university of groningen

Origin and regeneration of microglia, an overview.

Historical perspectives, current views and future perspectives.

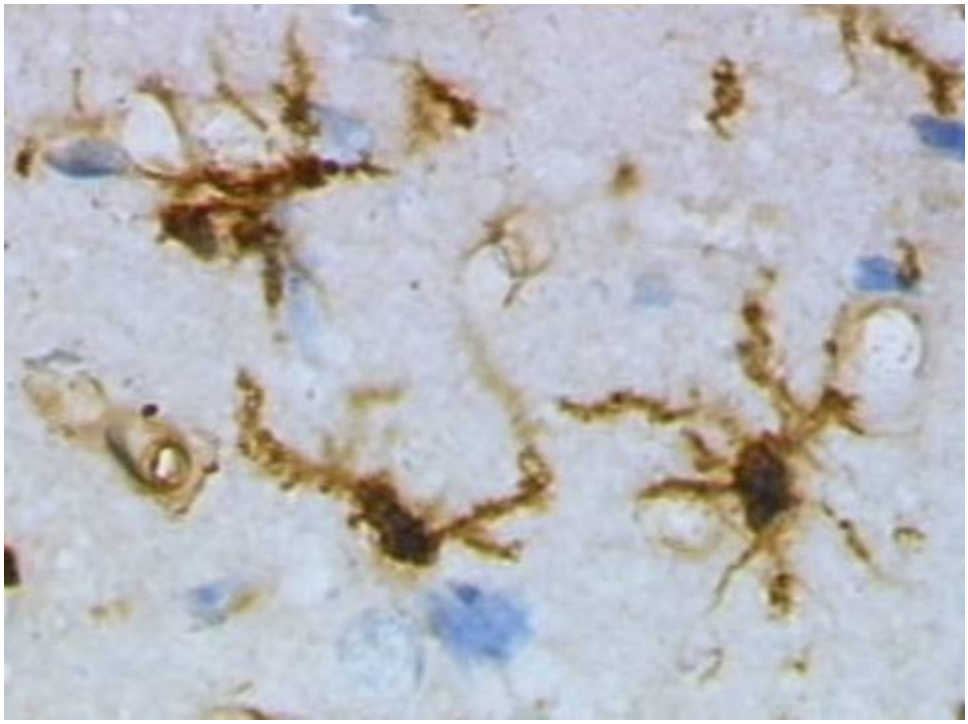

Figure 1: Microglia cells positive for lectins (brown). (Author: Grzegorz Wicher), Courtesy of Wikimedia commons.

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Datum: 29-01-2016
Bachelor Thesis

Abstract

The central nervous system has been long viewed as an immune privileged organ, this has placed microglia at the centre of attention in brain pathologies. Microglia are the principal immune effector cells of the central nervous system and were regarded as having a central role in neurodegenerative diseases. The origin of microglia has long been unknown; this also goes for the understanding of their function and if or how they are replenished. It was hypothesised that microglia could be replenished from the periphery and their main function was thought to phagocytose apoptotic cells or debris. Our current understanding of microglia is much more extensive, as recent findings have elucidated the origin of microglia, as well as established them to have important homeostatic functions in tissue remodelling and synaptic pruning. Moreover, the source of cells that repopulate the CNS after depletion of the microglial population has been established. These findings are paving the way for a better understanding of their implications in neurodegenerative diseases with possible therapeutic implications. This thesis will provide the background knowledge of microglia, in a historical perspective, what is currently known and what implications these findings might have for future studies and therapies.

Content

Introduction

The nervous system of animals regulates voluntary and involuntary actions and comprise the peripheral nervous system and the central nervous system (CNS). The CNS is part of the nervous system consisting of the brain and the spinal cord. Where the peripheral nervous system contains mostly neurons, the CNS has much more cell types to aid in supporting functions. Some of these supporting cell types are glial cells, named after the Greek word for glue as they were discovered when Ruldolf Virchow was looking for connective tissue in the brain. Now, we know that glial cells consist of many cell types, broadly classified in 2 types, macroglia and microglia. Macroglia in the CNS can be divided in a couple of other cell types, such as astrocytes, oligodendrocytes, ependymal cells and radial cells. Microglia are, as the name implies, classified as a type of glial cell and make up for 5-20% of total glial cell population (Hugh Perry 1998). This thesis will cover some of our current knowledge of microglia. In the CNS they act as tissue macrophages and are of the mononuclear phagocyte lineage (Hickey & Kimura 1988). They are the first and main form of active immune defence in the CNS, acting as sentinels, monitoring their environment for tissue damage or pathogenic invasion. In normal health, the CNS is an immune privileged site, this is caused by the blood-brain barrier (BBB). Recent studies have revealed that apart from functioning as macrophages, microglia also have an important role in CNS homeostasis and development of the brain (Cronk & Kipnis 2013). In figure 1, an overview of microglia function is given.

