Original article

Folia Neuropathologica

Expression of immunohistochemical markers on microglia in Creutzfeldt-Jakob disease and Alzheimer's disease: morphometric study and review of the literature

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Folia Neuropathol 2012; 50 (1): 74-84

Abstract

Introduction: *Microglia are the resident immune cells of the CNS. They are involved in the pathogenesis of diverse neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease, prion diseases as well as multiple sclerosis, amyotrophic lateral sclerosis and AIDS dementia complex. Activated microglia up-regulate many surface receptors such as the major histocompatibility complex (MHC) or complement receptors and secrete a variety of soluble biologically active factors, which are either neurotrophic (e.g. Glia-Derived Neurotrophic Factor [GDNF]) or proinflammatory and neurotoxic (e.g. tumour necrosis factor alpha [TNF-α], interleukin 1β [IL-1β], nitric oxide [NO], superoxide, eicosanoids, quinolinic acid).*

Aim: *The aim of this work was to assess differences in the expression of microglial markers (ferritin, CD68, and HLA-DR) between AD and Creutzfeldt-Jakob disease (CJD) brains.*

Material and methods: *Analyses were performed on 65 slices derived from 26 brains [46 CJD (20 brains), 12 AD (4 brains) and 7 controls (2 brains)]. Slices were labelled immunohistochemically using anti-ferritin, anti-HLA-DR and anti-CD68 antibodies. The nonparametric Mann-Whitney U test was used to assess quantitative differences between groups.*

Results: *The expression of microglia markers (HLA-DR and CD68) is more noticeable in CJD than in AD or control brains. There is no difference between AD and controls. The latter statement is only true in the case of using HLA-DR or CD-68 labelling. Furthermore, ferritin is not a recommended marker in this context.*

Conclusions: *CNS inflammation is more prominent in CJD than in AD or controls. The lack of differences between AD and controls may result from a relatively advanced neurodegeneration in AD brains. In late phases of AD, inflammation is no longer present, in contrast to the early stages of the disease.*

Key words: *AD, CJD, microglia, ferritin, CD68, HLADR.*

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Introduction

Microglia are resident immune cells of the central nervous system (CNS) which have the capacity to proliferate and transform into macrophages. They are derived from cells of monocytic lineage that enter the CNS during embryonic and early postnatal periods [12,14,21,48,54,63,80]. Phenotypically, they are ramified cells of "downregulated phenotype" [63] of small cell bodies with numerous slender branching processes. Microglia serve the role of immune surveillance and host defence. They are very sensitive to changes in their microenvironment and they "continually explore and sample the local environment" [63]. In response to neuronal injury or infection, ramified microglia transform into activated states – ameboid microglia [50]. Activated microglia up-regulate many surface receptors such as the major histocompatibility complex (MHC) or complement receptors and secrete a variety of soluble biologically active factors which are either neurotrophic (e.g. Glia-Derived Neurotrophic Factor [GDNF]) or proinflammatory and neurotoxic (e.g. tumour necrosis factor alpha (TNF-α), interleukin 1β (IL-1β), nitric oxide [NO], superoxide, eicosanoids or quinolinic acid) [8,58,80]. Therefore, it is widely accepted that microglia contribute to neurodegeneration in different diseases through the release of a variety of proinflammatory and potentially neurotoxic substances. They are often named as "sensors of pathology" [63].

Del Rio Hortega visualised microglia using the silver carbonate impregnation method. Nowadays, microglia are identified with immunohistochemical methods. The use of lectin immunochemistry greatly enhanced the ease of microglia identification. RCA (*Ricinus communis agglutinin*) and GSA I B4 (isolectin B4 from *Griffonia simplicifolia*) are typically employed for this purpose. Moreover, there are numerous antibodies to be used as immunohistochemical markers of microglia; those predominantly include antibodies against ferritin, CD68 as well as the major histocompatibility complex class II (MHC II) and many others [80].

