Microglial Polarization States: Implications for Astrocytes

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Microglia are the primary resident immune cells of the central nervous system. Astrocytes also contribute to central nervous system immunity; however, this role is often overshadowed by their broad range of non-immunological functions. Microglia are classified into functional subsets based on their altered expression profiles following activation. Microglial subsets are commonly described in terms of pro-inflammatory M1 microglia and anti-inflammatory M2 microglia – this M2 subset then diverging further into M2a, M2b, and M2c states. It has also been suggested that microglia do not maintain these subset populations strictly, but rather lie upon a dynamic spectrum where presence or absence of certain characteristics defines the microglia's main functional role. Upon activation astrocytes can also demonstrate pro- or anti-inflammatory phenotypes. They have shown to be integral to the pathology of certain neurodegenerative diseases, having a role just as or more important than that of microglia, such as their role in the progression of multiple sclerosis. It has recently been suggested that astrocytes also display polarization states similar to the microglial M1/M2 profiles, however these have not yet been defined in any great detail. Delineation of microglial polarization states has improved the understanding of functional microglial subsets and has allowed for the characterisation of the role of M1 and M2 microglia in physiology and pathology respectively. It therefore may be beneficial to delineate these polarization states in astrocytes also. Identifying the functional role of different astrocyte subsets would allow greater understanding of their contribution to neurodegenerative diseases. The idea of characterizing functional astrocyte polarization states has great potential which has yet to be truly realized.

Introduction – Macrophages, Microglia and Astrocytes

Macrophages are a leukocyte subset with phagocytic ability and are derived from circulating peripheral blood monocytes. The roles of the macrophage are primarily host defence, wound healing, and immune regulation functions (Mosser & Edwards 2008). Mosser and Edwards seek to ascribe each function to a specific macrophage subset, subset divergence being dependent on humoral factors acting to modify the macrophage phenotype. Different humoral factors affect macrophages in different ways by changing their functional role (Sica & Mantovani 2012).

These functional changes in response to humoral factors also occur in microglia, the macrophages of the central nervous system (CNS). Neural cells with similar characteristics to macrophages were identified in 1880 during histology experiments and these cells were first described as microglia by Río Hortega, the "Father of Microglia" in the 1920s, who was also the first to postulate that these cells may function similarly to macrophages. The main difference between macrophages and microglia lies in their ontogeny. Macrophages undergo traditional derivation from haematopoietic stem cells whereas microglia are derived from yolk sac stem cells (Prinz & Priller 2014). However, despite these ontogenetic differences, the phenotype and functional polarization states of macrophages have been shown to translate into microglia, at least *in vitro*. The vast range of markers identified which signify different macrophage states correlate with those described in microglia (Colton & Wilcock 2010). However it must be stated that despite similarities to extra-CNS macrophages, microglia remain a distinct cell type, maintaining their own specific markers and displaying unique expression patterns (Yamasaki *et al.* 2014). Overall the literature accepts that macrophage phenotypes translate well into microglia, as will this review, however the above caveats must be taken into consideration.

Neurons in the CNS are supported by several different types of neuroglia such as microglia, oligodendrocytes, and ependymal cells. The astrocyte is another important type of glial cell and our understanding of its functions has developed greatly in recent years. We have progressed from the long-held idea that these glia act as mere support cells, to the belief that they possess a more dynamic role in the CNS, this being supported by a plethora of evidence (Bayraktar *et al.* 2015, Khakh & Sofroniew 2015). The abundance and organized dispersal of these glial cells indicates a role more important than previously thought. The non-immunological roles of astrocytes in the CNS are broad and include a role in both foetal development, via synaptic pruning and trophic factor secretion, and in adulthood, contributing to ionic homeostasis, neurotransmitter metabolism, glutamatergic signalling, and structural contribution to the blood brain barrier (Sofroniew & Vinters 2010). However, astrocytes additionally have both pro- and anti-inflammatory roles in immune modulation (Min *et al.* 2006, Jang *et al.* 2013). Similar to macrophages, characterization of astrocyte polarization states relies on different cells having distinct immunological phenotypes. It may therefore be possible to move towards classifying and discussing astrocytes in terms of polarization states similar to the M1/M2 classification of macrophages.