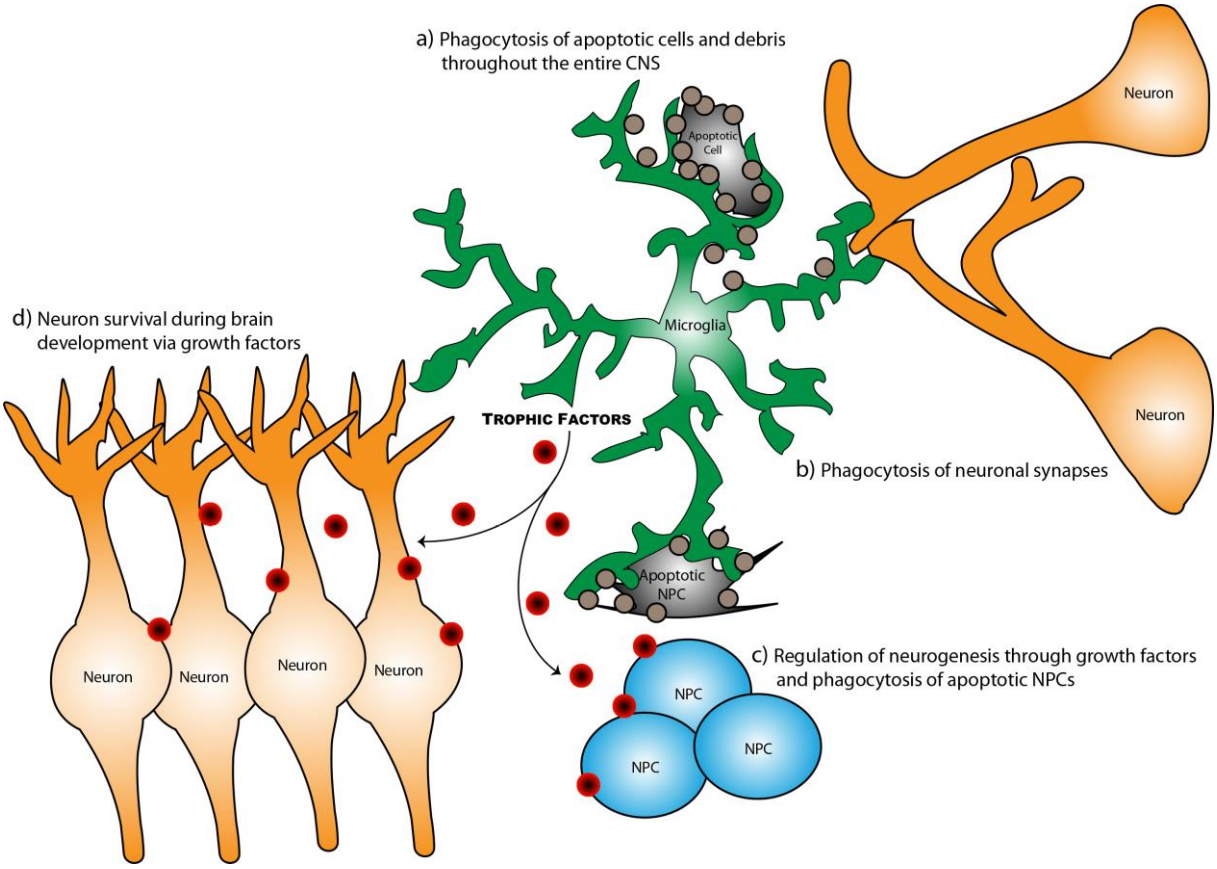

Figure 2: Microglia functions a) Phagocytosis of apoptotic cells in the CNS during adult life and postnatal development. b) Removal of neuronal synapses which are to be removed. c) Assisting in neurogenesis through growth factors and phagocytosis of apoptotic neural progenitor cells. d) Neuronal survival via release of trophic factors. Image from: Cronk & Kipnis, 2013, used with permission from the author.

Types of microglia

The neural-supporting role of microglia has only recently been established, as for years they were thought of as “evil”, being the primary cause of inflammatory damage and potentially causing disease (Cronk & Kipnis 2013). This is the reason that these functions are best characterized in comparison to their homeostatic activities. This is easily seen when looking at the types of microglia described in the literature (Gehrmann et al. 1995; Davis et al. 1994). *Amoeboid microglia* are more motile microglia, allowing them to function as scavenger cells and phagocytose cellular debris. They don’t have an antigen presenting function. *Ramified microglia* are monitoring their environment. They have small cellular bodies with long branching processes, allowing for better monitoring activity, this is the steady state condition of microglia. When microglia are activated in infection of neurodegeneration they can become phagocytic, and thus ramified microglia display very little antigen presenting surface molecules (Aloisi 2001). Ramified microglia can be activated, this causes them to move to the *non-phagocytic* stage, this is a transition stage caused by changes in the environment. In the non-phagocytic stage, the cellular body grows, takes up major histocompatibility complex (MHC) class I/II proteins and proliferates. This leads to the *phagocytic microglia*, which are larger than ramified microglia and antigen presenting because of their MHC proteins. They can also phagocytose foreign material and present the immunomolecules for immune cell activation. After phagocytosis microglia can become g*itter cells.* These are phagocytic microglia which are unable to phagocytose any further material. Then there are two more types of microglia distinguishable by their location; *perivascular microglia* and *juxtavascular microglia.* Both of these cells express MHC II proteins, but where perivascular microglia reside within the basal lamina, juxtavascular microglia reside adjacent to the basal lamina.

The blood-brain barrier

Just like any other organ, the brain needs nutrients and oxygen. Unlike other organs, the brain is not protected by the peripheral immune system, this is caused by the blood-brain barrier (BBB). The BBB allows for tight regulation of movement of ions, molecules and cells between the blood and the brain, it separates the brain parenchyma from the blood stream, protecting the CNS (Daneman & Prat 2015; Zlokovic 2008). The CNS endothelial cells (ECs), pericytes, astrocytes and microglia together determine the properties of the BBB . ECs in the brain are different from other ECs because they contain higher amounts of mitochondria and extremely low levels of leukocyte adhesion molecules (Daneman & Prat 2015). The last is important for regulation of the amount of immune cells entering the CNS. Moreover, ECs of the BBB also have tight junctions (TJs), making a continuous layer of non-fenestrated (no pores) ECs (Dudvarski Stankovic et al. 2015).

Origin of microglia (historically)

Comparison to macrophages

Microglia, as stated before, resemble other tissue macrophages. This has led to a discussion in the field of neurosciences, are microglia of myeloid origin or do they have their origin somewhere inside the brain? Microglia have originally been described by del Rio-Hortega in 1919 and he postulated them to have a mesodermal origin (Shemer et al. 2015). This would mean that the CNS is colonized with microglia before a myeloid line can infiltrate the CNS. Since we have a BBB, the myeloid origin seemed unlikely, but there is increasing evidence that this origin could be true. Circulating macrophages can infiltrate at a site of injury or inflammation and phagocytose debris and pathogens (Gordon et al. 2014). They also have a function in tissue development and remodelling (Pollard 2009) and act anti-inflammatory via the secretion of anti-inflammatory cytokines at the end of an inflammatory response (Fadok et al. 1998). These macrophages are derived from circulating monocytes, which have a myeloid origin and have an average half-life of about one day.

