Chapter 3

Defining Microglial Phenotypes in Alzheimer's Disease

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Additional information is available at the end of the chapter

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Abstract

The concept of activated microglia being associated with neurodegenerative pathological structures in aging and Alzheimer's disease (AD) has been well established, but questions remain about how well are we defining "*what are microglia **actually** doing*" when we look at diseased or aged brains? Most studies of microglia in human AD brains have employed a limited set of antigenic markers, particularly the major histocompatibility complex protein HLA-DR and ionized calcium-binding adaptor molecule IBA-1, along with cellular morphological criteria, but in recent years, it has been appreciated that microglial responses are very heterogeneous depending on their surrounding environment—*every microglia might be different*. Initial observations on human brain microglia associated with plaques and tangles suggested that microglial inhibition with broad spectrum anti-inflammatory drugs should slow down AD pathology, but clinical trials did not show this approach to be effective. In this article, we will consider the needs, challenges and benefits for refining how microglia are defined as they associate with pathological proteins. This may aid in defining which ones are accelerating neurotoxicity and which ones are performing reparative/phagocytic functions. More complete definition of microglial phenotypes offers the potential of developing ***targeted*** anti-inflammatory approaches for this disease.

Keywords: neuroinflammation, pathology, immunohistochemistry, antibodies, activation, microglia

1. Introduction

Progress in developing new treatment approaches for Alzheimer's disease (AD) has been slow. The goals of identifying key pathological events early in the disease and preventing them from progressing further has been investigated for many approaches; including preventing amyloid

formation and/or aggregation [1, 2], preventing tau phosphorylation and aggregation [3], reducing mitochondrial dysfunction and production of reactive oxygen species with various anti-oxidants [4, 5], enhancing autophagy [6], reducing the consequences of abnormal lipid metabolism [7], and targeting inflammation and microglia [8] amongst others with limited effectiveness. Since the identification of increased inflammation in AD brains, first characterized more than 30 years by increased expression by microglia of HLA-DR [9], and subsequently for other macrophage proteins, including beta II integrins and immunoglobulin Fc receptors CD16, CD32 and CD64 [10, 11], ferritin [12, 13], CD68 [14, 15] amongst others, led to the development of the inflammation hypothesis for AD that suggested that inflammatory products were driving the loss of synapses and neurons in this disease [16]. If this were true, reducing inflammation should have shown great potential for treating AD, however; this approach has not been successful to date [17, 18].

The initial studies identified several features about the microglia associated with AD plaque and tangle pathology. There was increased expression of the MHC class II marker HLA-DR, and morphologically the microglia were different with thicker cell processes and enlarged cell bodies [19]. From these initial observations, it was concluded that the microglia were in a pro-inflammatory state and thus must be producing neurotoxic cytokines that would be accelerating the progression of disease. This hypothesis is illustrated in **Figure 1**, where in this illustration aggregated amyloid beta (Aβ) peptide, produced as an initial feature of AD, activates the microglia, which can produce cytokines such as tumor necrosis factor (TNF)-α or interleukin (IL)-1β that accelerate neuronal dysfunction. This scheme was supported by experimental studies showing that aggregated/fibrillar Aβ had strong proinflammatory-inducing properties on microglia, including activation of the NADPH oxidase complex of myelocytic

