

## Microglia of teleosts: facing a challenge in neurobiology

**B. Cuoghi, L. Mola**

Department of Paleobiology Museum and Botanical Garden, Anatomical Museums, University of Modena and Reggio Emilia, Modena, Italy



©2007 European Journal of Histochemistry

This review is concerned with recent literature on teleost fish CNS microglia. It covers not only various aspects of these cells, notably comparing them with mammalian microglia, but also points out the several potentialities neural tissue of teleosts exhibits in neurobiological research. The relationships between neurons and glial cells are considered in fish, aiming at an integrated picture of the complex ways neurons and glia communicate and collaborate in normal and injured neural tissues. In addition, attention has been paid to different teleost models according to their availability, easy maintenance in experimental conditions, possibilities of embryos manipulation and sequenced genome. The recent setting up of successful protocols for fish glia and mixed neuron-glia cultures, together with the molecular facilities offered from genome knowledge, should provide a new boost to studies about microglia and neuron-microglia relationships.

Key words: teleost, microglia, zebrafish, puffer, medaka.

Correspondence: Lucrezia Mola,  
Dipartimento del Museo di Paleobiologia e  
dell'Orto botanico, Musei Anatomici,  
via Berengario, 14, I-41100 Modena, Italy  
Tel.: +39.059.2055535, +39.059.2057132.  
Fax: +39.059.2055548.  
E-mail: lucrezia.mola@unimore.it

**Paper accepted on October 5, 2007**

**European Journal of Histochemistry  
2007; vol. 51 issue 4 (October-December):231-240**

Microglial cells, the resident macrophages of the brain, are the main immune cells of the CNS and respond rapidly to injury, infection and inflammation. After insult to the CNS the resting, ramified microglia retract their branches, regain an activated, amoeboid phenotype, become motile and migrate to the site of damage. A further proof about microglia involvement in CNS immunoprotection is the expression of antigenic markers specific to the immune system (Kreutzberg, 1996). Indeed, cell surface antigens that have been demonstrated on microglia in normal brain include the Fc and complement type-three (CR3) receptors indicating that these cells play a role in CNS defence (Perry and Gordon, 1988). Resident microglial cells are therefore a key component of the CNS innate immunity to pathogens, cell injuries and toxic proteins. Microglia take part to this natural response providing, together with infiltrating macrophages, inflammatory mediators and act in neuroprotective and neurodegenerative processes (Glezer *et al.*, 2007).

Studies on the mammalian nervous system have indicated that microglia are also involved in a wide variety of processes, such as synaptic plasticity and maintenance of homeostasis in the brain, as well as in pathological conditions such as in Parkinson's and Alzheimer's disease, multiple sclerosis (Carmignoto, 2000; Vernadakis, 1996) and collaborate with astrocytes in producing soluble factors which contribute to the integrity of the local neuronal environment. Moreover, microglia-derived soluble factors seem to regulate neurogenic differentiation of neural stem/progenitor cells (NSPCs): molecules as IL-6 and LIF released by activated microglia promote astrocytic differentiation of NSPCs suggesting the importance of cell-cell interactions between glial cells and NSPCs (Nakanishi *et al.*, 2007).

Furthermore, microglial cells are the main source of interleukin (IL)-1 in the CNS and have also been

suggested as targets for its action (autocrine action) (Pinteaux *et al.*, 2002). IL-1 is one of the most important pro-inflammatory cytokine and is also a mediator of neurodegeneration and CNS inflammation (Pinteaux *et al.*, 2002). Presence of IL-1, was detected after activation *in vitro* also in invertebrate microglia, as other proinflammatory cytokines such as IL-6 and TNF- $\alpha$  as well (Sonetti and Peruzzi, 2004), demonstrating the production of these molecules is a primitive and well-conserved task of microglia during evolution.

On the other hand, it has to be underlined that the role of activated microglia in recovery still is controversial, these cells being responsible for production of both toxic or harmful molecules and trophic factors, including cytokines, nitric oxide, growth factors and extracellular matrix components (Chen *et al.*, 2000; Kreutzberg 1996; Nakajima *et al.*, 2001). The switch between neuroprotection or neurotoxicity, anyway in order to re-establish homeostasis in neural tissue, depends on microglia-glia and on microglia-neurons interactions, both in terms of signal molecules and of their concentration (Chen *et al.*, 2000).

It is clear that glial functions are so numerous and neuron-glia interactions so complicated that we still have a long way to go before we can fully appreciate the significance of these cells for the physiology and pathophysiology of the nervous system.