Tissue macrophages on the other hand, are found in nearly every tissue, they play a crucial role in development and homeostasis, and have an average lifespan of several months or even years. Tissue macrophages are important to the maintenance of tissues; it is their task to clear up apoptotic or necrotic cell debris. It has been established that microglia fall into this category of macrophages (Gordon et al. 2014). They play an important role in the maintenance of the brain by clearing unneeded neuronal synapses and clearance of apoptotic cell debris during development, and also in the adult brain. For years the origin of tissue macrophages was thought to be the bone marrow (BM) (van Furth & Cohn 1968; Sawyer et al. 1982; Volkman et al. 1983). This was widely accepted and would seem logical, since the BM is the largest producer of immune cells. However, because of the BBB it's more difficult to say what cells replace microglia, since under normal conditions white blood cells do not cross it.

Myeloid line hypothesis

The hypothesis that microglia are derived from monocytes arose because of the resemblance to them. Moreover, the discoverer of microglia, del Rio-Hortega, as stated before, postulated a mesodermal origin and that they could possibly be derived from circulating monocytes in adults. Several studies established homologies between monocytes/macrophages and microglia, making use of morphological features of both cell types (Murabe & Sano 1982) and immunohistochemical markers for F4/80 receptor and CD11b in mice (Perry et al. 1985). These studies led to the belief that microglia could be replenished by circulating monocytes after inflammation or other process leading to the formation of gitter cells. In mice, a knockout model for the macrophage colony-stimulating factor (MCSF) receptor (CD115), led to a near complete absence of tissue macrophages and severe growth and development defects (Dai et al. 2002; Li et al. 2006). For example, the near complete absence of microglia leads to disruption of normal brain formation causing enlarged ventricles and compressed parenchyma, most prominently in the olfactory bulb and cortex (Erblich et al. 2011). A knockout mice model for PU.1, a transcription factor crucial for myeloid cells, resulted in mice completely devoid of microglia (McKercher et al. 1996; Beers et al. 2006).

Another observation in the development of rodents supported the myeloid line hypothesis. Shortly after birth, the microglial population expands (Alliot et al. 1999) to an extent that it's hard to believe that these cells originate from the CNS alone. Studies using radiation and BM transplantation experiments provided further evidence for the myeloid regeneration of microglia population (Eglitis & Mezey 1997; Brazelton et al. 2000; Priller et al. 2001). These studies underlined the possible myeloid origin of microglia, but there hasn't always been a consensus in the field. Two studies published in 1979 found hardly any homology between monocytes and microglia (Oehmichen et al.

1979; Wood et al. 1979). In the study of Wood, Gollahon, Tilzer, Vatz & Morantz they even questioned the existence of microglia as a distinct cell type.

The evidence suggested by the BM transplant studies possibly did not represent normal physiological conditions. In these models, BM derived cells have the possibility to colonize the CNS and act as microglia. The main problem with these studies is that whole-body irradiation harms BBB integrity, possibly causing the infiltration of BM-derived myeloid cells into the CNS (Mildner et al. 2007). The knockout models only provide indirect evidence for the myeloid line hypothesis, just because the development of a certain cell line is dependent on a factor, does not mean that other cell lines are not dependent on it. The evidence provided by studying the immunohistochemical properties of microglia, especially CD11b, which is a receptor for iC3b of the complement system, is explained by the homeostatic properties of microglia. Considering microglia eliminate unneeded neuronal synapses via a complement dependent manner (Schafer et al. 2012; Stevens et al. 2007).

Yolk sac origin

The yolk sac "hypothesis" only applies to the embryonic stage, after all, adult humans, or other adult mammals, have no yolk sac. During embryonic development, the yolk sac is one of the first structures distinguishable via ultrasound in humans. It is important for early embryonic blood supply, being the first site of haematopoiesis and importantly, it is of mesodermal origin as postulated by del Rio-Hortega (Golub & Cumano 2013). This school emerged around 1990, when a study reported the presence of microglial cells in the foetal mouse cerebellum (Ashwell 1990) and later in the rat forebrain (Ashwell & Waite 1991). As it often goes with studies which contradict the current knowledge, the findings of these studies were not immediately recognized. Even as recent as 2007, microglia were still thought of as having peripheral mesodermal (myeloid) origin (Chan et al. 2007). However, more recent findings have confirmed the yolk sac origin of microglial precursors, as will be described below.

The other reason it is important to state that microglia have YS origin and are self-renewing is their role in the development of the brain, in which they have an important role as phagocytic cells aiding in tissue remodelling. These functions, studied using animal models, are aptly described in the review from Bilimoria and Stevens (Bilimoria & Stevens 2015). Microglia seem to play an important role in programmed cell death in the developing brain, in which they seem to appear in waves (Ashwell et al. 1989; Dailey et al. 2013; Dalmau et al. 1998) Since the BBB is closed at E13.5 (figure 3), colonization of the CNS must occur in time to make sure the brain will develop normally. All these findings give microglia a more central role in the CNS compared to the older view of them as being the "evil" phagocytic cell.