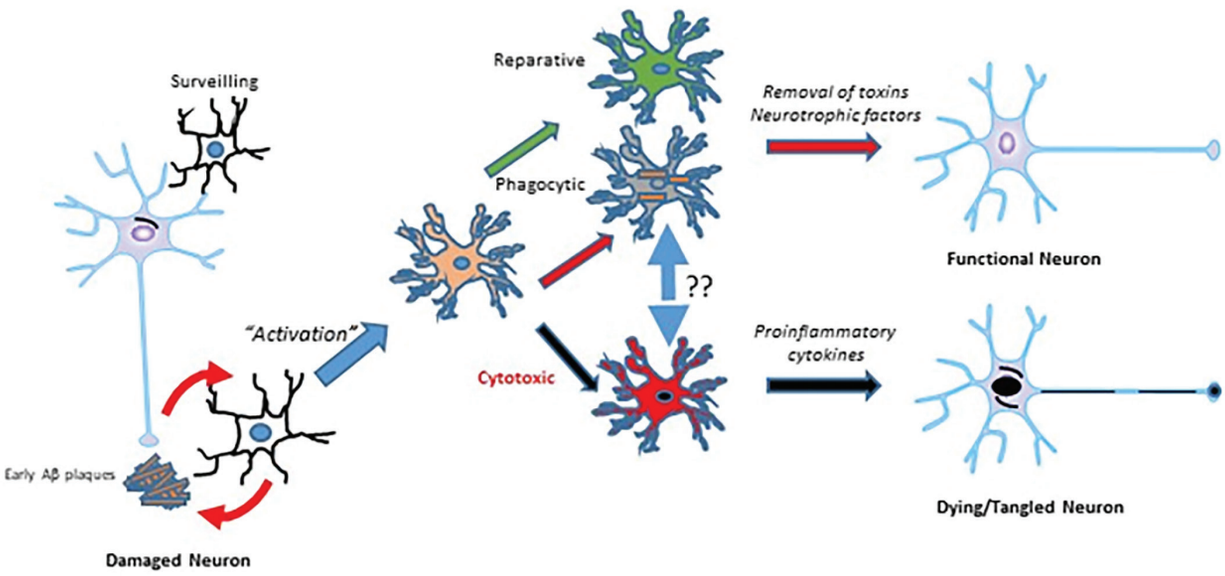

Figure 1. Microglia are performing multiple functions in Alzheimer's disease brains. How should microglia be classified in human brains. Scheme to illustrate the potential change in activation of microglia with development of disease. The morphologies of resting or surveilling microglia is established, but it is apparent that activated-cytotoxic-microglia in brain tissue may be indistinguishable from the activated-reparative or -phagocytic microglia. The observation of ameboid microglia in AD tissue without other pathology (infarcts/hemorrhage) involving lymphocyte infiltration is rare.

cells that leads to a burst of production of highly toxic reactive oxygen species [20, 21], as well as induction of many proinflammatory genes [22]. Another feature of this figure, which could be as important, is the other (non-proinflammatory) microglia that will be present in the AD pathological environment. There has been insufficient attention to describing these microglia, but they will play an essential role in phagocytosis of Aβ as well as performing other homeostatic functions. It has been hypothesized that one could distinguish between these microglia based mainly on cellular morphology; however there are limited data to support this. The presence of activated microglia in AD tissues provided the rationale for testing of "non-specific" non-steroidal anti-inflammatory drugs (NSAIDS) in AD patients. There had been supporting, though not unanimous, data that subjects with a history of long term anti-inflammatory drug usage also seemed to be protected from dementia [23–25]. Controlled drug trials of NSAIDs or related agents in AD patients have never shown significant effectiveness in slowing disease progression, and even these widely-used agents appear to have significant side effects in elderly subject. It has been argued that these trials failed because the disease was too far progressed to respond to therapy, but we will posit in this article that maybe our understanding of the unique features of inflammation in AD are still not complete enough for selecting appropriate drug treatments. This comes back to the central question of "*what are the activated microglia actually doing*" in the AD brain [17, 18]. Such studies need adequate numbers of quality tissue samples from control and AD cases. The presence of AD plaque and tangle pathology is also a common feature in aged brains without clinical dementia. These cases, called high pathology controls or high plaque non-demented, are particularly valuable for neuroinflammation studies as they provide material for studying what might be pre-AD stages of microglial activation. Being able to describe progression of inflammatory changes leading AD could be critical for identifying therapeutic targets.

2. Dichotomy of microglial function: do we know which microglia are producing damaging inflammation and which are performing phagocytosis of damaging abnormal proteins

