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*Review Article*

# Microglia, the Sentinel of Brain in the Evolution of Nervous System from Invertebrate to Vertebrate: A Short Review

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**Abstract**

The presence of microglial cells as resident macrophage population in the Central Nervous System (CNS) is well documented from the study of repairing of lesions in CNS that varies widely throughout the animal kingdom. The existence of neuroglia cells similar to vertebrate microglia and small mobile phagocytes and hemocytes were documented from ganglia of some invertebrate animal models like leech (*H. medicinalis*), insects (*P. americana and D. melanogaster*) and mollusca (*M. edulis*). Neuronal replacement and migration of immunocompetent cells (macrophage, microglia, ependymal cells etc.) after surgical lesions in CNS of non-mammals (fishes, reptiles and aves) are much restricted to specific neurogenic niches associated to the neural regeneration and migration of cells in invertebrates. Microglial presence is largely restricted in the optic tract of fish and amphibian ganglionic cells because they have a surprising capacity to regenerate their neurons after lesions. Hence the CNS of both invertebrates and vertebrates contain microglia like mononuclear phagocytes, ensheathing glia and reticular glia, which indicate about the evolutionary conserved innate immune response to maintain CNS development and health. But the presence and gradual changes in the structure and function of microglia and neuron-microglia relationship in the CNS along the phylogeny need to be focused thoroughly.

*Keywords*: Neuroglia cells; Hemocytes; Neurogenic niches; Lesions; Phylogeny.


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## 1. Introduction

The existence of vertebrate like immune system in invertebrates is the cynosure of interest among workers since few decades when it was observed that there exist several reactions in invertebrate animals against lesions and infections. Although analogies with the vertebrate immune response do exist, it is not easy to assume that all animals of

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invertebrates show similar specificity and sensitivity of immune cells in order to combat infections and lesions effectively. Mostly the innate immunity and role of phagocytic cells were observed in the invertebrate immune systems where some area specific macrophages like cells were found active [1,2]. In higher vertebrates, particularly in primates, we found a highly sophisticated and sensitive defence mechanism is active for the central nervous system (CNS), where microglia is the major immune-competent cell working as CNS tissue macrophage [3,4]. This brain structure appeared from simple nerve net and ganglion, and developed to complex cephalization forming the brain in higher vertebrates through the phylogeny (Fig. 1). However, the advent of microglia in the evolutionary hierarchy from lower invertebrates to higher vertebrates is not clearly evidenced and the evidences are quite sporadic in nature. Therefore, the development of immunity in brain from invertebrates to higher vertebrates needs to be focused in a broader sense which may reflect the general, conserved functions and deviation of the standard. The information and understanding acquired will help us to understand the disease response of microglia in diversified situation.

In discussing the invertebrate defence mechanism, one must consider both the diversity of immune cells exhibited by this group of animals and their phylogenetic linkages. The appearance of diverse type of phagocytic cells like macrophage, microglia, dendritic cells etc. during inflammatory reactions or lesions in various invertebrates show uniformity in development of immune mechanisms along the phylogenetic lineages. The basic function of any immune system can be considered in removal of infectious agents and debris, since cells specialized for phagocytosis occurs universally in animals [5]. In order to function effectively, the phagocytes must be able to distinguish unwanted matters from living tissue of the host. As the primary immune system of brain, the phagocytic and related effector functions of the cells in and around ganglia or primary nerve centres in invertebrates and cephalized CNS of vertebrates are considered primordial brain macrophages or microglia.

In the present review we will try to present the evidences of presence of such primordial microglia like cells or equivalents among invertebrates and vertebrates through the phylogeny to showcase their evolutionary continuity and conserved role in relation to the evolving nervous system. As most of our present day knowledge is derived from the mammalian brain, we have tried to search and highlight the findings which are scattered within non-mammalian taxa starting from annelids to aves as they are not commonly discussed, but possess immense importance in understanding the function of microglia in the models of higher taxa and human neuro-pathophysiology.