Current views

Origin

Our current views in understanding the origin of microglia has shifted, now it has been established that the original population of tissue macrophages are derived from uncommitted c-Kit$^+$ stem cells which are yolk sac erythro-myeloid progenitors (Perdiguero et al. 2014; Kierdorf et al. 2013). Overall, tissue macrophages have been found to be largely self-renewing, independently of haematopoietic stem cells (HSCs) (Ginhoux et al. 2010; Schulz et al. 2012; Hashimoto et al. 2013; Yona et al. 2013; Guilliams et al. 2013; Jakubzick et al. 2013; Epelman et al. 2014) with the exception of intestinal macrophages (Bain et al. 2014). Microglia are dependent on several factors for development: PU.1, Irf8 (Kierdorf et al. 2013) and the colony-stimulating factor 1 receptor (CSF1R) (Erblich et al. 2011; Ginhoux et al. 2010), these factors make the c-Kit$^+$ stem cells develop into CD45$^+$/c-Kit$^-$/CX3CR1$^+$ cells which migrate to the CNS, and this only happens after circulation is established. Only after migration of microglia progenitors to the CNS does the BBB form, effectively closing it off for the periphery. These findings mean that microglia are indeed of mesodermal origin, as postulated by del Rio-Hortega.

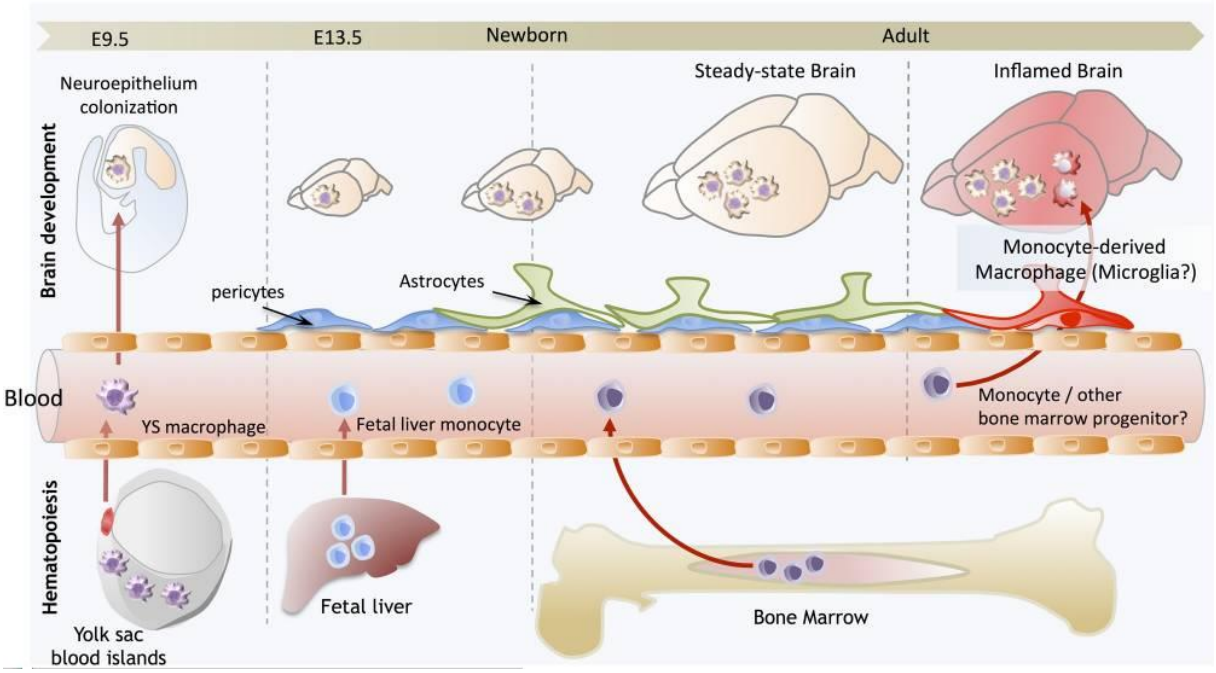

Figure 3: Schematic overview of the origin of microglia: c-Kit$^+$ stem cells migrate from the yolk sac blood islands to the neuroepithelium to develop into microglia. The BBB is formed, protecting the CNS from colonization by foetal (American English: fetal) liver macrophages. After birth microglia population expands and colonize the entire CNS, in normal steady-state conditions the population will maintain itself. Only during certain conditions, such as inflammation, can BM-derived monocytes enter the CNS to supplement the microglial population. Image from: Ginhoux, Lim, Hoeffel, Low, & Huber, 2013(Ginhoux et al. 2013), used with permission from the author.

Recent studies have used inhibitors for the factors described in the above paragraph to deplete the CNS in adult mice from microglia without the use of radiation. The article of Bruttger et al. (2015) used a system which allowed conditional depletion of microglia population without impairing BBB integrity. In this model they generated mice which, when injected with diphtheria toxin, were depleted of microglia making use of the CX3CR1 promoter. In the study of Elmore et al. (2014) they used a CSF1R inhibitor to conditionally deplete microglia population, also without impairing BBB integrity, which was checked by using Evans blue (Hawkins & Egleton 2006). Both studies found that after depletion of the residing microglia pool, the CNS was completely repopulated after 1 week when treatment was arrested. These findings implicate local regenerative

properties of microglia population, which is of great clinical significance. Importantly, no behavioural or cognitive abnormalities have been observed in mice devoid of microglia, providing opportunities for therapies in neuroinflammation by completely depleting the patient of microglia, effectively stopping inflammation (Elmore et al. 2014). Moreover, they tested the effect of immune changes in the microglia depleted brain by administering either LPS or PBS, this showed that no immune response was initiated by the remaining cells of the CNS (Elmore et al. 2014). Both studies also found that repopulation is dependent on the neuronal stem cell marker Nestin. Furthermore, Bruttger et al. showed that repopulation was dependent on interleukin-1 (IL-1) signalling, as administering an IL-1 receptor antagonist severely impaired microglia repopulation. They also found that morphologically the microglia were different: round instead of ramified. Plus it was found that BM derived macrophages, acting as microglia, display a different gene expression profile compared to microglia and keep expressing a different gene expression profile when populating the CNS (Bruttger et al. 2015).