Since the initial discoveries of activated microglia in AD and aging brains, the concepts and knowledge of what microglia are doing or could potentially be doing has progressed. The central role of microglia in brain, or macrophages in general, is to phagocytose and digest cellular waste products, which should include the extra-cellular Aβ that is deposited in AD and aging brains. An important question that is still unanswered today is "why are microglia not doing a better job of removing Aβ plaques?" Some concepts of microglial function in relation to AD came from transgenic mouse models using animals engineered to develop Aβ plaques in a manner similar to humans. These studies produced some conflicting results, but in general it was shown that microglia could be manipulated to achieve greater removal of plaque material, but in these mice, as in humans, microglial removal of plaque material is not efficient without some stimuli [26]. Again, we can ask does this apply to all microglia? Certain cytokine treatments affect microglia leading to reduced phagocytosis such that plaque material accumulates to a greater extent [27–29]. These studies illustrated how microglia can be

activated to be more or less efficient at Aβ removal [30, 31]. This was particularly shown in Aβ-peptide immunized mice, which had produced a specific antibody response to plaque material. The coating of plaques with anti-Aβ immunoglobulin appeared to promote phagocytosis through engagement of the microglial IgG Fc receptors. Overall, these studies showed that microglia of a particular phenotype have the potential to remove Aβ; similar observations have come from human pathological studies in certain subjects who had received the Aβ vaccine [14].

3. Schemes for defining microglial function: limitations of M1 and M2 activation state definitions

Phenotyping of macrophages and microglia progressed with the pioneering work of Gordon and colleagues who sought to develop schemes for classification of macrophages, first in mice and then in humans, by assigning activation states to the expression of different antigenic markers based on responses to defined activation stimuli [32, 33]. Much of this work employed gene expression profiling mRNA analysis since these techniques have fewer of the limitations associated with antibodies that will be discussed. These phenotyping schemes were also applied to microglia, both rodent and human. The scheme defined classical activation or M1 activation, as being the state of macrophages/microglia that have been stimulated with strong inflammatory agents such as lipopolysaccharide (LPS) and interferon gamma (IFN-γ). Such activated cells will be expressing increased levels of cytokines and enzymes such as TNF-α, IL-1β, IL6, matrix metalloproteinase (MMP)-3 and MMP-9. The corollary to this is alternative activation or M2, which defines the markers and products of cells responding to anti-inflammatory cytokines such as IL-4 or IL-13. These cells have a reparative/neurotrophic phenotype and can produce growth factors. Such reparative M2 microglia also show increased phagocytosis. The M2 scheme was further subdivided into M2a (responses to IL4 or IL13), M2b (responses to immune complexes in combination with IL-1β or LPS) and M2c (responses to anti-inflammatory IL-10, TGFβ or glucocorticoids). It was shown that increased expression of the scavenger receptor CD163, a marker for M2c was upregulated in microglia in AD and Parkinson disease dementia cases. This is the first study showing a type of alternative activation in AD tissues by immunohistochemistry [34].

Many studies have tried to apply these schemes to tissue microglia but their validity has been contested [35]. The schemes are dependent on using defined stimuli, while in the degenerating AD brain, there will be many different stimuli (Aβ and tau in different conformations, reactive oxygen, cytokines, bioactive lipids, ATP/ADP, DNA, etc.) that will account for the heterogeneity of microglia responses in tissue. In recent years, there has been criticism that the M1 and M2 scheme is not applicable for tissue microglia as such defined microglia do not seem to exist in brain [35]. This may be correct as the microenvironment around every plaque and every neuron will be different, but to attempt to profile microglia does require some form of scheme, even an imperfect one, to relate to function. It will also be proposed that the limitations of the M1 and M2 classification schemes could be due to technical reasons as much as biological reasons.