Evidence is mounting showing that astrocytes contribute to many neurodegenerative diseases such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS) – all of which affect members of the Irish population (Avila-Muñoz & Arias 2014, Meyer *et al.* 2014, Correale & Farez 2015). Over 700,000 people in Ireland are affected by a neurological condition with MS accounting for approximately 8,000 of these conditions and approximately 250 new MS cases being diagnosed each year (Neurological Association of Ireland 2014, Multiple Sclerosis Ireland n.d.). MS is a great burden on the exchequer with up to 80% of those diagnosed stopping work within 15 years and losing an average of 18 working years (The Work Foundation, 2011). Further research into the biology of astrocyte polarization states may help to further elucidate the mechanisms behind these debilitating neurodegenerative diseases and help to identify novel therapeutic avenues.

The Past – Classification of Macrophage Polarization States

Work on macrophage biology first looked towards defining two specific macrophage subsets: the classically activated pro-inflammatory M1 state macrophage and the alternatively activated anti-inflammatory M2 state macrophage. The M1 macrophage is activated by Toll-like receptor (TLR) agonists and interferon gamma (IFN-γ), and subsequently becomes pro-inflammatory. This inflammation is characteristic of the anti-microbial nature of M1 macrophages which was first described by Dalton in 1993 (Dalton *et al.* 1993). IFN-γ allows upregulation of the major histocompatibility complex II (MHC-II) and inducible nitric oxide synthase (iNOS) which signifies the priming of these M1 macrophages. MHC-II is a molecule which allows presentation of antigen to cells of the adaptive immune system, CD4+ T cells and iNOS is an enzyme that catalyses the production of nitric oxide, a reactive compound which can kill invading pathogens. MHC-II and iNOS are now seen as the archetypal markers of M1 macrophages in additional to a plethora of other receptors and pro-inflammatory secretions. The M1 macrophage amplifies the inflammatory response by production of cytokines, such as interleukin-1 (IL-1) or IL-12, chemokines, and free radicals, and by increasing antigen presentation to adaptive immune cells (Mosser & Edwards 2008).

Classification of the M1 state is straightforward when compared with the classification of M2 macrophages. It was first suggested in 2004 that M2 macrophages can be classified further into M2a, M2b, and M2c states (Mantovani *et al.* 2004). The first M2 state macrophage to be described was initially done in terms of IL-4 mediated upregulation of macrophage mannose receptor (MRC) and MHC-II (albeit a more restricted expression than that of M1) and was termed an M2a macrophage (Stein *et al.* 1992). It was then noted that IL-13 also modulated this macrophage subset, which is now known to occur through a common receptor chain, IL-4Rα (Doherty *et al.* 1993). The role of the MRC is the clearance of pro-inflammatory glycoprotein ligands, such as lysosomal hydrolases and tissue plasminogen activator (Gordon 2003). The

anti-inflammatory, resolving nature of M2a macrophages is also shown by their ability to express soluble factors which counteract pro-inflammatory mediators, for example arginase counteracts iNOS. Furthermore, M2a macrophages contribute to the repair of the extracellular matrix via factors such as collagenases and chitinases (Gordon 2003, Mosser & Edwards 2008).

M2a macrophages can be described as actively anti-inflammatory whereas M2c macrophages, which are also known as regulatory macrophages, have a deactivating function, comprehensively inhibiting production of, and antagonising pro-inflammatory factors and providing overall immunosuppression – a state termed "acquired deactivation" (Luo & Chen 2012, Fleming & Mosser 2011). Polarization to this state occurs primarily in response to IL-10 and transforming growth factor beta (TGF-β) (Colton 2009). IL-10 is also produced by M2c macrophages which has important contributions to immune tolerance/suppression, such as decreasing production of pro-inflammatory cytokines such as IFN-γ, and enhancing B-cell proliferation (Wang *et al.* 1995). This immunosuppressive state also causes tightening of vascular endothelial barriers thereby preventing leukocyte recruitment to sites of inflammation), increased growth factor production, and promotion of anti-apoptotic pathways; these homeostatic functions return the microenvironment to its normal physiological state.

