alzheimer's disease, a link was observed between all the inflammatory processes (microglia, IgG and C1q) and tau, supporting an active role for immune functions in the pathogenesis of Alzheimer's disease. The recent genetic data to emerge from the genome-wide association studies indicates that at least a component of the inflammation contributes towards disease pathogenesis and may be amenable to early intervention.

Our findings provide no evidence of a broad-spectrum long-term increase in microglial activation after immunotherapy despite the enhanced microglial phagocytosis of plaque-associated amyloid-β. Instead, we suggest that the microglial downregulation is likely to minimize bystander damage at least in the longer term. These observations highlight the complex nature of microglial reactions and the importance of attempting to assess not just the presence but also the functional state of microglia. The details revealed in this study highlight the importance of conducting neuropathological follow-up as part of clinical trials of new therapies in Alzheimer's disease.

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References

Ahmed Z, Shaw G, Sharma VP, Yang C, McGowan E, Dickson DW. Actin-binding proteins coronin-1a and IBA-1 are effective microglial markers for immunohistochemistry. J Histochem Cytochem 2007; 55: 687–700.

Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, et al. Inflammation and Alzheimer's disease. Neurobiol Aging 2000; 21: 383–421.

Albanese E, Banerjee S, Dhanasiri S, Fernandez J-L, Ferri C, Knapp M, et al. Dementia UK: a report into the prevalence and cost of dementia prepared by the Personal Social Services Research Unit (PSSRU) at the London School of Economics and the Institute of Psychiatry at King's College London, for the Alzheimer's Society. Alzheimer's Society; 2007.

Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, et al. Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. Nat Med 2000; 6: 916–9.

Bayer AJ, Bullock R, Jones RW, Wilkinson D, Paterson KR, Jenkins L, et al. Evaluation of the safety and immunogenicity of synthetic Abeta42 (AN1792) in patients with AD. Neurology 2005; 64: 94–101.

Boche D, Donald J, Love S, Harris S, Neal JW, Holmes C, et al. Reduction of aggregated Tau in neuronal processes but not in the cell bodies after Abeta42 immunisation in Alzheimer's disease. Acta Neuropathol 2010; 120: 13–20.

Boche D, Perry VH, Nicoll JA. Activation patterns of microglia and their identification in the human brain. Neuropathol Appl Neurobiol 2013; 39: 3–18.

Boche D, Zotova E, Weller RO, Love S, Neal JW, Pickering RM, et al. Consequence of Abeta immunization on the vasculature of human Alzheimer's disease brain. Brain 2008; 131 (Pt 12): 3299–310.

Bolmont T, Haiss F, Eicke D, Radde R, Mathis CA, Klunk WE, et al. Dynamics of the microglial/amyloid interaction indicate a role in plaque maintenance. J Neurosci 2008; 28: 4283–92.

Bouras C, Riederer BM, Kovari E, Hof PR, Giannakopoulos P. Humoral immunity in brain aging and Alzheimer's disease. Brain Res Brain Res Rev 2005; 48: 477–87.

Brazil MI, Chung H, Maxfield FR. Effects of incorporation of immunoglobulin G and complement component C1q on uptake and degradation of Alzheimer's disease amyloid fibrils by microglia. J Biol Chem 2000; 275: 16941–7.

Cagnin A, Brooks DJ, Kennedy AM, Gunn RN, Myers R, Turkheimer FE, et al. In-vivo measurement of activated microglia in dementia. Lancet 2001; 358: 461–7.

Chalmers K, Wilcock GK, Love S. APOE epsilon 4 influences the pathological phenotype of Alzheimer's disease by favouring cerebrovascular