Alzheimer's disease (AD) is a neurodegenerative disease characterized clinically by progressive cognitive decline and neuropathologically, by loss of neurons, primarily in the hippocampus and neocortical brain regions. Neuropathological diagnosis of AD is based on the presence of amyloid β (Aβ)-positive plaques, and neurofibrillary tangles (NFTs) in age-dependent quantity. Amyloid plaques originate from the extracellular deposition of Aβ which, in consequence, leads to neuronal loss through an unclear mechanism, probably apoptosis and autophagy [51,61,70].

The neuroinflammation hypothesis of AD stemmed from studies demonstrating the clustering of microglial cells within amyloid deposits in the human brain and suggests that the key pathomechanism of AD is the "activation of the microglial cell" [24,26, 33,34,40,61]. According to the neuroinflammatory hypothesis, microglia transform diffuse deposits of Aβ into compact senile (neuritic) plaques.

Transmissible spongiform encephalopathies (TSEs), also known as prion diseases [1,50], are fatal neurodegenerative and infectious disorders affecting humans (e.g. Creutzfeldt-Jakob disease [CJD], kuru and Gerstmann-Sträussler-Scheinker disease [GSS]) and animals (sheep scrapie, bovine spongiform encephalopathy [BSE], chronic wasting disease [CWD] in cervids and transmissible mink encephalopathy in ranch-reared mink). Neuropathological characteristics of CJD are spongiform change, neuronal vacuolation, astrogliosis, microglial proliferation, neuronal loss, microglial activation and pathological prion protein (PrP^d; d – for "disease") accumulation [3,5,39,53]. At the molecular level, TSEs are characterized by brain accumulation of a misfolded, protease-resistant isoform of the cellular prion protein (PrP^c) [65]. Neuronal loss in CJD is thought to be caused mainly by programmed cell death, including autophagy [28,30,42,51,52]. The time course of PrP^d deposition, appearance of activated microglia, and death of neurons in *animal* models suggest that microglial activation precedes the neuronal loss [4,9, 32,76,78] and microglia may be infected with scrapie [41]. However, the latter statement was challenged by others [64]. Resident microglia in the brain are replaced with the bone marrow microglia [64] and secrete cyto- and chemokines including TNF-α [46,47]. Furthermore, CCR1, CCR5, RANTES and fractalkine are upregulated in scrapie [64]. An earlier paper by Brown *et al*. [13] employing an *in vitro* model (using synthetic prion fragment P106-126) proved that activated microglia are necessary to cause the neuronal loss [13].

Marella and Chabry [55] have shown that microglial recruitment occurs *in vivo* within few days after inoculation with PrP^d-positive material. Such

a microglial recruitment results from a response of neurons and astroglia to TSE infection. PrP^d-stimulated neurons and astrocytes induced chemotaxis by the upregulation of chemokine expression [55].

Aim of the study

The aim of this study was to assess differences in the expression of microglial markers (ferritin, CD68, and HLA DR) between AD and CJD human brains.

Table I. Variation of microglia number analysis with diagnosis as a grouping variable

Labelling	Diagnosis	Number of slices	Mean rank	χ^2	*p*
Ferritin	CJD	38	28.3	1.037	0.595
	AD	12	33.9		
	Control	8	28.6		
HLA-DR	CJD	36	34.3	13.811	0.001
	AD	12	15.1		
	Control	8	22.4		
CD-68	CJD	36	32.4	5.857	0.053
	AD	12	20.8		
	Control	8	22.4		

Table II. Variation of microglia number analysis with place of sampling as a grouping variable

Labelling	Place of sampling	Number of slices	Mean rank	χ^2	*p*
Ferritin	Cerebral cortex	44	30.1	0.223	0.64
	Cerebellar cortex	14	27.6		
HLA-DR	Cerebral cortex	39	28.2	0.029	0.87
	Cerebellar cortex	17	29.1		
CD68	Cerebral cortex	39	29.4	0.423	0.52
	Cerebellar cortex	17	26.4		

Material and methods

The analysis was performed on 68 sections derived from 26 brains [48 CJD (20 brains), 12 AD (4 brains) and 8 controls (2 brains)]. However, the subtyping of sCJD was not performed.