M2b macrophages were originally described as type-II activated macrophages in reference to their ability to preferentially induce adaptive T-helper 2 cell responses (Mosser 2003). In contrast to the cytokine polarization signals of M2a and M2c macrophages, the M2b state is induced by engagement of the Fc gamma receptor on the macrophage surface by immunoglobulin G. Its functions are primarily mediated by IL-10 functions, similar to those carried out by M2c macrophages as mentioned above, but to a much greater extent. Interestingly M2b macrophages also secrete some of the same pro-inflammatory cytokines produced by classically activated M1 macrophages. Despite being considered an M2 macrophage, the M2b state shows distinct phenotypic differences in comparison to the other more traditional alternatively activated macrophages.

The Present – Accounting for Macrophage Plasticity in a Dynamic *in vivo* Environment

The current paradigm of M1/M2 macrophage polarization states was suggested by Mantovani *et al.* in 2004 and while the fundamentals of this paradigm are still viewed valid, there are growing concerns about the use of this information without considering its *in vivo* translation, and without immunological context (Mantovani *et al.* 2004, Sica & Mantovani 2012, Xue *et al.* 2014). Those suggesting a reassessment of how we consider macrophage states refer to the fact that an in vivo setting is a dynamic environment which will not maintain distinct macrophage types. Mosser and Edwards' spectrum of macrophage activation (Figure 1) takes this into account, suggesting that macrophages will not polarize into subsets with

explicitly defined features but rather gain or lose characteristics which deem them a macrophage primarily of a certain nature – be it host defence, wound healing, or immune regulation (Mosser & Edwards 2008). This spectrum also allows for the classification of "hybrid" macrophages, those which display characteristics from more than one subset. This spectrum is important in accounting for the ability of a macrophage to dynamically alter its expression of specific receptors and cytokines, i.e. its plasticity.

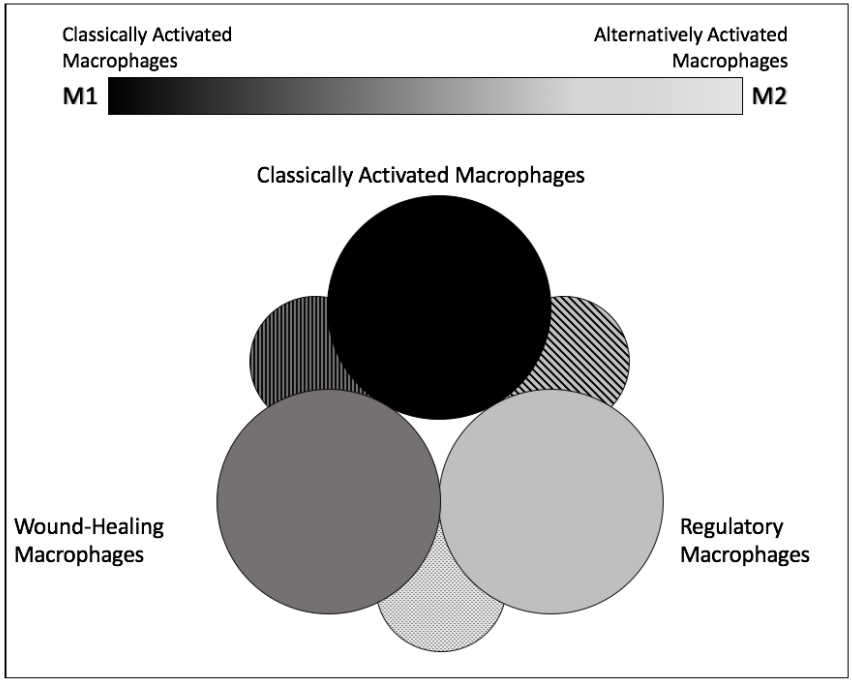

Figure 1. *The traditional linear M1/M2 model of macrophage polarization and the concept of a new polarization spectrum. The three primary colours represent the main groups of macrophage phenotype. The secondary colours in between groups illustrate the macrophage's potential to display characteristics from macrophages with different overall functions. For example, a macrophage falling into the orange area of the spectrum would be said to display functional characteristics from both classically activated and wound-healing macrophages. Adapted from Mosser & Edwards (2008).*