Fundamentals of glial cell behaviour and the roles of these cells in the nervous system are being studied mainly in mammals, particularly because the human CNS is the site of severe neuropathologies like Alzheimer's and Parkinson's disease. Nevertheless, some non-mammalian species make good models for studies of such fundamentals. Among these, teleosts fish are interesting as they represent intermediate stages between relatively simply structured invertebrates and the more complex mammals, rendering the opportunity to study neuronal and glial cell functioning and interactions at the single-cell level. Moreover, as underlined above, in contrast to mammals, teleosts exhibit an enormous potential to regenerate neural tissue upon injury.

In the present review we will focus on morphological and histochemical features of microglia in the teleost fish CNS for an exhaustive overview. Nevertheless till now fish glial cells lack of a consistent well-related amount of molecular and biochemical data, this topic is hot not only because of

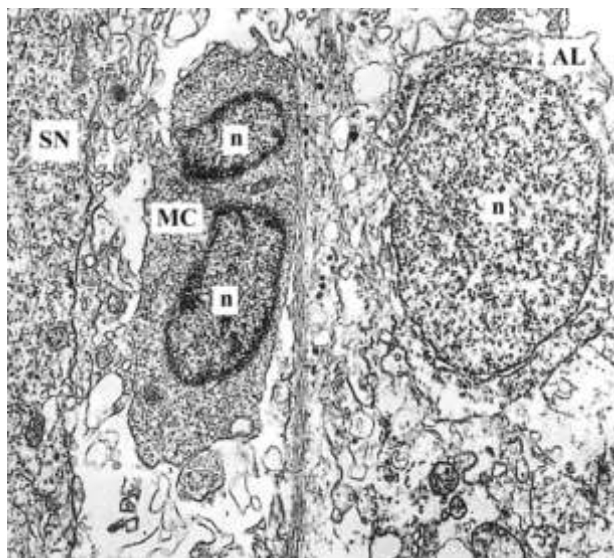
the increasing insight into the evolutionary aspects of glial cells, but also because teleosts fish have properties that make them relatively simple but very adequate models for neurobiologists to understand glia morphology and function. For instance, in carp and trout the continuously growth of the brain involves strong glial activity (Alunni *et al.*, 2005) and, compared to mammals, teleosts in general exhibit a much higher potential to regenerate neural tissue after injury, including strong neurogenesis to replace entire neurons (Zupanc, 1999). Since many years, the visual pathway is a useful model to study after injury interactions between glia and neurons in general, and microglial involvement in particular. This is because axons in fish optic nerve regenerate successfully and re-establish functional connection after injuries (Battisti *et al.*, 1995; Colavincenzo and Levine, 2000; Ghali *et al.*, 2000).

Other models are offered by some teleostean taxa, including puffer fish, which have a peculiar, well-defined cluster of giant Supramedullary Neurons (SN) with associated microglial cells and astrocyte-like cells, which form a suitable model to study neuron-glia interactions (Cuoghi, 2001; Mola and Cuoghi, 2004; Cuoghi and Mola, 2007).

### Morphological characteristics

Generally, reports about teleostean microglial cells are largely restricted to microglia in the optic tract; this is mainly because fish and amphibian ganglion cells, in contrast to those of mammals, have the surprising capacity to regenerate their axon after optic nerve lesion. The axons re-establish synaptic contacts with their original targets in the tectum, restoring vision (Battisti *et al.*, 1995; Velasco *et al.*, 1995). For this reason, the morphological features of microglial cells during optic nerve regeneration are well described for *Carassius auratus* (Battisti *et al.*, 1995; Springer and Wilson, 1989), also at electron microscopical level. Microglial cells resembled mammalian microglia, with heterochromatic nucleus and high content of dilated rough endoplasmic reticulum (Battisti *et al.*, 1995). *C. auratus* is also the elective non-mammalian model for the study of microglial role in optic nerve Wallerian degeneration (Moorhouse *et al.*, 1996; Colavincezo and Levine, 2000; Ghali *et al.*, 2000; Levin and Evans, 2002).

More recently, microglial cells were described at ultrastructural level in the supramedullary neurons



**Figure 1.** Microglial cell (MC) with polymorph nucleus (n) contacting a Supramedullary Neuron (SN) and located near to an astroglial-like cell (AL), with large oval-shaped nucleus (n). X 17.000.