All cases of CJD and AD were diagnosed based on the current neuropathological criteria: CJD – with the use of the immunohistochemical method to detect PrP^d [16], AD – according to the recommendations of experts from the National Institute of Aging (NIA) and the Reagan Institute [38].

Whole brains were fixed in 4% paraformaldehyde buffered to pH 7.4 with 0,1M TBS. Segments were cut into 4-5 µm sections and labelled immunohistochemically using anti-ferritin, anti-HLA DR and anti-CD68 antibodies (DakoCytomation). ChemMate™ Detection Kit, Peroxidase/DAB, Rabbit/Mouse Nr K 5001 were used in the labelling process. For every section, cells were counted under an optical microscope (Olympus BX41TF; magnification 400x). In every case, the counted cells came from the cortex or the cerebellum in 20 random high-power microscopic fields. Variation between the groups has been estimated by Kruskal-Wallis test with Mann-Whitney U test for direct *post hoc* comparisons.

Results

Mean numbers of microglia cells in 20 high power fields in the CJD group amounted to 219; 293.4 and 404.6 for immunohistochemical reactions with ferritin, HLA-DR and CD68, respectively. In the AD group, the corresponding values were 242.6, 86.6 and 244.7; while in the control group 202.4, 129.5 and 261.4. The dispersion of the mean numbers of the observed cells in consecutive labellings (ferritin, HLA-DR, CD68) was in the CJD group 26-810; 4-1414; 42-965, respectively; in the AD group 114-432; 20-308; 84-438 and in control slices 82-350; 56-218; 186-324. The values are visualised in Figs. 1-3 and in Table I.

As the sections were obtained from different brain regions (the cortex and the cerebellum), it was assessed whether the place of sampling (irrespective of the diagnosis) had any effect on the median number of the observed microglia in different labellings. For this purpose, the Kruskal-Wallis test was employed with the place of sampling used as a grouping variable. The results of this analysis are summarized in Table II. None of the three immunohistochemical reactions (ferritin, HLA-DR or CD68)

provided relevant statistical differences in the numbers of microglia depending on the place of sampling, suggesting that there is no difference between the material taken from the cortex and the cerebellum.

Secondly, the subgroups were compared: CJD vs. AD, CJD vs. control group, AD vs. control group. The Mann-Whitney U test – a non-parametric alternative of the Student t test for independent groups – was used.

1. CJD versus the control group

Mean ranks in ferritin labelling were 23.38 for CJD and 24.06 for the control group, in HLA-DR 24.38 and 14.06 and in CD68 24.03 and 15.63, respectively.

Mean ranks vary significantly between the CJD group and the control group when HLA-DR ($p = 0.04$) was used (Fig. 2). A similar difference, however, only at the level of a statistical trend ($p = 0.09$), could be observed for CD68 (Fig. 3). No difference was observed for Ferritin ($p = 0.89$) (Fig. 1).

2. CJD versus AD

Mean ranks in ferritin labelling were 24.42 for the CJD group and 28.92 for AD, in HLA-DR: 28.44 and 12.67, while in CD68: 26.89 and 17.33, respectively.

Mean ranks vary significantly between CJD and AD in HLA-DR ($p = 0.001$) (Fig. 2) and CD-68 labelling ($p = 0.04$) (Fig. 3). However, similarly to the comparison of the CJD and control groups there was no difference when ferritin was used ($p = 0.35$) (Fig. 1).