4. Practical issues involved in microglial phenotyping in human autopsy brains

Success in classifying microglia in postmortem human autopsy tissue sections is primarily dependent on the antibodies being used for this purpose, but also the manner in which the brain tissue being studied was preserved. Many published studies of microglial markers for immunohistochemistry have been restricted to antibodies that produce strong immunoreactivity on extensively fixed tissue sections. This is particularly true for HLA-DR, which is the most widely used for human microglial studies, as available antibodies can produce vivid results on a wide range of preserved brain tissue. The following references are the first for HLA-DR and the most recent, spanning 30 years of studies [36, 37]. The function of HLA-DR in AD microglia is still unclear. This protein functions to present processed antigens to T lymphocytes that are not present in the AD microenvironment. The signaling that leads to upregulation of HLA-DR in AD microglia has not been defined. In recent years, the marker IBA-1, which recognizes an actin-binding protein involved in cytoskeletal reorganization and cell motility, has also been extensively used to identify microglia because of the availability of robust staining antibodies [38]. IBA-1 antibodies seem to recognize all microglia with limited upregulation in activated microglia, though this interpretation is also dependent on observations related to microglial morphology. The use of antibodies that produce strong results in tissue sections may have biased our understanding of microglial function in disease as many other antigenic markers are present, but suitable antibodies to reveal them in tissue are not available. Most useful markers of function are cell-surface glycosylated proteins whose antigenicity become significantly affected by fixation conditions and also by the degree of glycosylation. The most widely available tissues for many researchers are tissue blocks that have been formalin-fixed for extended periods and then embedded in paraffin (FFPE); this process includes treatments with xylene. These preservation methods strongly affect the ability of antibodies to recognize many antigens, but in particular cell-surface glycoproteins. The numbers of antibodies that are effective at antigen recognition on FFPE tissue are a small percentage of available antibodies. In addition, the use of FFPE tissue usually requires the application of antigen retrieval techniques for most antibodies to work; there are a range of these methods but their successful application is dependent on operator skill and can lack reproducibility. As mentioned, the applicability of M1 and M2-like schemes to classify microglia in human brain samples has been criticized as many of the classification antigens have not been proven in tissue microglia [35], however such schemes may have been prematurely discarded due to the lack of panels of antibodies functional on available brain tissue samples.

4.1. Previous studies of microglial functional proteins in AD

Since the initial studies of increased HLA-DR expression by microglia in AD brains, in areas associated with pathology [9, 19, 36, 39–42], expression of a range of macrophage markers have been applied to AD brain tissues. These include beta II integrins (CD11a, b, and c and CD18—complement and phagocytic receptors), immunoglobulin Fc receptors (CD16, CD32, CD64) [11], lipopolysaccharide receptor CD14 [43], macrophage colony stimulating factor receptor-1 (CSF-1R; CD115) [44], type B scavenger receptor CD36 [45, 46], ferritin [47], signal regulatory

protein beta-1 (SIRPβ-1) [48] and progranulin [49]. The markers CD43 and TMEM106B were shown to be downregulated in AD microglia compared to controls [50, 51]. This represents an incomplete list due to space limitations but many of these markers are related to phagocytic function rather than cytotoxicity. Ferritin has unique properties in relation to microglial activation as it is a ubiquitous iron transport protein but in tissue seems to selectively identify a population of activated microglia [12, 13]. To directly demonstrate potential cytotoxicity, the demonstration of increased levels of cytokines in microglia is needed. Over the last 30 years, there have been few studies using immunohistochemistry to profile cytokines in tissue sections. A series of studies by Griffin and colleagues showed IL-1α-expressing microglia were associated with different types of plaques and tangles. Diffuse neuritic and non-neuritic plaques had the most IL-1α positive microglia, while dense core neuritic and non-neuritic plaques had significantly few IL-1α positive microglia. These results suggest that this population of microglia were involved at early stages of plaque formation [52]. Use of this marker demonstrated that IL-1α positive microglia were involved in the generation of neurofibrillary tangles in the parahippocampal gyrus [53]. In another study, it was shown that IL-1β and TNF-α could be localized to microglia in human AD tissue [42]. The limited numbers of studies do highlight the technical difficulties of detecting secreted proteins such as cytokines. Griffin and colleagues employed FFPE tissue for immunohistochemistry. We have attempted a number of times using our short-fixed microtome cut sections to localize cytokines to tissue and have never been successful. As these molecules are secreted rather than membrane localized, it is possible the hard fixation involved in FFPE is needed to anchor them, and then antigen retrieval to allow antibody access. With short fixed brain tissues materials, these soluble proteins might not be adequately fixed *in situ* for localization.

4.2. Selecting antibodies for microglial phenotyping

The whole field of human brain immunohistochemistry has several limitations when it comes to selection of suitable antibodies needed to reveal location of proteins of interest. Firstly, the antibody, usually a monoclonal antibody of mouse or rabbit origin, thus specific to an epitope representing a small portion of the target protein, has to be able to show specificity—namely it is actually recognizing the target protein *in situ* and not cross reacting with other proteins. Secondly, the antibody, if it can be validated to recognize the target protein in tissue, its specificity and sensitivity can be affected by the fixation conditions. In our experience, the study of microglial antigens with a wider range of antibodies has been less problematic using brain tissue fixed for a short period (48 h) in paraformaldehyde (not formalin) and then cryoprotected and sectioned using a freezing microtome. This process avoids the harsh treatments involved in paraffin embedding of tissue. Over the years, we have successfully identified microglial proteins CD87 [54], RAGE, CD33 [55], TREM-2 [56], TLR-2, -3, -4 along with HLA-DR, IBA-1, CD68 in AD tissues.