(SN) cluster in the pufferfish *Diodon holacanthus* and *Tetraodon fluviatilis* (Figure 1) as small cells (maximum diameter 5-10  $\mu\text{m}$ ), round or irregularly shaped, having a nucleus with dense clumps of chromatin and a cytoplasm containing vacuoles, dense bodies, lysosomes and inclusions that may represent residual bodies (Cuoghi and Marini, 2001; Cuoghi, 2001). These features strongly indicate that they are microglial cells similar to those described in mammals (Cuoghi 2001; Murabe and Sano, 1982; Pannese, 1994).

In *C. auratus* optic nerve, rapidly after the injury, microglia show morphological changes suggesting activation, for example, after one hour since the crash injury microglial cells begin phagocytosis of debris from degenerating axons and the number of microglial cells engaged in phagocytosis increased one day postoperative and remain phagocytically active almost six week postoperative. As effect of the activation, microglia become also migrating (Battisti *et al.*, 1995). The same changes are reported in light microscopic study of degenerating optic axons: as first appear as round cells and, after nerve lesion, become activated, obtaining many elongate processes and starting to migrate towards the injury site. This migration might be enabled by their cytoplasmic processes. Concomitant with this morphological change and mobility, activated microglia reveal phagocytosis to remove degenerat-

ed tissue (Colavincenzo and Levine, 2000; Springer and Wilson, 1989).

It was hypothesized that the degree of microglial cell arborisation may be related to the degree of phylogenetic development; in fact, the arborization seems to be greater in *Rana* and even stronger in mammalian microglia (Cammermeyer, 1970).

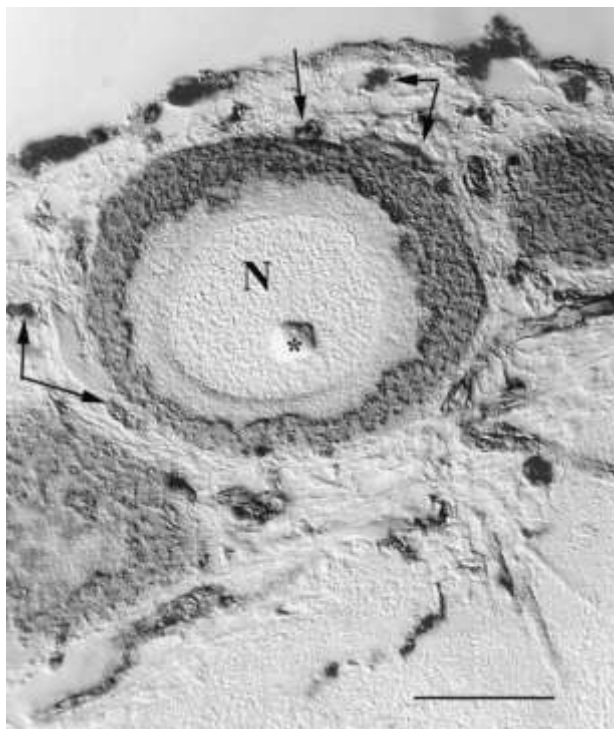
### Cytochemical features

In *C. auratus* optic nerve, microglia are identified as monocyte-derived cells immunopositive to OX-42 antibodies, directed against the complement component receptor C3bi, a well conserved antigen present on monocyte-derived cells, i.e. macrophages and microglia, in the CNS (Battisti *et al.*, 1995). Moreover, in different species of *Oreochromis*, microglial cells have also been immunocytochemically characterized by the FL.1 antibody, which proved to be homologous to CD45, the leukocyte-common antigen in mammals (Dowding *et al.*, 1991, Dowding and Scholes, 1993). The immunostaining specifically recognizes resident macrophages, including Kupffer's cells, peritoneal macrophages and microglial cells but does not mark circulating monocytes. FL.1 shows that the microglial cells are very numerous in the whole CNS, and in particular, constitute almost one-third of all glia in the optic nerve. Moreover, in the spinal cord, they occur almost 10 times as frequently as in mammals. After injury of the optic nerve, FL.1 positive microglia proliferates throughout the optic tract. Newly formed cells occur near the degenerating axons and synaptic terminals. Most of the microglial cells have an amoeboid shape (Dowding *et al.*, 1991, Dowding and Scholes, 1993).