Recently, Martinez and Gordon (2014) identified what they believe to be the key limitations of the current M1/M2 model of macrophage activation namely; its ignorance of the source and context of polarization stimuli, its lack of consideration of co-existing M1/M2 polarization stimuli, and that the model doesn't account for the fact that macrophages may not differentiate into set subsets. While they do not officially propose a new model, the authors argue that future models should

consider the polarizing stimuli present at different levels of immunity in an *in vivo* environment. It suggests that these stimuli should be grouped based on their overall immunological role (Figure 2) instead of individual stimuli being directly compared with their antagonistic molecule. Doing so would permit a more comprehensive portfolio of polarizing stimuli to be created, thereby allowing identification of more specialized macrophage populations.

Growth and Survival Factors Maturation and Differentiation • Macrophage Colony Stimulation Factor Survival and Recruitment • Adhesion Molecules, Chemokines Other • Vitamin D3, Retinoic Acid	**Cytokines** Classic Activation • IFN-γ Alternative Activation • IL-4, IL-13 Pro-Inflammatory • IL-1β, IL-6 Anti-Inflammatory • IL-10, Tumour Growth Factor β
Pathogen Interaction Mechanisms Cell Receptors • TLRs, Nucleic Acid Sensors, NOD-like receptors Humoral Factors • Immunoglobins – IgA, IgE, IgG • Complement, Lectins, Ficolins	**Resolving Factors** Systemic • Glucocorticoids Local • Proteoglycans • Fatty Acid Derivatives i.e. Resolvins, Maresins

Figure 2. *The four hypothesised levels of immunological macrophage polarization stimuli. The different levels, and they subcategorisations, seek to provide a more comprehensive classification of macrophage polarization stimuli. Adapted from Martinez and Gordon (2014).*

Having described the functional polarization subsets of macrophages, it is important to reiterate how these phenotypes have been translated into microglia. While the two cell types may be ontogenetically distinct, the polarization states and specific subsets described for macrophages are now routinely referred to in microglia and both generally correlate in terms of M1 and M2 markers (Wilcock 2012, Chhor *et al.* 2013, Colton & Wilcock 2010). Macrophage polarization states can therefore, for the most part, be considered applicable to microglia meaning that we can use our understanding of macrophages to improve how we understand CNS immunity.

M1 microglia are generally seen as the primary mediators of neurodegenerative diseases such as AD, ALS, and Parkinson's disease while M2 microglia are usually downregulated during pathologies (Tang & Le 2015, Qin *et al.* 2015). Potential therapeutic avenues that have been identified include inhibiting the M1

polarization of microglia or re-inducing the expression of M2 microglia (Kobayashi *et al.* 2013, Cherry *et al.* 2014). Classification of the microglial polarization states allowed these avenues to be explored. Translating these states into astrocytes may therefore further progress our understanding of the role of the astrocyte in neurodegenerative diseases