Other findings

Another recent study has provided evidence for the existence of a CNS lymphatic system, which was believed to be absent (Louveau et al. 2015). Previously it was thought that the CNS had another route for antigen delivery, namely via drainage to the cerebrospinal fluid, which would allow meningeal macrophages and other antigen presenting cells (APCs) in the subarachnoid space to sample all CNS antigens (Ransohoff & Engelhardt 2012). This newly discovered lymphatic system is actually connected to the peripheral lymphatic system. The presence of a lymphatic system in the CNS as stated by the authors: “may call for a reassessment of basic assumptions in neuroimmunology and sheds new light on the aetiology of neuroinflammatory and neurodegenerative diseases associated with immune system dysfunction (Louveau et al. 2015).” This finding is important in light of the APC role of microglia, since presenting antigens to immune cells might be a much smaller step for microglia than previously anticipated. This would eventually lead to antibody production and better opsonisation and phagocytosis of whatever antigen was presented. This review will not go further into detail about the mechanisms of APCs, but this discovery is probably too significant not to mention.

Relevance

Why is the origin and the proliferation of microglia an interesting subject? The reason is that inflammation of the CNS has been observed in multiple degenerative diseases such as Alzheimer’s disease (AD), Parkinson’s disease (PD), multiple sclerosis (MS), HIV-induced dementia and stroke (Kim & de Vellis 2005). As stated before, inflammation of the CNS is primarily mediated by microglial activation, making microglia an important target in fighting these diseases. If microglia were in fact BM derived they could be used therapeutically by exploiting their ability to colonize the CNS, which would be a non-invasive means for cell therapy. Since this is not the case, it would seem this route can’t be utilized for means of therapy. However, BBB integrity is lowered in patients suffering from MS (Larochelle et al. 2011) and Alzheimer’s disease (Brkic et al. 2015), so these might be an exception. Though BBB integrity is impaired in these diseases, the origin of microglia should still be taken into account, as the myeloid contribution to the CNS immune system might be very low.

In AD, the clearance of amyloid-β plaques (Aβ) is impaired, and a central role for microglia is possible. Since normal human monocytes or macrophages have the ability to clear Aβ very well, this ability seems to be lost in AD (Fiala et al. 2005). For example, higher expression of CD33 in microglia seem to correlate with an inhibition to uptake Aβ in mice (Griciuc et al. 2013). The same goes for TREM2 in late-onset AD (Guerreiro et al. 2013). Recently big European consortium studies are being performed to find genetic associations with AD (Cacace et al. 2015; Cuyvers et al. 2015). This shows

that this is a field of ongoing studies, with microglia at the centre of attention. However, another study shows that depletion of microglia population did not affect Aβ formation and maintenance (Grathwohl et al. 2009). All in all, it's hard to give microglia a causal role in brain pathologies. Although microglia are increasingly implicated in a variety of neurodegenerative conditions, their exact role is still unclear. In certain conditions, like obsessive compulsive behaviour (Chen et al. 2010) and neuropathic pain (Inoue & Tsuda 2009), microglia are causally linked to the disease phenotype, where in other conditions, microglia dysfunction might be a consequence of the disease and contribute to its progression.

Conclusion

In this thesis the developments in the field of microglia were reviewed together with the history and other important findings. Microglia are the principal immune effector cells of the CNS and have important functions in tissue homeostasis. The origin of microglia has been discussed, together with the replenishment after microglia depletion. This all gives us insight into our current knowledge of the role microglia in health and disease. Recent studies using fate-mapping techniques have elucidated the origin of microglia, as well as other tissue macrophages (Ginhoux et al. 2010; Schulz et al. 2012; Hashimoto et al. 2013; Yona et al. 2013; Guilliams et al. 2013; Jakubzick et al. 2013; Epelman et al. 2014). Findings of these studies suggest that microglia and other tissue macrophages are derived from yolk-sac erythro-myeloid progenitors. Replenishment of tissue macrophage and thus microglial population was found to happen locally and not from circulating monocytes., with exception of the intestines (Bain et al. 2014).

Recent findings have caused for a rapid expansion in the field of neurosciences, especially influencing the way we look at neuroinflammation and neurodegeneration. Since the origin of microglia has been established and the fact that they're replenished locally and not from circulating monocytes, we can eliminate the possibility to use these monocytes as a therapeutic target or for use as a Trojan horse for administering drugs in the CNS. The microglia pool can be regenerated locally indicating that, with a functional BBB in place, possible drugs should possibly be administered to the CNS directly for maximum effect, this of course has other difficulties. However, it also ensures that other tissue macrophages are not affected. How repopulation is triggered after cell death is also a mystery, especially in steady state conditions. This repopulation could be caused by the sensing of an empty niche by other microglia. Though important in maintaining the microglia population, it is unknown what the role of IL-1 and CSF1 are in the homeostasis of the normal adult brain. Thought of as being a marker for developing BM macrophages, Nestin was found on proliferating microglia (Elmore et al. 2014; Bruttger et al. 2015). This would mean that Nestin is expressed on myeloid cells regardless of their place of origin. The speed at which the microglia population is re-established means that they're most likely indispensable for brain integrity in steady state conditions. This is confirmed by studies in mice where an absence of microglia resulted in rapid regeneration of microglial population (Elmore et al. 2014; Bruttger et al. 2015).

The role of the CNS lymphatic system is also to be assessed, since it questions the role of microglia in the development of autoimmune disorders such as MS and possibly amyotrophic lateral sclerosis, though the last isn't always considered as an autoimmune disorder. The presence of this lymphatic system could mean that antigen presenting (phagocytic) microglia have a shorter route to the periphery for activating T-cells than previously thought, opening up possibilities for therapy. The findings of this system could mean that the peripheral immune system has a larger role in brain pathologies than previously thought. Actually, even the exact role of microglia in disease has not been completely established, some studies underline the homeostatic role of microglia more than the phagocytic role. Though recently large consortia in the field of Alzheimer's disease have put microglia at their centre of attention, other fields studying CNS pathologies have shifted away from microglia showing a more central role for cells from the peripheral immune system (Wraith & Nicholson 2012; Mosley et al. 2012).