Various methods have been performed to identify teleost fish microglial cells (Cuoghi, 2001; Cuoghi and Marini 2001; Dowding *et al.*, 1991; Springer and Wilson, 1989; Velasco *et al.*, 1999), among these lectins histochemistry has been successfully applied as a powerful tool. Lectins from *Lycopersicon esculentum* (LEL) have affinity for poly-N-acetyl lactosamine sugar residues and permit the identification of amoeboid and ramified microglial cells in postnatal and adult rat brain, of microglial cells in normal and injured fish retina, optic nerve and optic tectum, and of microglia in *T. fluviatilis* SN cluster, also in *in vitro* experiments (Acarin *et al.*, 1994; Velasco *et al.*, 1995; Cuoghi 2001; Cuoghi *et al.*, 2003). Since LEL histochemistry revealed that these residues both in the teleost

visual pathway and in the SN cluster are associated with a specific population of glial cells identified as amoeboid and ramified microglial cells, and since this method did not label other glial cell types or neurons, LEL is an excellent teleost microglial marker (Cuoghi, 2001; Velasco *et al.*, 1995; Zupanc *et al.*, 2003).

Furthermore, immunohistochemical tests performed on the *T. fluviatilis* SN cluster pointed out anti-ACTH positivity in SN and in microglia, both in sections (Figure 2) and in cultured cells (Cuoghi *et al.*, 2003). As to teleost neural districts, ACTH occurs not only in the hypothalamus, but also in the *nucleus lateralis tuberis*, in fibers running towards the olfactory lobes and in fibers of the optic tectum. In these brain areas ACTH could act as neuromodulator (Olivereau and Olivereau, 1990) as well as in the SN clusters of *T. fluviatilis* (Cuoghi *et al.*, 2003). The same role was proposed for others POMC-derived peptides ( $\alpha$ -MSH and  $\beta$ -End) in the elasmobranch brain (Chiba, 2001; Vallarino *et al.*, 1988; 1989) and in the lizard CNS (Vallarino, 1984).



**Figure 2.** Transverse section of dorsal spinal cord with the cluster of *Tetraodon fluviatilis*. View of single SN with nucleus (N) and nucleolus (\*). SN cytoplasm and surrounding glial cells (arrows) are immunopositive to anti-ACTH, BAS technique. Nomarski interference. Bar = 25  $\mu$ m.

Double labelling experiments have shown that a considerable part of the microglial cells emigrating from cultured SN explants is ACTH-positive (Cuoghi *et al.*, 2003). A few ACTH-immunoreactive irregularly shaped cells resembling microglia were also detected in the neural lobe of the pituitary gland of the teleost *Diplodus sargus*, where microglia might exert macrophage-like actions (Ferrandino *et al.*, 2000). ACTH and other POMC-derived peptides have broad and potent anti-inflammatory effects. Since activated microglial cells are an important source for the CNS of cytokines and other inflammatory agents, melanocortins may be important to control glial function in order to preserve neural cells (Delgado *et al.*, 1998). The hypothesis that ACTH molecules represent a language shared by SN and microglia which permits communication between these different cellular types appears intriguing.

### Microglial cells and neuronal repair

In *C. auratus* and generally in fish, the rapid response to injury of non-neuronal cells like astrocytes and microglia, their rapid phagocytic activity and their secretion of growth-promoting factors, contribute to a tissue environment that promotes robust regeneration by optic axons (Battisti *et al.*, 1995; Colavincenzo and Levine, 2000; Lillo *et al.*, 2001). In particular, a number of studies in the *C. auratus* CNS demonstrated a large microglial response to Wallerian degeneration after optic nerve section, underlying that the majority of reactive cells in the visual path after axotomy are local microglia (Colavincenzo and Levine, 2000; Ghali *et al.*, 2000; Levine and Evans, 2002).

Moreover, although there are differences between mammalian and lower vertebrates in the time-scale by which microglia and macrophages process degenerative material and produce cellular debris, the role of both cell types in gliogenesis and neuronal repair is substantial. In mammals, they modulate astroglial cell proliferation by releasing factors like cytokines (Giulian *et al.*, 1986, 1988; Kloss *et al.*, 1997; Kreutzberg, 1996). Conversely, they are also affected themselves by such factors and by other agents like ATP, which control their proliferation and activation. For this purpose, microglial cells possess a wide variety of cell surface receptors, reflecting their multifunctional nature (Altman, 1994; Davalos *et al.*, 2005; Kreutzberg, 1996; Ling and Wong, 1993; Moore

and Thanos, 1996; Narasimhan, 2005; Perry and Gordon, 1988).