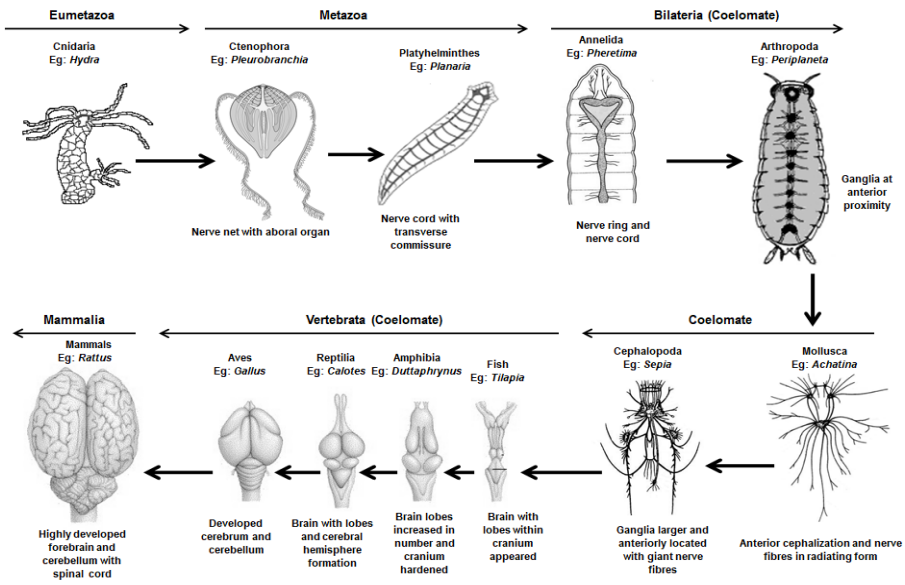


Fig. 1. Development of nervous system towards cephalization and brain formation (adapted from https://cronodon.com/BioTech/Ctenophores.html and http://www231.pair.com/fzwester/courses/bis10v/week10/12nervevolution.html

## 2. Microglia: An Essential Outsider in the Nervous System

Various experiments of Metchnikoff's (1905) developed the idea that amoeboid cell like macrophages, hemocytes, amoebocytes are involved in intracellular digestion which are retained throughout the evolution of more advanced animals, since they are capable of removing the inert particles, noncellular debris and pathogens, thereby contributing to the cellular immunity and host defence mechanism [6]. Gradually, the concept of tissue resident macrophages was established and it was found that myeloid lineage cells may have a wide range of morpho-functional forms [7,8]. Such myeloid cells which are found entangled with the ganglionic structures of nervous systems or embedded in the developed CNS tissue had been designated as brain resident phagocytic cells in general and for vertebrate brain they are microglia.

The first description of microglia like cells although started much earlier [9], their exact structure and functions were still not well established. These CNS-resident immune cells were first named "microglia" by del Rio-Hortega who studied them in the medicinal leech using the silver carbonate staining method [10]. Resident macrophages and microglia present in the CNS of the invertebrates and higher vertebrates as part of the mononuclear phagocyte system play a vital role in injury repair, pruning of neuronal digits and contribute to sterile physiological inflammation, thereby regulating the homeostatic maintenance of a healthy organism [11-13]. In general microglial populations are the sentinel of central nervous system and are involved in any neuropathological condition.

However, microglia is not derived from neuro-ectodermal progenitor cells which are the sources of all neuro-glial cells in brain, but they are derived from mesodermal myeloid lineage cells [14,15]. From the experimental evidences of mice models it is now believed that microglia or brain resident macrophages enter within the developing CNS tissue in early embryonic days as primitive macrophages, reside there, proliferate and colonize to form microglia in post-natal and adult phases (Fig. 2). Conversely, some believe that the progenitor myeloid lineage cells are highly dynamic and are capable to repopulate in CNS even later in ontogenic development apart from this early embryonic phase [16-18].