Our experience with antibodies when using these tissues identified some features that help increase the chances of successful immunolocalization. One company—R & D Systems—Biotechne, Minneapolis, MN—have produced many of their antibodies using a relatively unique strategy for the industry. Many of their antibodies were prepared from proteins of interest expressed in eukaryotic cells. These proteins will be glycosylated in a similar manner

to what might be expected in human tissues. In addition, the immunizing proteins usually cover the majority of the native protein, and thus preserve some of the secondary protein structure that affects antigenicity, along with containing multiple antigenic epitopes. These proteins produce antibodies with immunogenicity superior to the strategy of many companies that use short synthetic peptide sequences of 10–20 amino acids as immunogens, and then conjugated to a carrier prior to animal immunization. Our experience with R & D Systems affinity purified polyclonal antibodies has generally been favorable for use on lightly-fixed tissues. These antibodies will contain a range of epitopes that can increase the likelihood of identifying epitopes on proteins not severely affected by fixation. The use of large protein antigens to prepare polyclonal does have some drawbacks as there is the potential for cross reactivity with other related proteins. Quality control is dependent on being able to carry out protein absorption of antibody to show removal of tissue reactivity, along with western blot detection of specific protein bands.

Figure 2 (panels A and B) illustrates our experience using an R & D Systems antibody to Toll-like receptor (TLR)-3 (AF1487) to identify microglia in AD brains, and an R & D Systems polyclonal antibody to CD206 (AF2535), which failed to identify microglia (panels D and E). The TLR-3 polyclonal antibody could identify structures in human brain microglia (**Figure 2**, panels A and B). One comment is that if western blots are carried out using complex material such as brain material, the presence of other protein bands, besides the full length protein should be anticipated (**Figure 2**, panel C). Most functional proteins are either cleaved during their normal function, for example loss of leader sequences, cleaved to produce secreted forms, or broken down as part of cellular metabolism. Interestingly, a monoclonal antibody to TLR-3 produced with the same immunizing protein could not stain microglia in tissue, but this antibody will be specific for only a single epitope present in the immunizing protein. We have had similar experience with an R &D systems antibody to CD206 (**Figure 2**, panels D and E), also known as macrophage mannose receptor, produced against a eukaryotic cell expressed protein. This protein has been defined as a prototypical marker for M2a alternative activation as its expression is induced in the presence of IL-4. We used this antibody to determine if there was evidence for alternative activation microglia in human brains. Using this antibody, which on western blots could detect specific bands on brain samples, did not identify microglia in any of the control or AD tissue sections we stained. Noticeable however was the strong CD206 staining of round cells (perivascular macrophages) located within or around the vessels present in the brain sections. This is similar to a published finding [34]. This seems to indicate alternative activated macrophages are common in vessels, while alternative activated microglia are not present in neuropil. In human brains, identifying expression of inflammatory associated molecules at the RNA or protein level using brain homogenates need to be interpreted with caution as significant numbers of blood cells can be trapped within the tissue [57]. Confirmation of findings by immunohistochemistry is needed when making observations relevant to microglia. The absence of alternative activated markers in AD brain samples was confirmed for the CD200 receptor (CD200R). This is a myeloid specific receptor that is activated by the ligand CD200 to induce anti-inflammatory signaling. We showed that it was induced by IL-4 and IL-13 and fit the classical definition of an M2a marker, similar to CD206. Using several antibodies, including R&D Systems polyclonal antibody (AF3414) and a custom peptide antibody, we could not localize CD200R