Similarly, microglia in the fish visual system secretes particular factors in response to optic nerve injury (Giulian, 1984). In *C. auratus* this response involves the glial release of tenascin, a matrix molecule implicated in neuronal development, and of TGF- $\beta$ 1, an evolutionarily well-conserved growth factor. TGF- $\beta$ 1 acts on astrocytes, regulating their activity by stimulating synthesis of NGF and tenascin. In turn, astrocytes are closely associated with regenerating axons and likely synthesize other important growth-related factors, including tenascin, TGF- $\beta$ 1 and laminin. In fact, macrophages and microglia are the two known sources of TGF- $\beta$ 1 in the CNS. Clearly, non-neuronal cells and their secretions may play a role in the co-ordination of regenerative events in the fish optic nerve (Battisti *et al.*, 1995).

In the *T. tinca* visual pathway, microglia appear as fusiform cells with short processes together with round cells in the optic nerve, and ramified cells in the encephalic visual pathway (retina, optic nerve, optic tract and optic tectum). Fusiform and ramified cells have been classified as resident/ramified microglia, while round cells with granules and vacuoles (granular cells) observed in lesioned optic nerve, correspond to microglia/macrophages (Velasco *et al.*, 1995). After optic nerve crush in *T. tinca* a dramatic increase in the number of microglia/macrophages, as well as changes in the morphology, were observed. LEL binds to granular cells which have been considered as activated or reactive microglia on the basis of their similar morphology as such glia in mammals (Velasco *et al.*, 1995). Ramified microglia has also been detected in the diencephalic nuclei (*nucleus pretectalis superficialis pars parvocellularis* and *nucleus opticus dorsolateralis*) of *T. tinca* and may represent an initial reaction to the lesion, while amoeboid microglia could be related to subsequent debris removal and preparation of damaged tissue for regeneration (Velasco *et al.*, 1995).

In contrast to mammals, adult teleosts exhibit enormous capacities to replace damaged neural cells for new cells throughout the CNS, and this capacity does not only include re-growth of axonal processes but also the replacement of entire cell bodies. LEL has been used to study the role of microglia and macrophages in this neuronal regen-

eration, in the *corpus cerebelli* of *A. leptorhynchus*. The results confirm that one of the main functions of microglia and macrophages might be to remove debris of cells that have undergone apoptosis at the lesion site. It has been hypothesized that this clean type of cell death is a major factor in the fast and, sometimes, complete regeneration of neural tissue (Zupanc *et al.*, 2003). The same holds for Wallerian degeneration in *C. auratus* visual pathway (see above) and, after spinal cord transection, for regenerating axons in zebrafish. In this case, a macrophage/microglia response within two days of spinal cord transection, along with phagocytosis of myelin, was observed caudal to the transection by immunohistochemistry and electron microscopy (Becker and Becker, 2001).

Interestingly, immunocytochemistry and confocal microscopy indicated that following mechanical lesioning of the *corpus cerebelli* of adult *A. leptorhynchus*, granule neurons, astrocytes and microglia display a similar temporal pattern of expression of the neuropeptide somatostatin. Therefore, the idea has been raised that somatostatin is involved in regulation of neurogenesis in response to injury (Zupanc, 1999).

Another molecule which appears involved in neuronal repair is nitric oxide (NO): their synthesis is elicited in microglia-like cells as a response to axotomy of optic nerve in the tench (Clemente *et al.*, 2003). NO production by microglia is reported also in invertebrates (Chen *et al.*, 2000; Peruzzi *et al.*, 2004) and NO is also known to act on motile cells in both vertebrates and invertebrates. For example, it inhibits migration of rat vascular smooth muscle cells (Sarkar *et al.*, 1996) and of active amoeboid invertebrate immunocytes and microglia (Magazine *et al.*, 1996). In fish NO produced by SN clusters could be responsible of part of the bi-directional communications between SN and glia (Cuoghi *et al.*, 2003). For a complete view, it has to be considered that NO actions are not limited to microglia because, for instance, it influences also the movement of growing axons in nerve repair and regeneration. Moreover, the role of NO is not always unequivocally determined, being a migration promoter or stopper, depending on its concentration. Anyway, since microglial cells themselves are a source of NO, they seem to be able to mould repair by both promoting and inhibiting axon growth (Chen *et al.*, 2000).