The establishment of the homeostatic role of microglia could possibly mean that we've not yet identified all phenotypes. Ramified microglia could be a diverse population, currently found to be just one population. It is possible that they have different roles in different areas of the CNS, perhaps cerebellum microglia have a different phenotype compared to those in the cerebrum. Also, we do not know the microglia half-life, which has been studied in mice using radioactive isotopes (Lawson

et al. 1992). Radioactivity causes disruption of BBB integrity, plus mice do not represent humans in the best possible way. It is important to know the life span of microglia before making them a therapeutic target to prevent over-dosage of drugs or possibly even inducing disease itself. It has been established that IL-1 and CSF1 are important for maintaining microglial population, which both might have their own therapeutic implications (Bruttger et al. 2015; Elmore et al. 2014). Moreover, the establishment that depleting microglial population has no effect on mice cognitive or other behaviour might be important in therapy also (Elmore et al. 2014). This combined with the finding that replacing microglial population with a myeloid line cured obsessive grooming in mice, might even suggest that microglia could be a target for psychological disorders, but this is a dangerous field (Chen et al. 2010).

Microglia are important cells in the CNS, with roles in development, maintenance and clearance of debris. Their exact role in homeostasis, pathology and development is still to be elucidated, but recent findings have paved the way. A lot of questions regarding microglia have been answered, regarding their origin and how the population is replenished after inflammation or other injury. Their possible central role in brain homeostasis and tissue maintenance makes them an interesting target for studies regarding CNS pathology and homeostasis. Future research will focus on their role in disease and their homeostatic function, as well as their role in tissue remodelling in the developing brain. More research will clear up questions regarding these cells.

References

Alliot, F., Godin, I. & Pessac, B., 1999. Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. *Brain research. Developmental brain research*, 117(2), pp.145–52. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10567732 [Accessed December 20, 2015].

Aloisi, F., 2001. Immune function of microglia. *Glia*, 36(2), pp.165–179. Available at: http://doi.wiley.com/10.1002/glia.1106 [Accessed January 11, 2016].

Ashwell, K., 1990. Microglia and cell death in the developing mouse cerebellum. *Brain research. Developmental brain research*, 55(2), pp.219–30. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2253324 [Accessed January 20, 2016].

Ashwell, K.W. et al., 1989. The appearance and distribution of microglia in the developing retina of the rat. *Visual neuroscience*, 2(5), pp.437–48. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2487081 [Accessed January 20, 2016].

Ashwell, K.W. & Waite, P.M., 1991. Cell death in the developing trigeminal nuclear complex of the rat. *Brain research. Developmental brain research*, 63(1-2), pp.291–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1724212 [Accessed January 20, 2016].

Bain, C.C. et al., 2014. Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nature immunology*, 15(10), pp.929–37. Available at: http://www.nature.com.proxy-ub.rug.nl/ni/journal/v15/n10/full/ni.2967.html [Accessed December 11, 2015].

Beers, D.R. et al., 2006. Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. *Proceedings of the National Academy of Sciences of the United States of America*, 103(43), pp.16021–6. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1613228&tool=pmcentrez&rendertype=abstract [Accessed January 20, 2016].

Bilimoria, P.M. & Stevens, B., 2015. Microglia function during brain development: New insights from animal models. *Brain research*, 1617, pp.7–17. Available at: http://www.sciencedirect.com/science/article/pii/S0006899314016163 [Accessed November 11, 2015].

Brazelton, T.R. et al., 2000. From marrow to brain: expression of neuronal phenotypes in adult mice. *Science (New York, N.Y.)*, 290(5497), pp.1775–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11099418 [Accessed January 20, 2016].

Brkic, M. et al., 2015. Amyloid Oligomers Disrupt Blood-CSF Barrier Integrity by Activating Matrix Metalloproteinases. *Journal of Neuroscience*, 35(37), pp.12766–12778. Available at: http://www.jneurosci.org.proxy-ub.rug.nl/content/35/37/12766 [Accessed September 17, 2015].

Bruttger, J. et al., 2015. Genetic Cell Ablation Reveals Clusters of Local Self-Renewing Microglia in the Mammalian Central Nervous System. *Immunity*, 43(1), pp.92–106. Available at: http://www.cell.com/article/S1074761315002563/fulltext [Accessed September 25, 2015].

Cacace, R. et al., 2015. Rare Variants in PLD3 Do Not Affect Risk for Early-Onset Alzheimer Disease in a European Consortium Cohort. *Human mutation*, 36(12), pp.1226–35. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26411346 [Accessed January 26, 2016].

Chan, W.Y., Kohsaka, S. & Rezaie, P., 2007. The origin and cell lineage of microglia: new concepts. *Brain research reviews*, 53(2), pp.344–54. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17188751 [Accessed November 27, 2015].

Chen, S.-K. et al., 2010. Hematopoietic origin of pathological grooming in Hoxb8 mutant mice. *Cell*, 141(5), pp.775–85. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2894573&tool=pmcentrez&rendertype=abstract [Accessed January 27, 2016].

Cronk, J.C. & Kipnis, J., 2013. Microglia - the brain's busy bees. *F1000prime reports*, 5, p.53.

Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3854698&tool=pmcentrez&rendertype=abstract [Accessed December 17, 2015].

Cuyvers, E. et al., 2015. Genetic variability in SQSTM1 and risk of early-onset Alzheimer dementia: a European early-onset dementia consortium study. *Neurobiology of aging*, 36(5), pp.2005.e15–22. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25796131 [Accessed January 27, 2016].

Dai, X.-M. et al., 2002. Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects. *Blood*, 99(1), pp.111–20. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11756160 [Accessed December 13, 2015].

Dailey, M.E. et al., 2013. Imaging microglia in brain slices and slice cultures. *Cold Spring Harbor protocols*, 2013(12), pp.1142–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24298036 [Accessed January 20, 2016].