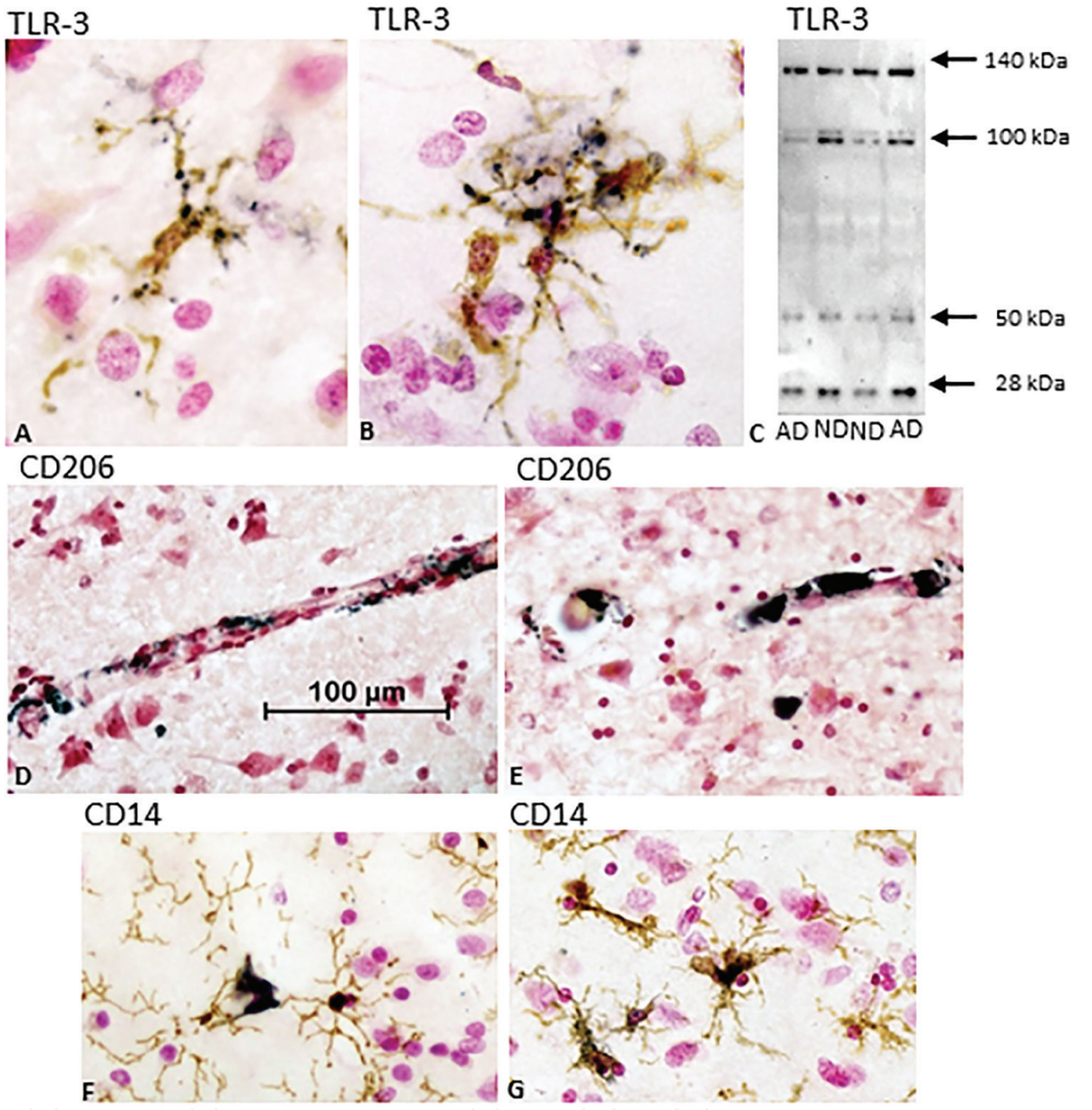

Figure 2. Approaches to microglial phenotyping in Alzheimer's disease brains. (A) and (B) Immunohistochemistry for a new marker for microglia (toll-like receptor-3: TLR-3 in human brains. Double immunostaining for TLR-3 (purple) colocalizing with IBA-1 microglia in (A) non-demented control middle temporal gyrus and (B) Alzheimer's disease case. See text for further explanation. These findings were obtained using R&D Systems antibody (AF1868). (C) Western blot of human brain samples for TLR-3. This panel illustrates that protein bands other than full length peptides can be present in biological samples. (D) and (E) Absence of alternatively activated microglia expressing CD206 in ND (D) or AD (E) temporal cortex brain sections but positive expression in perivascular/vascular macrophages. (F) and (G) The proinflammatory marker CD14 does show increased expression by microglia in AD cases (purple) colocalizing with IBA-1 immunoreactivity brain Strong positive staining is present in perivascular/vascular macrophages (purple) is also a feature.

immunoreactivity to brain microglia even though protein and mRNA expression of CD200R are detectable in human brains [58].