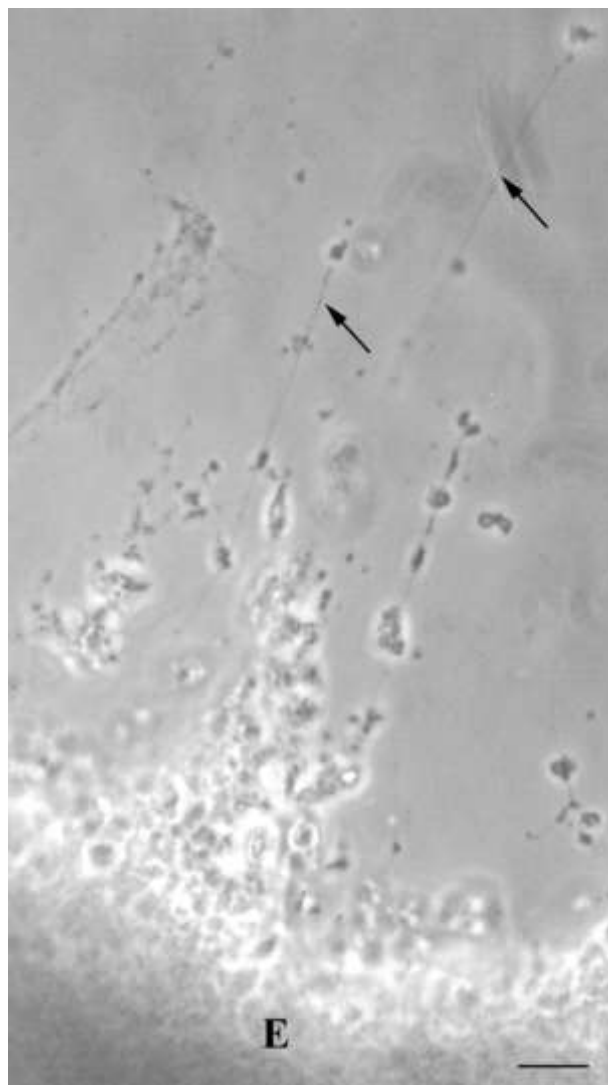
### Microglial cell cultures

Primary cultures of glial cells can be prepared relatively easily from brains of foetal or newborn rat and mice or embryonic chicken. However, fish glial cell cultures are mainly derived from the optic nerve or the retina (Ankerhold *et al.*, 1998; Bastmeyer *et al.*, 1989; 1993) and only recently some reports have appeared about microglial cell cultures from the brain of Tetraodontiform species (Bradford *et al.*, 1997; Cuoghi *et al.*, 2003) and *C. auratus* (Houalla and Levine, 2003). To unravel the functional roles of fish glial cells, *in vitro* cell cultures are necessary. They may involve glial monocultures or microglial cells that are mixed with astrocytes or identified neurons. The culture protocols should not only permit to test microglial cells for specific antigens or mRNAs (Cuoghi *et al.*, 2003; Houalla and Levine, 2003) but also enable the analysis of the microglial response under controlled experimental conditions. In this way, for example, the phagocytic activity of goldfish microglia becomes activated *in vitro* upon introducing latex microspheres and opsonized myelin into the culture dish (Houalla and Levine, 2003).

In order to define the chemical and functional nature of the neuron-glia cross-talk in SN, recently a protocol was set up for primary cell cultures prepared from brain tissue of adult puffer fish (Cuoghi *et al.*, 2003). The culturing of the puffer fish SN cluster is successful with respect to both SN and microglia. The latter cells show a remarkable phenomenon, in that they egress from the explant within a few hours of culture (Figure 3). Conformational changes in microglial cells during prolonged culture were observed, from a stellate to a rounded shape (Cuoghi *et al.*, 2003) in accordance with the changes reported for invertebrate and vertebrate microglia migrating *in vitro* (Dickson *et al.*, 1991; Rieseke *et al.*, 1989; Sonetti *et al.*, 1994). The microglial nature of these migrating cells was confirmed by their staining with LEL. The cytoarchitectural dynamics in SN explants during culturing were studied with confocal imaging of the LEL-positive microglial cells. This showed that some microglial cells do not migrate but remain *in situ*, surrounding the SN (Cuoghi *et al.*, 2003). Possibly, these cells are actively engaged in processes like maintenance of cellular homeostasis and removal of debris from injured tissue areas in the explant, which fits with roles traditionally attributed to mammalian microglia (Moore and Thanos, 1996; Vernadakis, 1996).