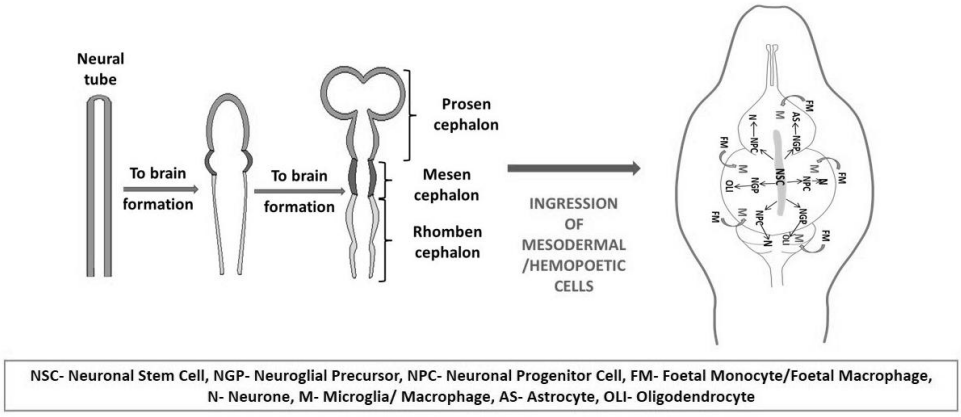


Fig. 2. Gradual development of brain from neural tube and incorporation of mesoderm derived foetal haemopoietic cells in developing brain.

## 3. Microglia and Similar Cells in Phylogeny

### 3.1. *Microglia in annelids*

The first specimen in which microglia was identified was the medicinal leech, *Hirudo medicinalis* when del Rio Hortega described microglia as a distinct and separate cellular population in the CNS [27]. The nerve cord of *Hirudo* sp having mid body segmental ganglia which consists of neurons and large population of microglial cells [28]. Unlike mammals, the leech CNS has demonstrated the capacity to repair and restore its function after injury [29] and the specimen showed microglia accumulation at the site of repair *in vivo* [30,31]. In another experiment, the immunoreactivity in leech CNS after axonal injury showed the accumulation of microglia as mobile phagocytes at the site of new laminin (extracellular matrix molecule) appearance and axonal sprouting [32]. The neurons and microglia were found to synthesize intrinsic antimicrobial peptides that exert neutrophilic properties against microbial exposure after CNS injury [33].

### 3.2. *Microglia in molluscs*

The presence of distinctive class of neuroglial cells in comparison to the vertebrate microglial cells was studied from the ganglionic excision of two different molluscs (*Planorbarius corneus*) and (*Mytilus edulis*), and a in an insect species (*Leucophaea maderae*). The emergence of small mobile cells were observed when the structures were maintained in incubation media. From stellate appearance, the cells transformed to rounded structure with some amoeboid movements. Functional characteristics of immunocytes similar to microglia in these species involved the translocation of the cells, phagocytic activity and cell adherence to culture dish [34].

Aplysia was also used as a suitable model as it has a simpler immune system with large and well-characterized neurons. Therefore, its CNS tissue preparation served to investigate responses to nerve injury [35]. The behaviours like hemocyte interaction with growth cones and release one or more factors that control neuronal inflammation *in vitro* that can recapitulate those of inflammatory properties *in vivo* [36-38]. The hemocytes population in Aplysia and some other invertebrates showed different cellular morphologies and can be distinguished sufficiently for their contents as well as by using monoclonal antibodies [40-42]. Such hemocytes around ganglionic structures resemble with the structure and functional properties of brain macrophages and microglia. More recent study in Aplysia showed that hemocytic influence on the fate of neurons through direct contact/ interactions after axotomy [43]. In another model, the injury response of the nervous system of the pond snail *Lymnaea stagnalis* showed that the Arg-Gly-Asp (RGD) motif/ peptide (an integrin recognition sequence) can modulate various attributes of the phagocytic activation of which showed particle involvement, oxidative burst and other responses both *in vitro* and *in vivo* resulting in significant modulation of regeneration of nerve cells [44].