One marker that seems to have been overlooked in microglial profiling in tissue is CD14, the LPS co- receptor. This receptor is a classical M1-like activation marker with upregulation

associated with proinflammatory activation. There has only been a single study describing microglial immunohistochemistry in human AD brains for CD14 [43]. Using short fixation tissue, we reexamined expression of this marker (**Figure 2**, panels F and G). It is strongly expressed by most vascular macrophages of all cases (**Figure 2**, panel F—ND case), but increased expression was readily detectable in subsets of IBA-1 microglia in AD cases (**Figure 2**, panel G—purple). As CD14 can bind Aβ with proinflammatory activation through interaction with TLR-2 or TLR-4, increased CD14 expression could be a more used marker for defining cytotoxic microglia.

4.3. Profiling TREM-2 microglia in human brains

This discussion is of particular relevance for considering microglial phenotyping of TREM-2 positive microglia. Considerable interest in the role of TREM-2 in AD has spurred new interest in neuroinflammation and AD. A single nucleotide polymorphism (SNP) in the TREM-2 gene (rs75932628) that results in a mutation in the TREM-2 protein (R47H) can increase the risk of developing AD by 2- to 11-fold depending on the population studied [59, 60]. Mutations in TREM-2 or its adaptor protein DAP12 were first identified in Nasu-Hakola disease, which leads to early onset dementia amongst other symptoms [61]. The mutation appears to lead to loss of function of the TREM-2 protein, whose normal function is to promote phagocytosis of apoptotic neurons through binding to heat shock protein 70 (hsp70) or different conformations of lipids. There have been few studies of immunohistochemistry of TREM-2 in human AD brains, which appears mainly due to lack of robust antibodies for pathological work. We published one of the first studies that showed plaque- and tangle-associated microglia were positive for TREM-2 [56]. In this study, we had to screen a number of antibodies for specificity and sensitivity in human brain tissue. The best results were obtained with an R&D Systems polyclonal antibody to TREM-2 (AF1828) prepared using a eukaryotic cell expressed protein corresponding to 75% of the protein and to the complete extracellular domain. A recent study of TREM-2 expression in AD frontal cortex using an antibody prepared using a peptide corresponding to N-terminal amino acids 29–59 of human TREM2 (ab175262, Abcam, Cambridge, MA) showed specificity by western blots, but these authors presented no data on TREM2 immunohistochemistry [62]. TREM-2 expression is restricted to dendritic/myeloid cells and is high in brain microglia. Specificity of commercial antibodies has been an issue, but also the sensitivity of detection. Two studies have concluded that TREM-2 was not expressed by microglia in brain, but both studies employed FFPE tissue samples with antigen retrieval [63, 64]. One study showed that the R&D antibody was specific for TREM-2, similar to our published work, but they could not demonstrate microglial TREM-2 immunoreactivity [63]. Similar to our previous studies, we employed lightly fixed brain tissues that were not paraffin-embedded [56]. With these sections, we could demonstrate specific TREM2 localization to microglia [56]. Our finding is reasonable as TREM-2 has been localized to plaque-associated microglia in AD model transgenic mice [65].