The Immunological Role of Astrocytes in the CNS

Evidence is mounting illustrating the important immunological role of this multifunctional glial cell (Jang *et al.* 2013, Jensen *et al.* 2013). The immune response of the astrocyte is considered to be mainly pro-inflammatory. A hallmark reaction of this pro-inflammatory state is reactive astrogliosis, which can be defined as a spectrum of changes that occur in astrocytes in response to all forms of CNS perturbations, with the degree of change correlating with the severity of perturbation (Sofroniew 2009). Reactive astrogliosis can lead to the upregulation of many pro-inflammatory genes, leading to enhanced production of chemokines, cytokines, and growth factors. Other molecular changes include aberrant neurotransmitter synthesis/release and alterations in fluid/ion homeostasis, as well as morphological changes which prevent the spread of any threats to tissue integrity (Sofroniew & Vinters 2010). A pro-inflammatory astrocyte can help to further evolve the immune response by signalling for the recruitment of adaptive immune cells. A recent study has also suggested that astrocyte activation may cause the activation of microglia, the opposite of what is currently considered normal (Jang *et al.* 2013). The pro-inflammatory state of astrocytes is well classified as an evident component of several neurodegenerative diseases, such as Amyotrophic Lateral Sclerosis, Alzheimer's Disease, and Parkinson's Disease (Maragakis & Rothstein 2006). However, perhaps less well described is the anti-inflammatory component of astrocyte responses. While their immune function is seen to be primarily pro-inflammatory, anti-inflammatory cytokine secretions have also been reported (Jensen *et al.* 2013). Studies have identified known mediators, such as the complement-5a fragment, and unknown mediators which exert anti-inflammatory activity (Gavrilyuk *et al.* 2005, Min *et al.* 2006). This activity however tends to be overshadowed by the pro-inflammatory role of astrocytes, despite providing novel therapeutic opportunities.

MS, for example, is an autoimmune disease whose detrimental effects are mainly mediated by T-cells. However new research has suggested that astrocytes may also contribute to this chronic, demyelinating disease (Brosnan & Raine 2013, Correale & Farez 2015). The majority of current therapies are immune modulators or monoclonal antibodies which target T-cells. Therapies in general are expensive and impractical with regards to method and frequency of dose (Goldenberg 2012). Astrocytes may present a new therapeutic target. The active role of astrocytes in MS is only recently becoming a research focus and elucidation of their role in MS pathology indeed their role in any of the many other neurodegenerative diseases could benefit from an understanding of astrocyte polarization states.

The Future – Emerging Astrocyte Immune Functions & The Importance of Defining Astrocyte Polarization States

Classification of macrophage subsets relies on their different immune phenotypes. Astrocytes also express different immune phenotypes, pro- and anti-inflammatory, yet despite this, their specific polarization states have not been defined (Jensen *et al.* 2013). Data has indicated that astrocytes can also affect immunity indirectly by modulating pro- and anti-inflammatory microglial polarization states via inducing or inhibiting the release of certain inflammatory mediators (Bianco *et al.* 2005, Aloisi *et al.* 1997). Astrocytes may also exert this effect by upregulating microglial antioxidant enzymes such as heme oxygenase-1 (Min *et al.* 2006). TGF-β was discarded as a potential mediator of this microglial change by Min *et al.* in 2006 however recent new evidence exists which disputes this claim (Norden *et al.* 2014).

Irrespective of whether astrocytes affect CNS immunity directly or indirectly via modulation of microglia, it is clear that classification of specific astrocyte polarization states is the next logical step in elucidating the extensive role of astrocytes in neurodegenerative diseases. The role of astrocytes has been proven to cause immunopathology in many neurodegenerative diseases and delineation of their subsets would potentially allow for more focused research into their physiology and pathophysiology to take place . The changes in the expression profiles of pro- vs anti-inflammatory astrocytes could be determined, helping to identify either the specific molecules contributing to pathologies, or the defensive mediators present in normal physiology which become downregulated. Our knowledge of the astrocyte's contribution to CNS immunity has developed greatly over the past decade. We have moved from considering this cell to have only a filler function to a recently hypothesis suggesting that astrocytes have specific polarization states similar to those of macrophages (Jang *et al.* 2013). Mainly focusing on the pro-inflammatory astrocyte state, a basic M1 astrocyte molecular profile was postulated. Further studies in the area remain to be carried out, however may hold great potential for the field of neuroimmunology.