### Conclusions and future perspectives

Microglial cells are the resting guardians of the CNS, downregulated and adapted to that particular environment. However, one of their characteristics is their quick response to various signalling molecules indicating damage to brain structural integrity, but also to subtle homeostasis variations of their micromilieu (Kreutzberg, 1996). In the last decade, many studies were performed in mammals, but also teleost models emerged because of fish brain's peculiarities, *in primis* its recover/regenerative capabilities. In these processes microglia act by removing cellular debris (Colavincenzo and Levine, 2000; Zupanc and Zupanc, 2006), but likely also by producing and responding to a plethora of sig-



**Figure 3.** *Tetraodon fluviatilis* cluster explant and migrating cells. After 20 h of culture, actively migrating cells from the explant (E) on the substratum, following guide-lines (arrows). Bar = 10  $\mu$ m.

nalling molecules, NO and cytokines among others, as already documented in mammals (Pinteaux *et al.*, 2002; Sarkar *et al.*, 1996). Beside mammals, nowadays teleosts are the vertebrate group in which most information about the immune system is available, due to the great impetus on the discovery of genes homologous or orthologous to mammalian immunomodulatory molecules (Scapigliati *et al.*, 2006). For example, in comparative genomic studies, unambiguous fish orthologues have been found for only a few mammalian IL-1 family members; interestingly, the puffer *Fugu rubripes* (Tetraodontiformes) wholly sequenced genome did reveal teleosts orthologues for IL-18 and its putative receptor complex (Huising *et al.*, 2004).

On the contrary, the knowledge about biological activities exerted by cytokines, both produced by and acting on teleostean microglia is not so deep and not organized in an integrated picture. Such a prospect of survey appears even more attractive now that protocols for culturing teleosts microglia and microglia-neurons are tested (Cuoghi *et al.*, 2003; Houalla and Levine, 2003). For instance, a detection of IL-1 $\beta$  would be interesting also in view of the phylogenetic implications, since it has already been reported both in mammalian (Pinteaux *et al.*, 2002) and invertebrate microglia (Sonetti and Peruzzi, 2004).

Moreover, the intensity of microglial response in the fish visual system is much higher than that seen in mammalian CNS and may partially explain the capability of teleosts fish to regenerate axons. In this view, the identification of the cytokines networks that activate endogenous microglia, avoiding a massive influx of peripheral cells, will be even more appealing (Levine and Evans, 2002).

Following this line, also the identification of receptor expression on teleost microglia is an important, necessary step. For example, it is known that mammalian microglia have receptors for signalling molecules, produced both by glia itself and by neurons, such as ATP, acetylcholine and noradrenaline (NA) (Kreutzberg, 1996). An interesting work proposal could be to test microglial cells of *T. fluviatilis* SN cluster, also *in vitro*, for the presence of NA receptors, since SN were found to be immunopositive for NA but, till now, this production has been interpreted merely as classical neurotransmission (Mola *et al.*, 2002).

In general, whatever the molecules involved, intercellular cross-talk between glia and neurons is

the modern challenge in neurobiology and is crucial for our understanding of cellular communication in CNS. More than ten years ago, a famous review on mammalian microglia ended with the statement that the study of such complex relationship between microglia and neurons needed suitable models (Kreutzberg, 1996). Since that time, some fish models demonstrated to be particularly suitable for various experimental aims and, we think, should be even more attractive to neurobiologists now. This holds for zebrafish (*Danio rerio*), whose embryos can be obtained in large numbers and are accessible at all stages of development. Moreover, zebrafish embryos and larvae are optically clear and develop rapidly, being amenable to a wide variety of experimental approaches revealing the functions of individual cells or neural circuits (Lewis and Eisen, 2003). The possibility of following step by step details of cell processes in living zebrafish embryos using Differential Interference Contrast microscopy, together with single-cell tracing with fluorescent dyes, provides researchers with a powerful tool (Herbomel *et al.*, 1999). For instance, zebrafish embryos were successfully used in an exhaustive and elegant study about early macrophage spread, including early microglia, in the whole cephalic mesenchyme (Herbomel *et al.*, 2001). Beside the description of phenotypic transformation of these macrophages into early (amoeboid) microglia in the brain and retina, the study presents convincing evidence against the classical view which stated that the first tissue colonization by macrophages occurred via blood vessels as circulating monocytes. The early macrophage lineage is rather responsible for an invasive process, which comprehends the crossing of brain, retinal and epidermal basal lamina (Herbomel *et al.*, 2001).

A second model are small pufferfish like some *Tetraodon* species, which combine the advantages of easy availability and maintenance in the laboratory (Cuoghi 2001; Jaillon *et al.*, 2004) with a relatively simple, but complete genomic structure, and the presence of a well-defined cluster of giant SN with associated microglial cells and astrocyte-like cells, which form a suitable model to study neuron-glia interactions (Cuoghi 2001; Mola and Cuoghi, 2004; Cuoghi and Mola, 2007). Moreover, another advantage is the accessibility and large dimensions of SN, reaching 100  $\mu\text{m}$  in diameter in *T. fluviatilis* (Cuoghi 2001). Both these species are particularly suitable because the complete sequencing of

zebrafish is in progress, and puffer's genome is fully sequenced and available, making easier approaches at molecular level.