### 3.3. *Insect microglial system*

Various scientists have chosen insects as a model to study the role of glial cells in repair mechanism notably for structural simplicity, its accessibility to electrophysiological study, and the possibility of extensive experimental manipulations [45]. Earlier investigations on general structural changes of nervous system in insects was studied primarily [19-21] which was followed by the study of glial system of cockroach (*Periplaneta americana* L.) central nervous connectives consisting of a superficial layer of interdigitating cells, the perineurium, and an underlying complex of neuroglial cells [46]. Glial cells disruption using glial toxin studied the role of glial repairing mechanism [47] and several studies were intended to find out the role of microglia in neuronal damage and regeneration in insect nervous system [22-26]. The appearance of granule containing cells (probably hemocytes) were found to involve in phagocytic activity in close adherence to the damaged area of CNS followed by increase in sub-perineural spaces, filled with infiltration precipitation protein from circulatory body fluid and glial cells [45]. Thus the

slow restoration within a month re-establishes the blood-brain barrier at the site of neuronal damage by glial repair mechanism.

Modelling innate immunity in *Drosophila melanogaster* produces several important insights in the field of evolutionary immunobiology. The initial study in *Drosophila melanogaster* provided mechanistic insight into peripheral immune response by hemocytes and fat bodies [48,49] and presence of glial cell population within the mushroom body [50,51]. Macrophages, the brain-resident myeloid cells [52] proliferate in the brain during naturally occurring cell death [53] and its neutrophilic role has been suggested [54]. The different cell types of glia-ensheathing glia and reticular glia, show microglia like behaviours where the former is a major sentinel cell type in *D. melanogaster* CNS [55]. Three basic classes of glial presence in insects (surface, cell body and neuropil glia) and glial developmental dynamics were studied elaborately in *Drosophila* sp and progenitor origin of these different populations of cells were possible to study due to specific markers and genetic tool availability [56].

### 3.4. *Microglia in fish*

Resident macrophage and microglial existence and their role in scavenging in the CNS of vertebrate innate immune system follows two basic approaches – first, lesioning and experimental manipulations *in vivo* [57,58] and second, the powerful combination of cultured cell lines with monoclonal antibody and biochemical techniques [59]. In lamprey spinal cord, microglia/macrophages distributed throughout the neural parenchyma were activated and increased in numbers after injury in the spinal cord and this response continued for several weeks. Microglial cells that expressed Semaphorin-3 were located on the surface of the spinal cord which acts as a diffusible attractant for neuronal repair and regeneration after spinal transaction [60].

The fish microglial population is not restricted only as the resting guardian of CNS; rather they are mobile for ongoing maintenance in the CNS [61,62]. Glioma cell biology produce a new opportunity to use different teleost models due to their easy availability, information on sequenced genome, manipulative capability of embryo in experimental condition and easy maintenance. Zebra fish (*Danio rerio*) was a suitable model in teleost within which neuronal insult in the brain elicits increase in microglial/ macrophage population [63] other than olfactory bulb [64]. Microglia have been identified previously in other areas of adult zebra fish brain such as optic tectum, telencephalon and the optic nerve [65-67] which has the ability to recover rapidly from injury [65,68,69].

It was found that teleost brain has much higher capability of neuronal regeneration and stronger glial activation in comparison to mammals after any neuronal injury. In carps and trouts, such phenomena was observed clearly [70,71]. Neuron- glial [72-74] studied in suitable model like puffer fish, showing supra-medullary neuronal association with microglial cells and astrocyte-like cells. Moreover fish glial cell culture derived from optic nerve/ retina [75-77] and microglial cell culture was studied from the brain of Tetraodontiform species [78,79]. Different studies on fish models of previous and present

days not only revealed several facts of the system but also had been instrumental to know the mammalian innate immune activities of brain. Some idea about how microglia acts in mammals and how they are developmentally associated with mesodermal cells, were the major aspects of research in neurobiology using the neuronal tissue of teleosts. The neuro-glial interrelation are considered in fish, particularly teleosts, and this model has been exploited to understand the neuronal tissue injury in several studies [80,81].