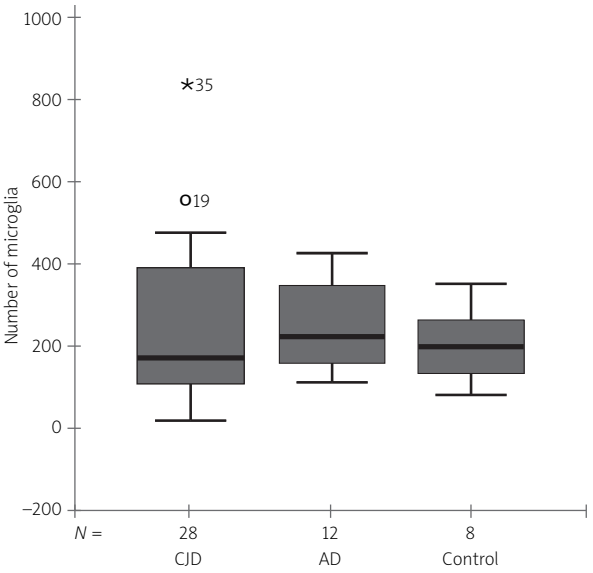
3. AD versus the control group

Mean ranks in ferritin labelling were 11.50 for the AD group and 9.0 for controls, in HLA-DR 8.92 and 12.88; in CD68 labelling 10.00 and 11.25, respectively.

AD and control subgroups showed no difference irrespective of the labelling.

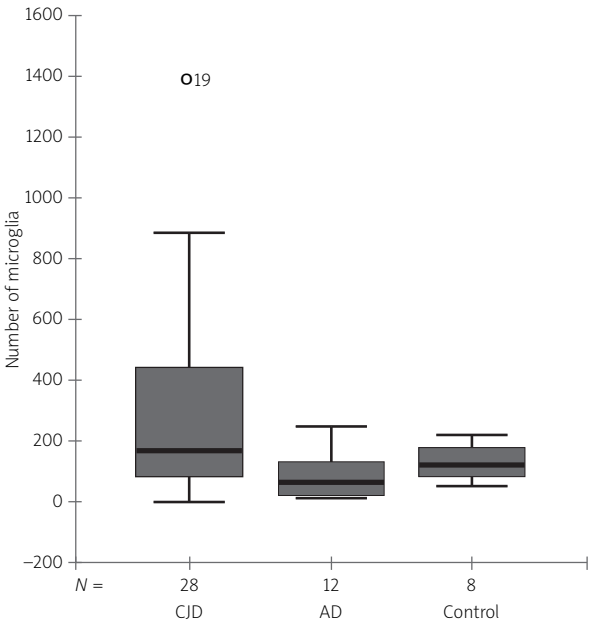
Comparison of the HLA-DR/CD68 ratio between the groups

MHC II antigen expression is enhanced by microglia activation in the cortex [56]. Therefore, HLA-DR



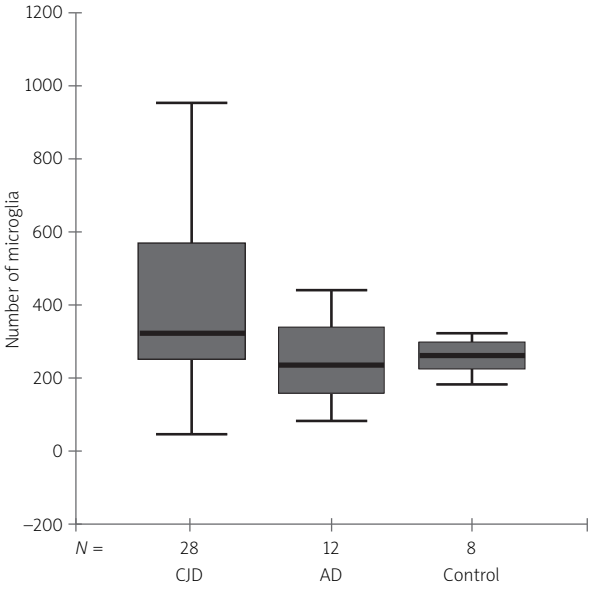
Ferritine	CJD	AD	Control group
CJD	*	$p = 0.931$	$p = 0.896$
AD	$p = 0.931$	*	$p = 0.354$

Fig. 1. A comparison of the mean number of the observed microglia cells in the immunohistochemical reaction to ferritine. Circles and stars have been used to mark cases, which were statistical outliers by means of being out of 5% variation from trimmed mean values.