Conclusions

It is now clear that microglia are not the only important CNS immune cell. While the immune function of astrocytes in the CNS is still seen as secondary to that of microglia, the importance of these dynamic, multifunctional glia has seen more acceptance in recent years. There now exist a multitude of studies illustrating the pro- and anti-inflammatory roles of astrocytes, as previously described. Our knowledge of the astrocytic contribution to neurodegenerative diseases continues to grow and is helping to make the idea of defining specific astrocyte polarization states all the more relevant.

The M1/M2 macrophage paradigm has been suggested as a framework to aid the classification of astrocyte polarization states, important consideration must be made. The hypothesis that astrocytes "exhibit M1/M2-like functional polarization" similar to that of macrophages may technically be correct, however one must remember the recent paradigm shift towards a macrophage spectrum and one must consider the modulating effects of an *in vivo* environment (Jang *et al.* 2013). Astrocyte subsets may also exist on a similar spectrum and be affected by different levels of immune stimuli. It may prove beneficial for research into astrocyte polarization to consider a spectrum right away, rather than discuss a linear scale which may soon become obsolete.

Astrocytes are often seen as secondary CNS immune cells in comparison to microglia. Classifcation of astrocyte polarization states would help delineate the specific functional astrocyte populations involved in different neurodegenerative diseases. Combined with increased knowledge of their molecular profiles, astrocytes may provide a novel target in neurodegenerative diseases whereby targeting astrocytes modulates microglia, instead of targeting microglia directly. This knowledge may also help to clarify further the mechanisms by which these diseases manifest.

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References

ALOISI, F., PENNA, G., CERASE, J., MENÉNDEZ IGLESIAS, B. & ADORINI, L. (1997). IL-12 production by central nervous system microglia is inhibited by astrocytes. *The Journal of Immunology*, 159, 1604-12.

AVILA-MUÑOZ, E. & ARIAS, C. (2014). When astrocytes become harmful: Functional and inflammatory responses that contribute to Alzheimer's disease. *Ageing Research Reviews*, 18, 29-40.

BAYRAKTAR, O. A., FUENTEALBA, L. C., ALVAREZ-BUYLLA, A. & ROWITCH, D. H. (2015). Astrocyte development and heterogeneity. *Cold Spring Harbor perspectives in biology*, 7, a020362.

BIANCO, F., PRAVETTONI, E., COLOMBO, A., SCHENK, U., MÖLLER, T., MATTEOLI, M. & VERDERIO, C. (2005). Astrocyte-Derived ATP Induces Vesicle Shedding and IL-1β Release from Microglia. *The Journal of Immunology*, 174, 7268-7277.

BROSNAN, C. F. & RAINE, C. S. (2013). The astrocyte in multiple sclerosis revisited. *Glia*, 61, 453-465.

CHERRY, J. D., OLSCHOWKA, J. A. & O'BANION, M. K. (2014). Neuroinflammation and M2 microglia: the good, the bad, and the inflamed. *J Neuroinflammation*, 11, 98.

CHHOR, V., LE CHARPENTIER, T., LEBON, S., ORÉ, M.-V., CELADOR, I. L., JOSSERAND, J., DEGOS, V., JACOTOT, E., HAGBERG, H., SÄVMAN, K., MALLARD, C., GRESSENS, P. & FLEISS, B. (2013). Characterization of phenotype markers and neuronotoxic potential of polarised primary microglia in vitro. *Brain, Behavior, and Immunity*, 32, 70-85.

COLTON, C. & WILCOCK, D. M. (2010). Assessing activation states in microglia. CNS Neurol Disord Drug Targets, 9, 174-91.

COLTON, C. A. (2009). Heterogeneity of microglial activation in the innate immune response in the brain. *J Neuroimmune Pharmacol*, 4, 399-418.

CORREALE, J. & FAREZ, M. F. (2015). The role of astrocytes in Multiple Sclerosis progression. *Frontiers in Neurology*, 6.

DALTON, D. K., PITTS-MEEK, S., KESHAV, S., FIGARI, I. S., BRADLEY, A. & STEWART, T. A. (1993). Multiple defects of immune cell function in mice with disrupted interferon-gamma genes. *Science*, 259, 1739-42.