Dalmau, I. et al., 1998. Development of microglia in the postnatal rat hippocampus. *Hippocampus*, 8(5), pp.458–74. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9825958 [Accessed January 20, 2016].

Daneman, R. & Prat, A., 2015. The Blood–Brain Barrier. *Cold Spring Harbor Perspectives in Biology*, 7(1), p.a020412. Available at: http://cshperspectives.cshlp.org/content/7/1/a020412.long [Accessed January 11, 2016].

Davis, E.J., Foster, T.D. & Thomas, W.E., 1994. Cellular forms and functions of brain microglia. *Brain research bulletin*, 34(1), pp.73–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8193937 [Accessed January 18, 2016].

Dudvarski Stankovic, N. et al., 2015. Microglia-blood vessel interactions: a double-edged sword in brain pathologies. *Acta neuropathologica*. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26711460 [Accessed January 4, 2016].

Eglitis, M.A. & Mezey, E., 1997. Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. *Proceedings of the National Academy of Sciences of the United States of America*, 94(8), pp.4080–5. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=20571&tool=pmcentrez&rendertype=abstract [Accessed January 20, 2016].

Elmore, M.R.P. et al., 2014. Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. *Neuron*, 82(2), pp.380–97. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4161285&tool=pmcentrez&rendertype=abstract [Accessed November 24, 2015].

Epelman, S. et al., 2014. Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. *Immunity*, 40(1), pp.91–104. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3923301&tool=pmcentrez&rendertype=abstract [Accessed January 20, 2016].

Erblich, B. et al., 2011. Absence of colony stimulation factor-1 receptor results in loss of microglia, disrupted brain development and olfactory deficits. *PloS one*, 6(10), p.e26317. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3203114&tool=pmcentrez&rendertype=abstract [Accessed January 17, 2016].

Fadok, V.A. et al., 1998. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *The Journal of clinical investigation*, 101(4), pp.890–8. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=508637&tool=pmcentrez&rendertype=abstract [Accessed July 17, 2015].

Fiala, M. et al., 2005. Ineffective phagocytosis of amyloid-beta by macrophages of Alzheimer's disease patients. *Journal of Alzheimer's disease : JAD*, 7(3), pp.221–32; discussion 255–62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16006665 [Accessed January 27, 2016].

van Furth, R. & Cohn, Z.A., 1968. The origin and kinetics of mononuclear phagocytes. *The Journal of experimental medicine*, 128(3),

pp.415–35. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2138527&tool=pmcentrez&rendertype=abstract [Accessed November 10, 2015].

Gehrmann, J., Matsumoto, Y. & Kreutzberg, G.W., 1995. Microglia: intrinsic immuneffector cell of the brain. *Brain research. Brain research reviews*, 20(3), pp.269–87. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7550361 [Accessed January 11, 2016].

Ginhoux, F. et al., 2010. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science (New York, N.Y.)*, 330(6005), pp.841–5. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3719181&tool=pmcentrez&rendertype=abstract [Accessed February 6, 2015].

Ginhoux, F. et al., 2013. Origin and differentiation of microglia. *Frontiers in Cellular Neuroscience*, 7, p.45. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3627983&tool=pmcentrez&rendertype=abstract [Accessed October 5, 2015].

Golub, R. & Cumano, A., 2013. Embryonic hematopoiesis. *Blood cells, molecules & diseases*, 51(4), pp.226–31. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24041595 [Accessed January 15, 2016].

Gordon, S., Plüddemann, A. & Martinez Estrada, F., 2014. Macrophage heterogeneity in tissues: phenotypic diversity and functions. *Immunological Reviews*, 262(1), pp.36–55. Available at: /pmc/articles/PMC4231239/?report=abstract [Accessed January 12, 2016].

Grathwohl, S.A. et al., 2009. Formation and maintenance of Alzheimer's disease beta-amyloid plaques in the absence of microglia. *Nature neuroscience*, 12(11), pp.1361–3. Available at: http://www.nature.com.proxy-ub.rug.nl/neuro/journal/v12/n11/full/nn.2432.html [Accessed January 27, 2016].

Griciuc, A. et al., 2013. Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta. *Neuron*, 78(4), pp.631–43. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23623698 [Accessed June 2, 2015].

Guerreiro, R. et al., 2013. TREM2 variants in Alzheimer's disease. *The New England journal of medicine*, 368(2), pp.117–27. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3631573&tool=pmcentrez&rendertype=abstract [Accessed January 17, 2016].

Guilliams, M. et al., 2013. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. *The Journal of experimental medicine*, 210(10), pp.1977–92. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3782041&tool=pmcentrez&rendertype=abstract [Accessed November 5, 2015].

Hashimoto, D. et al., 2013. Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity*, 38(4), pp.792–804. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3853406&tool=pmcentrez&rendertype=abstract [Accessed July 13, 2014].

Hawkins, B.T. & Egleton, R.D., 2006. Fluorescence imaging of blood-brain barrier disruption. *Journal of neuroscience methods*, 151(2), pp.262–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16181683 [Accessed January 20, 2016].

Hickey, W.F. & Kimura, H., 1988. Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo. *Science (New York, N.Y.)*, 239(4837), pp.290–2. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3276004 [Accessed December 20, 2015].

Hugh Perry, V., 1998. A revised view of the central nervous system microenvironment and major histocompatibility complex class II antigen presentation. *Journal of Neuroimmunology*, 90(2), pp.113–121. Available at: http://www.sciencedirect.com/science/article/pii/S0165572898001453 [Accessed January 11, 2016].

Inoue, K. & Tsuda, M., 2009. Microglia and neuropathic pain. *Glia*, 57(14), pp.1469–79. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19306358 [Accessed January 28, 2016].

Jakubzick, C. et al., 2013. Minimal differentiation of classical monocytes as they survey steady-state tissues and transport antigen to lymph nodes. *Immunity*, 39(3), pp.599–610. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3820017&tool=pmcentrez&rendertype=abstract [Accessed October 27, 2015].