HLA-DR	CJD	AD	Control group
CJD	*	$p = 0.001$	$p = 0.040$
AD	$p = 0.001$	*	$p = 0.143$

Fig. 2. A comparison of the mean number of the observed microglia cells in the immunohistochemical reaction to HLA-DR.



CD-68	CJD	AD	Control group
CJD	*	$p = 0.041$	$p = 0.094$
AD	$p = 0.041$	*	$p = 0.643$

Fig. 3. A comparison of the mean number of the observed microglia cells in the immunohistochemical reaction to CD-68.

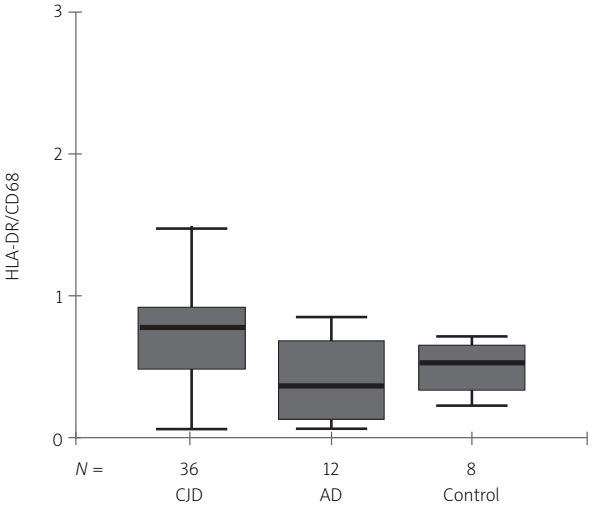


Fig. 4. Dispersion of the HLA-DR/CD68 quotients depending on the diagnosis.

is regarded as a microglia activation marker. Contrary to HLA-DR, the expression of CD68 does not change significantly during microglial activation.

Table III. Mean values of the HLA-DR/CD68 quotient, confidence intervals and median

Diagnosis	Mean value of HLA-DR/CD68 quotient	95% CI	Median
CJD	0.88	0.58-1.20	0.75
AD	0.38	0.20-0.57	0.23
Control	0.49	0.34-0.66	0.52

Therefore, in this technique all microglial cells, both resting and activated, are labelled. Dividing the number of HLA-DR+ cells by the number of CD68+ cells enables assessment of the immunologically activated part of all microglial cells.

We assessed the significance of the HLA-DR/CD68 ratio in different groups. Mean values of HLA-DR/CD68 quotients, confidence intervals and median are shown in Table III.

First, it was checked whether the place of sampling (cerebral cortex or the cerebellum) had any effect on the HLA-DR/CD68 ratio. Kruskal-Wallis test was used for this purpose. No significant influence of sampling on the HLA-DR/CD68 ratio was observed (Kruskal-Wallis test $\chi^2 = 0.00$; df = 1; $p = 0.99$). Therefore, this variable was not taken into consideration in further comparisons.

Next, HLA-DR/CD68 ratios were counted for every subgroup depending on the diagnosis and the non-parametric Mann-Whitney U test was used to compare them. Figure 4 shows the dispersion of HLA-DR/CD68 ratios.

1. CJD versus AD

Mean ranks amounted to 27.58 in CJD and 15.25 in AD. The difference was of statistical significance ($p = 0.008$) meaning that in CJD there is a significantly higher number of HLA-DR positive microglia than in AD.

2. CJD versus the control group

Mean ranks in CJD group were 24.14 and in the control group 15.13. The difference showed a trend for significance ($p = 0.07$). HLA-DR/CD68 ratio is higher in CJD.

3. AD versus the control group

Mean ranks in the AD group were 9.33 and in the control group 12.25. No statistical difference between the groups was observed ($p = 0.28$). AD and control groups have a similar HLA-DR/CD68 ratio.