over parenchymal accumulation of A beta protein. Neuropathol Appl Neurobiol 2003; 29: 231–8.
Christie RH, Freeman M, Hyman BT. Expression of the macrophage scavenger receptor, a multifunctional lipoprotein receptor, in microglia associated with senile plaques in Alzheimer's disease. Am J Pathol 1996; 148: 399–403.
Chung H, Brazil MI, Irizarry MC, Hyman BT, Maxfield FR. Uptake of fibrillar beta-amyloid by microglia isolated from MSR-A (type I and type II) knockout mice. Neuroreport 2001; 12: 1151–4.
D'Andrea MR. Evidence that immunoglobulin-positive neurons in Alzheimer's disease are dying via the classical antibody-dependent complement pathway. Am J Alzheimers Dis Other Demen 2005; 20: 144–50.
Deane R, Bell RD, Sagare A, Zlokovic BV. Clearance of amyloid-beta peptide across the blood-brain barrier: implication for therapies in Alzheimer's disease. CNS Neurol Disord Drug Targets 2009; 8: 16–30.
Deane R, Sagare A, Hamm K, Parisi M, LaRue B, Guo H, et al. IgG-assisted age-dependent clearance of Alzheimer's amyloid beta peptide by the blood-brain barrier neonatal Fc receptor. J Neurosci 2005; 25: 11495–503.
DiPatre PL, Gelman BB. Microglial cell activation in aging and Alzheimer disease: partial linkage with neurofibrillary tangle burden in the hippocampus. J Neuropathol Exp Neurol 1997; 56: 143–9.
Dodart JC, Bales KR, Gannon KS, Greene SJ, DeMattos RB, Mathis C, et al. Immunization reverses memory deficits without reducing brain Abeta burden in Alzheimer's disease model. Nat Neurosci 2002; 5: 452–7.
Edison P, Archer HA, Gerhard A, Hinz R, Pavese N, Turkheimer FE, et al. Microglia, amyloid, and cognition in Alzheimer's disease: an [11C](R)PK11195-PET and [11C]PIB-PET study. Neurobiol Dis 2008; 32: 412–9.
Edison P, Archer HA, Hinz R, Hammers A, Pavese N, Tai YF, et al. Amyloid, hypometabolism, and cognition in Alzheimer disease: an [11C]PIB and [18F]FDG PET study. Neurology 2007; 68: 501–8.
Eikelenboom P, Veerhuis R. The role of complement and activated microglia in the pathogenesis of Alzheimer's disease. Neurobiol Aging 1996; 17: 673–80.
El Khoury J, Hickman SE, Thomas CA, Cao L, Silverstein SC, Loike JD. Scavenger receptor-mediated adhesion of microglia to beta-amyloid fibrils. Nature 1996; 382: 716–9.
Ferrer I, Boada Rovira M, Sanchez Guerra ML, Rey MJ, Costa-Jussa F. Neuropathology and pathogenesis of encephalitis following amyloid-beta immunization in Alzheimer's disease. Brain Pathol 2004; 14: 11–20.
Fonseca MI, Zhou J, Botto M, Tenner AJ. Absence of C1q leads to less neuropathology in transgenic mouse models of Alzheimer's disease. J Neurosci 2004; 24: 6457–65.
Gilman S, Koller M, Black RS, Jenkins L, Griffith SG, Fox NC, et al. Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. Neurology 2005; 64: 1553–62.
Gray EG, Paula-Barbosa M, Roher A. Alzheimer's disease: paired helical filaments and cytomembranes. Neuropathol Appl Neurobiol 1987; 13: 91–110.
Griffin WS, Stanley LC, Ling C, White L, MacLeod V, Perrot LJ, et al. Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. Proc Natl Acad Sci USA 1989; 86: 7611–5.
Guerreiro R, Wojtas A, Bras J, Carrasquillo M, Rogaeva E, Majounie E, et al. TREM2 variants in Alzheimer's disease. N Engl J Med 2013; 368: 117–27.
Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science 1992; 256: 184–5.
Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet 2009; 41: 1088–93.
Herber DL, Maloney JL, Roth LM, Freeman MJ, Morgan D, Gordon MN. Diverse microglial responses after intrahippocampal administration of lipopolysaccharide. Glia 2006; 53: 382–91.