### 3.5. *Microglial in amphibians*

Within amphibians salamander served as a potent model system to work with the functions of cells in vertebrate wound healing and repair including brain lesions. It was found that macrophages were involved in early response of wound healing and they proliferate in brain where naturally occurring brain death was observed [53,82,83]. However, other studies showed that their recruitment in the injury site of CNS is scattered, and also some neutrophilic role of microglia were suggested earlier [54,84]. In adult frog spinal cord derived primary culture, macrophage like cells were found to interact with the growth cones for motor neurons and resulted in an elongation of the processes which eventually develop the motor neuron networks [85]. As the studies in amphibian model is scanty information is lacking regarding microglial action in guarding CNS microenvironment and immune clearance in the amphibian CNS.

### 3.6. *Microglial in reptiles*

The medial cortex similar to mammalian hippocampal fascia dentata [86-88] is the seat of microglial cell population in the lizards especially in the restricted areas of plexiform layers [89,90]. Medial cortex principal cell layer neurons lesioned by neurotoxin 3-acetyl pyridine (3AP); was followed by the development of new neurons regenerating in the new medial cortex with normal histological appearance [91,92]. The microglial role in the affected areas in dead cell clearance and regeneration activities was detected by labelling cells with nucleoside diphosphatase (NDPase) histochemical reaction [93,94]. Time lapse role in damage repair was studied with transitory disappearance of microglia [95] in the medial cortex in the early period of lesion (6-8 h to 15 days) which soon reappeared and scattered in form of ultrathin section within one month post-lesion period.

### 3.7. *Microglial in birds*

In comparison to other vertebrates, the brain of birds are much compact, distinct, short and broad. Cranial, pontine, cervical flexures are well marked and cerebral hemispheres are large due to enormous development of corpus striatum. Cerebellum is larger than other vertebrates except some mammals are an indicative of its physiological adaptation for aerial mode of flight. Optic nerves, chiasma and tracts are well developed and olivary nuclei enlarged within broad medulla. Several immunohistological techniques were used

in association with avian specific probes to determine oligodendrocyte presence and its distribution in the retina of chicken and quails with a central to peripheral gradient [96]. Microglial presence was detected by using monoclonal antibodies (QH1) in developing and matured quail brain [97]. *L. esculentum* lectin intensely stain microglial cells during the CNS development of *Gallus* sp showed similar location of ramified microglia as described in quail cerebellum and for cerebral hemisphere of chick embryo and chicks [98,99]. Lectin reactive cellular temporal spatial distribution showed microglial presence in cerebral hemisphere during early neurogenesis that tend to increase its' number during CNS differentiation [100]. According to Fujimoto, Miki and Mizoguti (1987) and Dalmau *et al*., (1997) have shown that amoeboid microglial cells after their phagocytic function undergo cell death to maintain good health of neural environment [98,101]. Hence during adverse condition in CNS, poorly ramified microglial cells are transformed into ramified form exhibiting cellular plasticity both in normal embryonic development and adult brain functioning.

The recent quantitative study on the total number of neurones and microglial cell populations in two migratory sandpipers in sub-arctic and mid-arctic tundra showed that although both the birds follow different migratory routes, their total hippocampal neurones are quantitatively same but differs in the size of hippocampus and in more hippocampal microglia [102]. The role of prostaglandin E2 (PGE2), a pro-inflammatory hormone and microglial influence in the sex specific brain sexual differentiation in the Japanese Quail showed a strong influence in the birds' reproductive behaviour [103]. Study on the virulent neurotropic strains of Newcastle disease virus (vNDV) infections in the poultry birds by double immunofluorescence (DIFA) techniques with biomarkers for neurones, astrocytes and microglia showed the NDV nucleoproteins presence in all the three cell types at similar levels [104]. This study is an indicative of the susceptibility of vNDV in all cell types (neurones and glial cells) similar to other paramyxovirus infections.