Discussion

Microglia play an important role both in diverse neurodegenerative disorders and in normal brain aging [71]. Nevertheless, investigators conducting research in the area of microglia biology are relatively scarce.

Data on the topic of the role of microglia in neurodegenerative diseases are not consistent, either. It is clear, however, that microglia activation plays a significant role in AD pathogenesis [24,26] as well as in prion diseases [11].

The main aim of this research was to assess whether the groups of brain sections with the diagnosis of CJD, AD and apparently normal controls differed in the expression of three microglia antigens (ferritin, CD68 and HLA-DR), and if the HLA-DR+/CD68+ ratio could differentiate these groups. The microglia were analysed in the cerebral cortex and the cerebellum.

Ferritin is one of the earliest described immunohistochemical markers of microglia; using anti-ferritin antibodies visualises both activated and resting cells [45]. CD68 is a marker of monocytes; cells are visualised irrespective to their activation [43]. HLA-DR is a marker the expression of which rises during the process of microglia activation [56]. In the natural physiological environment of the CNS, anti-HLA-DR antibodies label mainly the white matter cells; whereas in grey matter, HLA-DR expression may be observed as a consequence of pathological stimuli leading to microglia activation. Such a phenomenon may be explained by astroglial and neuronal inhibition of microglia. The commonly described histochemical reaction with agglutinin I *Ricinus communis* (*RCA I*) was not used in this study. The effectiveness of labelling with this technique changes with the time of fixation of the biological material: the older it is the less consistent the reaction might be. To avoid the influence of another variable, this method was abandoned.

The highest trimmed mean number of microglia was obtained in all three groups with the use of CD68. There are several putative explanations of such result.

1) CD68 antibodies react positively with all cells monocyte lineage irrespective of their current state of activation. While counting the cells, those placed in the proximity to vessels were omitted (to eliminate macrophages and pericytes) minimising the risk of analysing false-positive cells.
2) Anti-ferritin antibodies, although considered to detect one of microglial markers [44], are not specific. Anti-ferritin antibodies also label oligodendroglia, some astroglia and certain neurons as well as vascular wall cells. Only cells meeting morphological criteria of microglia were thus included in the analysis. Therefore, to reduce the risk of false cell identification, a certain fraction of microglia (e.g. morphologically similar to oligodendroglia) might have been omitted. This could explain the lack of major between-group differences with the use of ferritin. This may also explain why the mean microglial number in CD68 labelling was higher than that following ferritin labelling, despite the fact that both reactions label both activated and resting microglia.
3) Anti-HLA-DR antibodies label mainly activated microglia within the cerebral cortex and thus, only a limited proportion of all microglia cells. It is therefore reasonable to expect that the number of these HLA-DR-positive cells will be lower than that revealed by anti-CD68, a non-selective marker for all microglia [68].

As the analysed material was taken from the cortex and the cerebellum, it was necessary to check whether the area of sampling had any effect on the number of cells in the high power field. The statistical analysis excluded such a possibility, therefore this variable was not taken into consideration.

Another observation, covered in our research, is that there is a significantly higher number of microglia (both by anti-CD68 and anti-HLA-DR immunohistochemistry) in the CJD group in comparison to both AD and control groups.

A higher number of microglia in brains of patients with CJD in comparison to controls was described earlier [60,67,75]. Van Everbroeck *et al.* [75] reported the mean number of CD68+ cells 133 ± 49 cells/mm^2 (mean ± SD) in CJD and 64 ± 40 cells/ mm^2 in the control group. In our study, those values were as follows: in the CJD group 85.4 ± 35.8 cells/ mm^2 and in the control group 55.1 ± 10 cells/mm^2. In both papers, a statistically significant between-group difference was reported. The differences in the mean numbers of cells/mm^2 may be secondary to a variation of studied populations (age, e.g. polymorphism in codon 129 of *PRNP*) or different strains of CJD agent, or both.