DOHERTY, T. M., KASTELEIN, R., MENON, S., ANDRADE, S. & COFFMAN, R. L. (1993). Modulation of murine macrophage function by IL-13. *J Immunol*, 151, 7151-60.

FLEMING, B. D. & MOSSER, D. M. (2011). Regulatory macrophages: Setting the Threshold for Therapy. European *Journal of Immunology*, 41, 2498-2502.

GAVRILYUK, V., KALININ, S., HILBUSH, B. S., MIDDLECAMP, A., MCGUIRE, S., PELLIGRINO, D., WEINBERG, G. & FEINSTEIN, D. L. (2005). Identification of complement 5a-like receptor (C5L2) from astrocytes: characterization of anti-inflammatory properties. *Journal of Neurochemistry*, 92, 1140-1149.

GOLDENBERG, M. M. (2012). Multiple Sclerosis Review. *P t*, 37, 175-84.

GORDON, S. (2003). Alternative activation of macrophages. *Nat Rev Immunol*, 3, 23-35.

JANG, E., KIM, J. H., LEE, S., KIM, J. H., SEO, J. W., JIN, M., LEE, M. G., JANG, I. S., LEE, W. H. & SUK, K. (2013). Phenotypic polarization of activated astrocytes: the critical role of lipocalin-2 in the classical inflammatory activation of astrocytes. *J Immunol*, 191, 5204-19.

JENSEN, C. J., MASSIE, A. & DE KEYSER, J. (2013). Immune players in the CNS: the astrocyte. *J Neuroimmune Pharmacol*, 8, 824-39.

KHAKH, B. S. & SOFRONIEW, M. V. (2015). Diversity of astrocyte functions and phenotypes in neural circuits. *Nat Neurosc*i, 18, 942-952.

KOBAYASHI, K., IMAGAMA, S., OHGOMORI, T., HIRANO, K., UCHIMURA, K., SAKAMOTO, K., HIRAKAWA, A., TAKEUCHI, H., SUZUMURA, A., ISHIGURO, N. & KADOMATSU, K. (2013). Minocycline selectively inhibits M1 polarization of microglia. *Cell Death Dis*, 4, e525.

LUO, X. G. & CHEN, S. D. (2012). The changing phenotype of microglia from homeostasis to disease. *Transl Neurodegener*, 1, 9.

MANTOVANI, A., SICA, A., SOZZANI, S., ALLAVENA, P., VECCHI, A. & LOCATI, M. (2004). The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol*, 25, 677-86.

MARAGAKIS, N. J. & ROTHSTEIN, J. D. (2006). Mechanisms of Disease: astrocytes in neurodegenerative disease. *Nat Clin Pract Neuro*, 2, 679-689.

MARTINEZ, F. O. & GORDON, S. (2014). The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Re*p, 6, 13.

MEYER, K., FERRAIUOLO, L., MIRANDA, C. J., LIKHITE, S., MCELROY, S., RENUSCH, S., DITSWORTH, D., LAGIER-TOURENNE, C., SMITH, R. A., RAVITS, J., BURGHES, A. H., SHAW, P. J., CLEVELAND, D. W., KOLB, S. J. & KASPAR, B. K. (2014). Direct conversion of patient fibroblasts demonstrates non-cell autonomous toxicity of astrocytes to motor neurons in familial and sporadic ALS. *Proceedings of the National Academy of Sciences*, 111, 829-832.

MIN, K.-J., YANG, M.-S., KIM, S.-U., JOU, I. & JOE, E.-H. (2006). Astrocytes Induce Hemeoxygenase-1 Expression in Microglia: A Feasible Mechanism for Preventing Excessive Brain Inflammation. *The Journal of Neuroscience*, 26, 1880-1887.

MOSSER, D. M. (2003). The many faces of macrophage activation. *J Leukoc Biol*, 73, 209-12.

MOSSER, D. M. & EDWARDS, J. P. (2008). Exploring the full spectrum of macrophage activation. *Nat Rev Immuno*l, 8, 958-69.