Herber DL, Mercer M, Roth LM, Symmonds K, Maloney J, Wilson N, et al. Microglial activation is required for Abeta clearance after intracranial injection of lipopolysaccharide in APP transgenic mice. J Neuroimmune Pharmacol 2007; 2: 222–31.
Hock C, Konietzko U, Streffer JR, Tracy J, Signorell A, Muller-Tillmanns B, et al. Antibodies against beta-amyloid slow cognitive decline in Alzheimer's disease. Neuron 2003; 38: 547–54.
Holmes C, Boche D, Wilkinson D, Yadegarfar G, Hopkins V, Bayer A, et al. Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. Lancet 2008; 372: 216–23.
Honda M, Akiyama H, Yamada Y, Kondo H, Kawabe Y, Takeya M, et al. Immunohistochemical evidence for a macrophage scavenger receptor in Mato cells and reactive microglia of ischemia and Alzheimer's disease. Biochem Biophys Res Commun 1998; 245: 734–40.
Husemann J, Loike JD, Anankov R, Febbraio M, Silverstein SC. Scavenger receptors in neurobiology and neuropathology: their role on microglia and other cells of the nervous system. Glia 2002; 40: 195–205.
Hyman BT, Smith C, Buldyrev I, Whelan C, Brown H, Tang MX, et al. Autoantibodies to amyloid-beta and Alzheimer's disease. Ann Neurol 2001; 49: 808–10.
Hyman BT, Trojanowski JQ. Consensus recommendations for the postmortem diagnosis of Alzheimer disease from the National Institute on Aging and the Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer disease. J Neuropathol Exp Neurol 1997; 56: 1095–7.
Imai Y, Ibata I, Ito D, Ohsawa K, Kohsaka S. A novel gene iba1 in the major histocompatibility complex class III region encoding an EF hand protein expressed in a monocytic lineage. Biochem Biophys Res Commun 1996; 224: 855–62.
Imai Y, Kohsaka S. Intracellular signaling in M-CSF-induced microglia activation: role of Iba1. Glia 2002; 40: 164–74.
Iqbal K, Grundke-Iqbal I. Neurofibrillary pathology leads to synaptic loss and not the other way around in Alzheimer disease. J Alzheimers Dis 2002; 4: 235–8.
Ishizawa K, Dickson DW. Microglial activation parallels system degeneration in progressive supranuclear palsy and corticobasal degeneration. J Neuropathol Exp Neurol 2001; 60: 647–57.
Janus C, Pearson J, McLaurin J, Mathews PM, Jiang Y, Schmidt SD, et al. A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. Nature 2000; 408: 979–82.
Johnson-Wood K, Lee M, Motter R, Hu K, Gordon G, Barbour R, et al. Amyloid precursor protein processing and A beta42 deposition in a transgenic mouse model of Alzheimer disease. Proc Natl Acad Sci USA 1997; 94: 1550–5.
Jonsson T, Stefansson H, Steinberg S, Jonsdottir I, Jonsson PV, Snaedal J, et al. Variant of TREM2 associated with the risk of Alzheimer's disease. N Engl J Med 2013; 368: 107–16.
Kanazawa H, Ohsawa K, Sasaki Y, Kohsaka S, Imai Y. Macrophage/microglia-specific protein Iba1 enhances membrane ruffling and Rac activation via phospholipase C-gamma -dependent pathway. J Biol Chem 2002; 277: 20026–32.
Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet 2009; 41: 1094–9.
Landini G. How to correct background illumination in brightfield microscopy2006-2010. Available from: http://imagejdocutudorlu/dokuphp?id=howto:working:how_to_correct_background_illumination_in_brightfield_microscopy.
Li Y, Liu L, Barger SW, Griffin WS. Interleukin-1 mediates pathological effects of microglia on tau phosphorylation and on synaptophysin synthesis in cortical neurons through a p38-MAPK pathway. J Neurosci 2003; 23: 1605–11.