5. Does expression of antigen correlate with identifiable function

How does antigen expression relate to demonstrated microglial function? With the exception of HLA-DR and IBA-1, most studies of microglia in human brains have not been adequately

replicated. Immunohistochemistry is not a quantitative technique due to the amplification technologies used along with the non-availability of standards for comparison, but the studies by Boche and colleagues using semi-quantitative measures deserve mention [10, 14, 66, 67]. Using a large series of brain samples and the expression of different markers, including CD64, MSR-A, CD68, HLA-DR and IBA-1, it was shown that microglia could be subtyped depending on their profile. These studies set the standards for microglial profiling in human brains. These studies employed two measures for analysis; the area of immunoreactivity and the numbers of positive cells. These studies attempted to assign phagocytic function or activation function to the microglia in relation to the type of pathology. One interesting observation was the lack of significant correlation between expression levels of these different antigens by microglia. These markers are related to different functions, with CD64, MSR-1, and C68 being related to phagocytosis, HLA-DR with antigen presentation and IBA-1 with microglial motility. Studies of TREM-2 and CD33 in AD brains demonstrated upregulated expression in AD brains, but both receptors induce inhibitory signaling when activated [55, 56]. There is evidence that upregulation of such activated microglial proteins encountered in disease tissue could be to have an inhibitory effect on inflammation, not amplifying inflammatory responses.

5.1. Where are the non-activated microglia in AD or aging brains?

In recent years, gene discovery methodology (RNA sequencing, microarrays, single cell sorting) has been applied to studies of microglia. One particularly interesting marker identified is the purinergic ADP/ATP receptor P2RY12. This was shown to be highly expressed by microglia (human and rodent) compared to macrophages. In addition, it appears to be a marker of non-activated microglia as expression becomes rapidly downregulated upon inflammatory activation with LPS [68]. P2RY12 has been defined as a M2 marker as it is unregulated in vitro in human microglia by treatment with IL-4 [69]. A common concept over the years about neuroinflammation and AD is that widespread proinflammatory activation is a significant and extensive feature. The expression that the brain is on fire has been used more than once in review articles of AD inflammation. If inflammation is widespread in pathological affected tissues, one would expect that P2RY12 expression would be very low or absent in AD brains. Our preliminary findings show that this is not the situation; there were many P2RY12 microglia in regions with severe AD pathology. Although western blot and mRNA studies showed no difference in expression of this gene between AD and control samples, however by immunohistochemistry, there was a very specific distribution of P2RY12 positive and negative microglia in brains in relation to pathological structures.

6. Future directions

The potential benefits for complete definition of microglial phenotypes by immunohistochemistry in human brains could be significant. The development of effective inflammation-focused therapies for AD requires the identification of therapeutic targets that are relevant

to the human disease, not to models of disease in a transgenic animal or cell culture. AD is a uniquely human disease of the elderly, with pathology having developed for years before dementia becomes observable. In transgenic models, disease pathology can develop over weeks. There have been many agents that can reverse plaque development and inflammation in AD mice models that have failed to be effective in humans. There are many challenges involved in human focused studies, but the benefits of having human disease targets validated in human tissue could involve significant saving in time and resources from pursuing the wrong approaches. Immunohistochemistry is not considered a state of art technique in the twenty-first century as the technologies have not much changed in 30 years, but ultimately it is required to show that gene discovery findings are valid. The need for large numbers of high quality human tissue samples has been one limitation, but this can be addressed by collaborative studies. Brain tissue that has been consistently prepared with appropriate clinical and pathological records allow studies involving progressive changes in pathology from negligible to severe rather than the less useful classification of control or AD. There is also a need for improved expectations on the performance of antibodies. The performance and reproducibility of antibodies in all biological experiments has been a growing concern [70], but ultimately it is the responsibility of the experimenter/pathologist to determine the suitability of antibodies used to make a unique observation. The field of neuroinflammation in AD has been reinvigorated with discoveries about TREM-2 having a direct link to AD risk. To be able to reliably identify TREM-2 positive microglia in brain is needed to fully understand its role in sporadic AD, and validate the large numbers of model studies that have proposed therapeutic strategies for AD focused on TREM-2.

7. Conclusions

Microglia represent approximately 10% of the total cell population in human brain, but it is now appreciated how complex their responses are to pathological stimuli and for maintaining healthy neurons. Treating pathological inflammation in AD with broad spectrum agents (e.g., cyclooxygenase inhibitors) may do more harm than good. If the microglial responses to pathology are highly dependent on the microenvironment; for example one microglia may be producing excess TNF-α while an adjacent one is attempting to remove the pathological stimuli, then treatments need to be targeted appropriately. This will only be possible if the microglia actively involved in AD can be adequately profiled.

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