Multiple Sclerosis and MS Ireland Fact Sheet. Multiple Sclerosis Ireland. http://www.msreadathon.ie/uploads/File/MS%20and%20MS%20Ireland%20Fact%20Sheet%20-%20Media.pdf.

Neurological Association of Ireland Annual Report (2014). Neurological Association of Ireland. http://www.nai.ie/assets/84/288B4BA9-B338-CB71-541B29E-4602D742A_document/NAI_Annual_Report_2014.compressed.pdf.

NORDEN, D. M., FENN, A. M., DUGAN, A. & GODBOUT, J. P. (2014). TGFβ produced by IL-10 redirected astrocytes attenuates microglial activation. *Glia*, 62, 881-895.

PRINZ, M. & PRILLER, J. (2014). Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. *Nat Rev Neurosci*, 15, 300-12.

QIN, H., LIU, Y., FOX, T., HARMS, A., STANDAERT, D. & BENVENISTE, E. (2015). Function of M1-polarized macrophages in the pathogenesis of Parkinson's disease (INC6P.300). *The Journal of Immunology*, 194, 192.2.

Ready to Work? Meeting the Employment and Career Aspirations of People with Multiple Sclerosis. The Work Foundation. http://www.theworkfoundation.com/downloadpublication/report/289_289_ms3.pdf.

SICA, A. & MANTOVANI, A. (2012). Macrophage plasticity and polarization: in vivo veritas. *The Journal of Clinical Investigation*, 122, 787-795.

SOFRONIEW, M. V. (2009). Molecular dissection of reactive astrogliosis and glial scar formation. *Trends in Neurosciences*, 32, 638-647.

SOFRONIEW, M. V. & VINTERS, H. V. (2010). Astrocytes: biology and pathology. *Acta Neuropathol*, 119, 7-35.

STEIN, M., KESHAV, S., HARRIS, N. & GORDON, S. (1992). Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. The *Journal of Experimental Medicine*, 176, 287-292.

TANG, Y. & LE, W. (2015). Differential Roles of M1 and M2 Microglia in Neurodegenerative Diseases. *Molecular Neurobiology*, 1-14.

WANG, P., WU, P., SIEGEL, M. I., EGAN, R. W. & BILLAH, M. M. (1995). Interleukin (IL)-10 inhibits nuclear factor kappa B (NF kappa B) activation in human monocytes. IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. *J Biol Chem*, 270, 9558-63.

WILCOCK, D. M. (2012). A changing perspective on the role of neuroinflammation in Alzheimer's disease. *International Journal of Alzheimer's Disease*, 2012.

XUE, J., SCHMIDT, SUSANNE V., SANDER, J., DRAFFEHN, A., KREBS, W., QUESTER, I., DE NARDO, D., GOHEL, TRUPTI D., EMDE, M., SCHMIDLEITHNER, L., GANESAN, H., NINO-CASTRO, A., MALLMANN, MICHAEL R., LABZIN, L., THEIS, H., KRAUT, M., BEYER, M., LATZ, E., FREEMAN, TOM C., ULAS, T. & SCHULTZE, JOACHIM L. (2014). Transcriptome-Based Network Analysis Reveals a Spectrum Model of Human Macrophage Activation. *Immunity*, 40, 274-288.

YAMASAKI, R., LU, H., BUTOVSKY, O., OHNO, N., RIETSCH, A. M., CIALIC, R., WU, P. M., DOYKAN, C. E., LIN, J., COTLEUR, A. C., KIDD, G., ZORLU, M. M., SUN, N., HU, W., LIU, L., LEE, J.-C., TAYLOR, S. E., UEHLEIN, L., DIXON, D., GU, J., FLORUTA, C. M., ZHU, M., CHARO, I. F., WEINER, H. L. & RANSOHOFF, R. M. (2014). Differential roles of microglia and monocytes in the inflamed central nervous system. *The Journal of Experimental Medicine*, 211, 1533-1549.