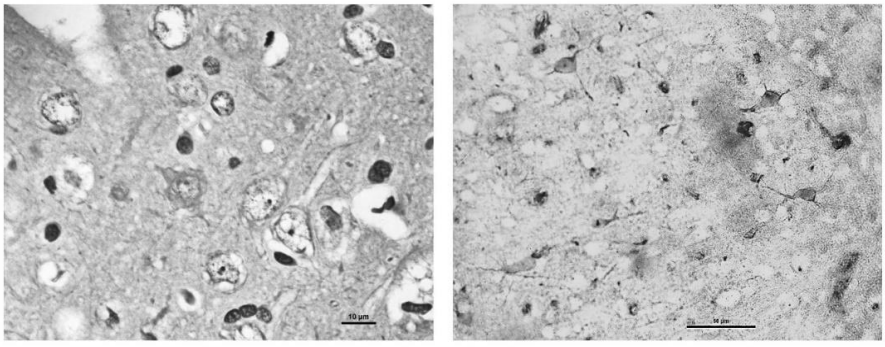


Fig. 3. Microglia in mammalian brain.

### 3.8. *Microglial in mammals*

The work on CNS macrophages, microglia or MPS cells are plenty in mouse, rat and human samples or models covering a hugely diverse functional aspects at present. Majority of our present knowledge on microglia and its function in different neuropathological conditions are derived from the mammalian system. Part of this knowledge in developmental aspects had been described in Fig. 2. Above are the microscopic images of microglia in rat brain (Fig. 3). Other aspects of mammalian microglia in CNS physiology and diseases are large enough to discuss in this review and beyond the focus of current discussion [11,18,105].

## 4. Conclusion

Present review showed that immune competent cells appeared and showed their immune efficacy early in the course of evolution. But, primarily these cells were not distinguishable with peripheral haemocytes in the invertebrates. They were associated closely with the ganglionic formations of diffused nervous system of invertebrates and were transient, i.e., not restricted to the nervous system of annelid, molluscs and in insects. However, they were found exerting innate immune functions locally and in Drosophila and medicinal leech models; such microglia like cells showed their active participation in tissue repairing and neuronal regeneration. These are the processes well documented in higher vertebrates. Also such nervous system associated cells in Drosophila showed primary evidences of cellular morpho-functional heterogeneity which are observed in fish and profoundly documented in higher vertebrates [55,81,106,107]. Within the limited experimental evidences in amphibians, reptiles and birds we can observe that a specialized cell population emerged gradually in the course of evolution which are restricted within the CNS as cephalization or formation of brain proceed. With evolved brain through phylogenetic lineage these brain restricted cells, which were previously transient, had become CNS specific resident immune cells with a restricted route of entry in early development of embryonic brain. They become more specialized and efficient with diverse surveillance and effector functions to protect the complicated brain tissue of higher vertebrate including primates where maximized development of cerebral cortex and higher brain centres are found [106,108]. Recent studies revealed a core conserved genetic programme persists within the microglial evolutionary precursors and the highly efficient, diverse and chief immune-competent cell in primate brain including human [109]. Therefore, microglial morpho-functional diversity, sensitivity, immune-efficacy and response to various neuro-glial disorders can be traced back to its evolutionary history. However, the studies on microglia or brain macrophages in invertebrates and lower vertebrates are much less and detailed phylogenetic studies are required to decipher the detailed functional evolution of this important CNS components which in turn will help us to know the cell function in our higher and complex brain.

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