Lowe J, Mirra SS, Hyman BT, Dickson DW. Ageing and dementia. In: Love S, Louis DN, Ellison DW, editors. Greenfield's neuropathology. 8th edn. London: Edward Arnold (Publishers) Ltd.; 2008. p. 1031–152.
Lue LF, Walker DG. Modeling Alzheimer's disease immune therapy mechanisms: interactions of human postmortem microglia with antibody-opsonized amyloid beta peptide. J Neurosci Res 2002; 70: 599–610.
Maarouf CL, Daugs ID, Kokjohn TA, Kalback WM, Patton RL, Luehrs DC, et al. The biochemical aftermath of anti-amyloid immunotherapy. Mol Neurodegener 2010; 5: 39.
Malm TM, Magga J, Kuh GF, Vatanen T, Koistinaho M, Koistinaho J. Minocycline reduces engraftment and activation of bone marrow-derived cells but sustains their phagocytic activity in a mouse model of Alzheimer's disease. Glia 2008; 56: 1767–79.
Mattiace LA, Davies P, Yen SH, Dickson DW. Microglia in cerebellar plaques in Alzheimer's disease. Acta Neuropathol 1990; 80: 493–8.
McGeer PL, Itagaki S, Tago H, McGeer EG. Reactive microglia in patients with senile dementia of the Alzheimer type are positive for the histocompatibility glycoprotein HLA-DR. Neurosci Lett 1987; 79: 195–200.
Menendez-Gonzalez M, Perez-Pinera P, Martinez-Rivera M, Muniz AL, Vega JA. Immunotherapy for Alzheimer's disease: rational basis in ongoing clinical trials. Curr Pharm Des 2011; 17: 508–20.
Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, et al. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. Nature 2000; 408: 982–5.
Nicoll JA, Barton E, Boche D, Neal JW, Ferrer I, Thompson P, et al. Abeta species removal after abeta42 immunization. J Neuropathol Exp Neurol 2006; 65: 1040–8.
Nicoll JA, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO. Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. Nat Med 2003; 9: 448–52.
Nimmerjahn F, Ravetch JV. Fcgamma receptors: old friends and new family members. Immunity 2006; 24: 19–28.
Ohsawa K, Imai Y, Kanazawa H, Sasaki Y, Kohsaka S. Involvement of Iba1 in membrane ruffling and phagocytosis of macrophages/microglia. J Cell Sci 2000; 113 (Pt 17): 3073–84.
Orgogozo JM, Gilman S, Dartigues JF, Laurent B, Puel M, Kirby LC, et al. Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. Neurology 2003; 61: 46–54.
Overmyer M, Helisalmi S, Soininen H, Laakso M, Riekkinen P Sr, Alafuzoff I. Reactive microglia in aging and dementia: an immunohistochemical study of postmortem human brain tissue. Acta Neuropathol 1999; 97: 383–92.
Paresce DM, Chung H, Maxfield FR. Slow degradation of aggregates of the Alzheimer's disease amyloid beta-protein by microglial cells. J Biol Chem 1997; 272: 29390–7.
Paresce DM, Ghosh RN, Maxfield FR. Microglial cells internalize aggregates of the Alzheimer's disease amyloid beta-protein via a scavenger receptor. Neuron 1996; 17: 553–65.
Peress NS, Fleit HB, Perillo E, Kuljis R, Pezzullo C. Identification of Fc gamma RI, II and III on normal human brain ramified microglia and on microglia in senile plaques in Alzheimer's disease. J Neuroimmunol 1993; 48: 71–9.
Perry VH, Nicoll JA, Holmes C. Microglia in neurodegenerative disease. Nat Rev Neurol 2010; 6: 193–201.
Rabinowitz S, Gordon S. Differential expression of membrane sialoglycoproteins in exudate and resident mouse peritoneal macrophages. J Cell Sci 1989; 93 (Pt 4): 623–30.
Rabinowitz SS, Gordon S. Macrosialin, a macrophage-restricted membrane sialoprotein differentially glycosylated in response to inflammatory stimuli. J Exp Med 1991; 174: 827–36.
Ravetch JV, Bolland S. IgG Fc receptors. Annu Rev Immunol 2001; 19: 275–90.
Ravetch JV, Kinet JP. Fc receptors. Annu Rev Immunol 1991; 9: 457–92.
Rogers J, Luber-Narod J, Styren SD, Civin WH. Expression of immune system-associated antigens by cells of the human central nervous system: relationship to the pathology of Alzheimer's disease. Neurobiol Aging 1988; 9: 339–49.
Rozemuller JM, Eikelenboom P, Pals ST, Stam FC. Microglial cells around amyloid plaques in Alzheimer's disease express leucocyte adhesion molecules of the LFA-1 family. Neurosci Lett 1989; 101: 288–92.
Ryu JK, Cho T, Wang YT, McLarnon JG. Neural progenitor cells attenuate inflammatory reactivity and neuronal loss in an animal model of inflamed AD brain. J Neuroinflammation 2009; 6: 39.