Kierdorf, K. et al., 2013. Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nature neuroscience*, 16(3), pp.273–80. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23334579 [Accessed December 1, 2015].

Kim, S.U. & de Vellis, J., 2005. Microglia in health and disease. *Journal of neuroscience research*, 81(3), pp.302–13. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15954124 [Accessed January 7, 2016].

Larochelle, C., Alvarez, J.I. & Prat, A., 2011. How do immune cells overcome the blood-brain barrier in multiple sclerosis? *FEBS letters*, 585(23), pp.3770–80. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21550344 [Accessed December 23, 2015].

Lawson, L.J., Perry, V.H. & Gordon, S., 1992. Turnover of resident microglia in the normal adult mouse brain. *Neuroscience*, 48(2), pp.405–15. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1603325 [Accessed September 25, 2015].

Li, J. et al., 2006. Conditional deletion of the colony stimulating factor-1 receptor (c-fms proto-oncogene) in mice. *Genesis (New York, N.Y. : 2000)*, 44(7), pp.328–35. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16823860 [Accessed January 18, 2016].

Louveau, A. et al., 2015. Structural and functional features of central nervous system lymphatic vessels. *Nature*, 523(7560), pp.337–341. Available at: http://www.nature.com.proxy-ub.rug.nl/nature/journal/v523/n7560/full/nature14432.html [Accessed June 1, 2015].

McKercher, S.R. et al., 1996. Targeted disruption of the PU.1 gene results in multiple hematopoietic abnormalities. *The EMBO journal*, 15(20), pp.5647–58. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=452309&tool=pmcentrez&rendertype=abstract [Accessed January 20, 2016].

Mildner, A. et al., 2007. Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. *Nature neuroscience*, 10(12), pp.1544–53. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18026096 [Accessed December 7, 2015].

Mosley, R.L. et al., 2012. Inflammation and adaptive immunity in Parkinson's disease. *Cold Spring Harbor perspectives in medicine*, 2(1), p.a009381. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3253034&tool=pmcentrez&rendertype=abstract [Accessed January 28, 2016].

Murabe, Y. & Sano, Y., 1982. Morphological studies on neuroglia. VI. Postnatal development of microglial cells. *Cell and tissue research*, 225(3), pp.469–85. Available at: http://www.ncbi.nlm.nih.gov/pubmed/6290069 [Accessed January 15, 2016].

Oehmichen, M. et al., 1979. Features and distribution of intracerebrally injected peritoneal macrophages. *Experimentelle Pathologie*, 17(2), pp.71–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/446592 [Accessed January 20, 2016].

Perdiguero, E.G. et al., 2014. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature*, 518(7540), pp.547–551. Available at: http://dx.doi.org/10.1038/nature13989 [Accessed December 3, 2014].

Perry, V.H., Hume, D.A. & Gordon, S., 1985. Immunohistochemical localization of macrophages and microglia in the adult and developing mouse brain. *Neuroscience*, 15(2), pp.313–26. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3895031 [Accessed January 15, 2016].

Pollard, J.W., 2009. Trophic macrophages in development and disease. *Nature reviews. Immunology*, 9(4), pp.259–70. Available at: /pmc/articles/PMC3648866/?report=abstract [Accessed January 14, 2016].

Priller, J. et al., 2001. Targeting gene-modified hematopoietic cells to the central nervous system: use of green fluorescent protein uncovers microglial engraftment. *Nature medicine*, 7(12), pp.1356–61. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11726978 [Accessed January 20, 2016].

Ransohoff, R.M. & Engelhardt, B., 2012. The anatomical and cellular basis of immune surveillance in the central nervous system. *Nature reviews. Immunology*, 12(9), pp.623–35. Available at: http://www.nature.com.proxy-ub.rug.nl/nri/journal/v12/n9/full/nri3265.html [Accessed September 5, 2015].

Sawyer, R.T., Strausbauch, P.H. & Volkman, A., 1982. Resident macrophage proliferation in mice depleted of blood monocytes by strontium-89. *Laboratory investigation; a journal of technical methods and pathology*, 46(2), pp.165–70. Available at: http://www.ncbi.nlm.nih.gov/pubmed/6174824 [Accessed January 20, 2016].

Schafer, D.P. et al., 2012. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron*, 74(4), pp.691–705. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3528177&tool=pmcentrez&rendertype=abstract [Accessed May 18, 2015].

Schulz, C. et al., 2012. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science (New York, N.Y.)*, 336(6077), pp.86–90. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22442384 [Accessed January 30, 2015].

Shemer, A. et al., 2015. Microglia Plasticity During Health and Disease: An Immunological Perspective. *Trends in immunology*, 36(10), pp.614–24. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26431939 [Accessed January 4, 2016].

Stevens, B. et al., 2007. The classical complement cascade mediates CNS synapse elimination. *Cell*, 131(6), pp.1164–78. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18083105 [Accessed August 24, 2015].

Volkman, A. et al., 1983. Differential effects of chronic monocyte depletion on macrophage populations. *Laboratory investigation; a journal of technical methods and pathology*, 49(3), pp.291–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/6887784 [Accessed January 20, 2016].

Wood, G.W. et al., 1979. The failure of microglia in normal brain to exhibit mononuclear phagocyte markers. *Journal of neuropathology and experimental neurology*, 38(4), pp.369–76. Available at: http://www.ncbi.nlm.nih.gov/pubmed/448398 [Accessed January 20, 2016].

Wraith, D.C. & Nicholson, L.B., 2012. The adaptive immune system in diseases of the central nervous system. *The Journal of clinical investigation*, 122(4), pp.1172–9. Available at: http://www.jci.org/articles/30010 [Accessed January 28, 2016].

Yona, S. et al., 2013. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity*, 38(1), pp.79–91. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3908543&tool=pmcentrez&rendertype=abstract [Accessed July 10, 2014].

Zlokovic, B. V, 2008. The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron*, 57(2), pp.178–201. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18215617 [Accessed July 15, 2015].