In vivo research shows that microglia activation precedes neuronal death [27,76,79]. Activated microglia secrete various humoral substances (cytokines, free radicals) which influence neurons and glia [85]. Activation and accumulation of microglia in pathologically changed brains are also characteristic of other neurodegenerative diseases such as AD or Parkinson's disease [34,57,66] and general aging [12].

According to the neuroinflammation hypothesis of AD, the key element underlying pathogenesis of AD is microglia activation [24, rev. 73]. Activated microglia secrete potentially neurotoxic substances such as cytokines, complement proteins, active forms of oxygen and nitrogen or proteolytic enzymes [29,31, 59,66]. The earliest epidemiological studies substantiated this etiological hypothesis. In particular, a chronic usage of non-steroid anti-inflammatory drugs (NSAIDs) seems lowered the risk of developing AD [rev. 2]. However, prospective studies have not substantiated that the use of NSAIDs has any protective effect on AD development.

Another concept in the neuroinflammation hypothesis was the assumption that microglia transform diffuse deposits of Aβ into amyloid (neuritic) plaques [34,40]. In recent years this opinion has been questioned and alternative ways of plaque formation, e.g. the lysis of Aβ-loaded cells (neurons and astroglia) or a vascular origin, have been proposed. [rev. 80]. Of note, no correlation was found *in vivo* between ^{3}H-PIB (Pittsburgh Component B) binding and binding of ^{3}H-PK11195, a marker of activated microglia in the first patient with Alzheimer's disease evaluated by this novel methodology [44].

Many experiments on humans, transgenic mice, and mice vaccinated with Aβ did not substantiate the possibility of Aβ phagocytosis by microglia within plaques and blood vessels. On the other hand, studies on transgenic mice demonstrated that after microglial activation, the CNS amyloid burden was diminished [6,17,23]. In addition, the latter observation of the activation of microglia leading to a reduction of amyloid plaques was confirmed *in vivo* with the use of multiphoton microscope techniques [7]. The latter phenomenon was also reported in AD patients vaccinated with Aβ [62]. The experiments of Wyss-Coray *et al.* [82] also suggested that the activation of microglial phagocytosis is associated with a lower amyloid burden in transgenic mice. An increased expression of transforming growth factor β (TGFβ1) in murine glia was observed. Despite the fact that this cytokine is considered one of the strongest anti-inflammatory substances, its higher secretion reduced the number of Aβ plaques and strengthened the activation of microglia. TGFβ1 improves the production of complement protein C3 which leads to a higher Aβ opsonisation and promotes Aβ phagocytosis. The inhibition of C3 conversion resulted in the lack of Aβ opsonisation and prevented microglia from Aβ clearance and, as a result, doubled the amyloid burden in murine brains [81].

The following may be responsible for the inconsistency in the results of experiments on microglia behaviour in contact with Aβ:

- immunomodulation of astroglia,
- immunomodulation of neurons,
- senescence of microglia.

Microglia activation is followed by astrocyte activation. Astrocytes phagocyte and degrade Aβ. *In vivo* experiments suggest that the coexistence of microglia and astrocytes diminishes the ability of microglia to digest and degrade plaques and Aβ [22,69]. Astrocytes cultured *in vitro* with microglia secrete molecules sensitive to glycosaminoglycans which inhibit microglial Aβ clearance. Moreover, astrocyte-derived IL-4 can also inhibit microglia activation *in vitro* [72]. Therefore, activated astrocytes may exert a regulative effect (negative feedback) on the phagocytic activity of microglia.

Another factor influencing microglia activation is the neuronal expression of cyclooxygenase-2 (COX-2). COX-2 participates in prostaglandin production and its expression is usually elevated in places of inflammation. In AD, initially COX-2 expression is evident in pyramidal neurons particularly involved in AD [10]. COX-2 expression rises at the onset of the disease and then declines in the advanced stages of AD [35, 84]. Of note, the expression of COX-2 correlated positively with the level of prostaglandin E2 (PGE2) in cerebrospinal fluid (CSF). CSF PGE2 levels are clearly higher in people with mild dementia and decrease in the late stages of AD [20].