Sasaki Y, Ohsawa K, Kanazawa H, Kohsaka S, Imai Y. Iba1 is an actin-cross-linking protein in macrophages/microglia. Biochem Biophys Res Commun 2001; 286: 292–7.
Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 1999; 400: 173–7.
Serrano-Pozo A, Mielke ML, Gomez-Isla T, Betensky RA, Growdon JH, Frosch MP, et al. Reactive glia not only associates with plaques but also parallels tangles in Alzheimer's disease. Am J Pathol 2011; 179: 1373–84.
Shen Y, Lue L, Yang L, Roher A, Kuo Y, Strohmeyer R, et al. Complement activation by neurofibrillary tangles in Alzheimer's disease. Neurosci Lett 2001; 305: 165–8.
Simard AR, Soulet D, Gowing G, Julien JP, Rivest S. Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. Neuron 2006; 49: 489–502.
Soscia SJ, Kirby JE, Washicosky KJ, Tucker SM, Ingelsson M, Hyman B, et al. The Alzheimer's disease-associated amyloid beta-protein is an antimicrobial peptide. PLoS One 2010; 5: e9505.
Sperling RA, Jack CR Jr, Black SE, Frosch MP, Greenberg SM, Hyman BT, et al. Amyloid-related imaging abnormalities in amyloid-modifying therapeutic trials: recommendations from the Alzheimer's Association Research Roundtable Workgroup. Alzheimers Dement 2011; 7: 367–85.
Streit WJ, Braak H, Xue QS, Bechmann I. Dystrophic (senescent) rather than activated microglial cells are associated with tau pathology and likely precede neurodegeneration in Alzheimer's disease. Acta Neuropathol 2009; 118: 475–85.
Ulvestad E, Williams K, Matre R, Nyland H, Olivier A, Antel J. Fc receptors for IgG on cultured human microglia mediate cytotoxicity and phagocytosis of antibody-coated targets. J Neuropathol Exp Neurol 1994a; 53: 27–36.
Ulvestad E, Williams K, Vedeler C, Antel J, Nyland H, Mork S, et al. Reactive microglia in multiple sclerosis lesions have an increased expression of receptors for the Fc part of IgG. J Neurol Sci 1994b; 121: 125–31.
Veerhuis R, Van Breemen MJ, Hoozemans JM, Morbin M, Ouladhadj J, Tagliavini F, et al. Amyloid beta plaque-associated proteins C1q and SAP enhance the Abeta1-42 peptide-induced cytokine secretion by adult human microglia in vitro. Acta Neuropathol 2003; 105: 135–44.
Webster SD, Galvan MD, Ferran E, Garzon-Rodriguez W, Glabe CG, Tenner AJ. Antibody-mediated phagocytosis of the amyloid beta-peptide in microglia is differentially modulated by C1q. J Immunol 2001; 166: 7496–503.
Webster SD, Park M, Fonseca MI, Tenner AJ. Structural and functional evidence for microglial expression of C1qR(P), the C1q receptor that enhances phagocytosis. J Leukoc Biol 2000; 67: 109–16.
Wilcock DM, DiCarlo G, Henderson D, Jackson J, Clarke K, Ugen KE, et al. Intracranially administered anti-Abeta antibodies reduce beta-amyloid deposition by mechanisms both independent of and associated with microglial activation. J Neurosci 2003; 23: 3745–51.
Wilcock DM, Gordon MN, Ugen KE, Gottschall PE, DiCarlo G, Dickey C, et al. Number of Abeta inoculations in APP + PS1 transgenic mice influences antibody titers, microglial activation, and congophilic plaque levels. DNA Cell Biol 2001; 20: 731–6.
Wilcock DM, Munireddy SK, Rosenthal A, Ugen KE, Gordon MN, Morgan D. Microglial activation facilitates Abeta plaque removal following intracranial anti-Abeta antibody administration. Neurobiol Dis 2004a; 15: 11–20.

Wilcock DM, Rojiani A, Rosenthal A, Levkowitz G, Subbarao S, Alamed J, et al. Passive amyloid immunotherapy clears amyloid and transiently activates microglia in a transgenic mouse model of amyloid deposition. J Neurosci 2004b; 24: 6144–51.

Wilcock DM, Zhao Q, Morgan D, Gordon MN, Everhart A, Wilson JG, et al. Diverse inflammatory responses in transgenic mouse models of Alzheimer's disease and the effect of immunotherapy on these responses. ASN Neuro 2011; 3: 249–58.

Zotova E, Holmes C, Johnston D, Neal JW, Nicoll JA, Boche D. Microglial alterations in human Alzheimer's disease following Abeta42 immunization. Neuropathol Appl Neurobiol 2011; 37: 513–24.

Zotova E, Nicoll JA, Kalaria R, Holmes C, Boche D. Inflammation in Alzheimer's disease: relevance to pathogenesis and therapy. Alzheimers Res Ther 2010; 2: 1.