These changes are consistent with the sequence of events observed by Hoozemans *et al.* [37] in their neuropathological study with an initial microglia activation that correlates well with the Braak's staging up to grades III-IV, followed by a less prominent microglia activation in later stages of the disease. The results of other previously published papers are in line with this report [15,18,19,25,74,77].

The aforementioned studies suggest the fluctuating course of microglia activation in AD, declining with a disease progression [36]. In our study, the AD group did not differ from the control group in relation to the number of microglia either in CD68 or in HLA-DR labelling. The material was derived from patients who died of AD, suggesting they had already progressed to the advanced stages of AD in which neuroinflammation was declining. Of note, Hoozemans *et al.* [36] reported that neuroinflammation, embracing also microglial markers, decline with age. It would be interesting to compare the degree of inflammation observed in our group with that of patients who died in earlier stages of the disease.

In our study, microglia activation was compared between groups with two different methods: as a raw number of cells per mm^2 and as HLA-DR/CD68 quotients providing information about a proportion of activated cells in the whole microglia population. HLA-DR/CD68 ratios amounted to 0.88 for CJD; 0.38 for AD and 0.49 for the control group. Physiological differences between HLA-DR expression in white and grey matter have already been described. In a healthy grey matter, the microglial HLA-DR expression is weak so the HLA-DR/CD68 quotient is low [56]. A slightly higher percentage of HLA-DR+ microglial cells was described in a very specific part of the CNS – normal retina [83] where 56.8% CD68+ cells (microglia) showed a positive reaction with anti-HLA-DR. A lower HLA-DR/CD68 quotient in the control group in our work may result from a different research protocol and from a different CNS sampling (cerebral grey matter and the cerebellum vs. retina), where the inhibiting effect of neurons and astroglia on microglia activation was stronger.

The comparison of HLA-DR/CD68 quotients reveals that its value is significantly higher in CJD than in AD and still higher (statistical trend) than in the control group. Compared to other studied groups, CJD brains not only demonstrate a higher number of microglia per mm^2, but also a higher proportion of HLA-DR-positive (activated) cells.

It is noteworthy that the AD group did not differ from the control group in the HLA-DR/CD68 ratio, i.e. the proportion of microglia showing HLA-DR expression was not higher in comparison to brains from the control group, as expected from the results of earlier studies [29,31,59,66]. This may result from the advanced neurodegeneration observed in our AD brain samples, which is why the inflammatory process may have already extinguished [25,36]. Additionally, it may be a consequence of the technique of counting cells: 20 high power fields chosen at random. In AD chemotaxis and microglia activation are present mainly in mature plaques which, due to the random choice, may not have been adequately represented under the microscope.

This study has certain limitations which could have influenced its results. Only partial demographic data on the patients with CJD were available, the clinical history and the dominant symptoms were unknown. Therefore, no analysis of clinical variables was performed, the research only focused on neuropathology. The majority of the material was derived from archives of the Department of Molecular Pathology and Neuropathology, Medical University of Lodz.

Conclusions

1. The expression of microglia markers (HLA-DR and CD68) as well as the HLA-DR/CD68 quotient is more prominent in CJD than in AD or control brains, reflecting more intensive CNS inflammation in CJD.
2. No difference between AD and controls was observed with HLA-DR and CD68 labelling or HLA-DR/CD68 quotient. This may be due to a relatively advanced neurodegeneration in our AD sample. In late phases of AD, CNS inflammation is no longer present in contrast to early stages of the disease.
3. There is no difference between groups in ferritin labelling. Thus, ferritin is not a useful marker of microglia.
4. The area of sampling (the brain vs. the cerebellum) had no effect on the number of microglia cells.

Acknowledgements

Prof. P. Eikelenboom and Prof. A.J.M. Rozemuller are kindly acknowledged for critical reading of the manuscript.

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