Also a high-quality draft genome sequence of medaka fish (*Oryzias latipes*) has been recently published (Kasahara *et al.*, 2007) and this species is actually used in various fields of biology, in particular for developmental genetic studies, also for their easy maintenance in the laboratory and many experimental explorations on embryos (Ishikawa, 2000).

For all these species with known genome, the increased availability of specific molecular probes for *in situ* hybridization, specific primers for PCR and sequencing, transcripts sequences, and fish-specific antisera against key proteins and peptides, would be an extraordinary opportunity for researchers in the field of neuron-microglia interactions.

### Acknowledgments

The authors are greatly indebted to Prof. E.W. Roubos for his help and advice throughout the manuscript's drafting. We also express our thanks to Dr. M. Mandrioli for his precious suggestions and comments about genomic and molecular aspects and to Mrs. K. Bevini for critical reading and for corrections to the text.

### References

- Acarin L, Vela JM, González B, Castellano B. Demonstration of poly-N-acetyl lactosamine residues in ameboid and ramified microglial cells in rat brain by tomato lectin binding. *J Histochem Cytochem* 1994;42:1033-41.
- Altman J. Microglia emerge from the fog. *Trends Neurosci* 1994; 17:47-9.
- Alunni A, Vaccari S, Torcia S, Meomartini ME, Nicotra A, Alfei L. Characterization of glial fibrillary acidic protein and astroglial architecture in the brain of a continuously growing fish, the rainbow trout. *Eur J Histochem* 2005;49:157-66.
- Ankerhold R, Leppert CA, Bastmeyer M, Stuermer CAO. E587 antigen is upregulated by goldfish oligodendrocytes after optic nerve lesion and supports retinal axon regeneration. *Glia* 1998;23:257-70.
- Battisti WP, Wang J, Bozek K, Murray M. Macrophages, microglia and astrocytes are rapidly activated after crush injury of the goldfish optic nerve: a light and electron microscopic analysis. *J Comp Neurol* 1995;354:306-20.
- Bastmeyer M, Bähr M, Stuermer CAO. Fish optic nerve oligodendrocytes support axonal regeneration of fish and mammalian retinal ganglion cells. *Glia* 1993;8:1-11.
- Bastmeyer M, Beckmann M, Nona SM, Cronly-Dillon JR, Stuermer CAO. Identification of astrocyte- and oligodendrocyte-like cells of goldfish optic nerves in culture. *Neurosci Lett* 1989;101:127-32.
- Becker T, Becker CG. Regenerating descending axons preferentially reroute to the gray matter in the presence of a general macrophage/microglial reaction caudal to a spinal transection in adult zebrafish. *J Comp Neurol* 2001;433:131-47.
- Bradford CS, Müller AE, Toumadje A, Nishiyama K, Shirahata S, Barnes DW. Characterization of cell cultures derived from Fugu, the Japanese pufferfish. *Mol Mar Biol Biotechnol* 1997;6:279-88.
- Cammermeyer J. The life history of the microglial cell: a light microscopic study. In: Ehrenpreis S, Solnitzky DC eds. *Neurosciences Research*, vol. 3. Academic Press, New York, 1970, pp. 43-129.
- Carmignoto G. Reciprocal communication systems between astrocytes and neurones. *Progr Neurobiol* 2000;62:561-81.
- Chen A, Kumar SM, Sahley CL, Muller KJ. Nitric oxide influences injury-induced microglial migration and accumulation in the leech CNS. *J Neurosci* 2000;20:1036-43.
- Chiba A. Marked distributional difference of  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH)-like immunoreactivity in the brain between two elasmobranchs (*Scyliorhinus torazame* and *Etmopterus brachyurus*): an immunohistochemical study. *Gen Comp Endocrinol* 2001; 122:287-95.
- Clemente D, Porteros A, Alonso JR, Weruaga E, Aijón J, Arévalo R. Effects of axotomy on the expression of NADPH-diaphorase in the visual pathway of the tench. *Brain Res* 2002;925:183-94.
- Colavincenzo J, Levine RL. Myelin debris clearance during Wallerian degeneration in the goldfish visual system. *J Neurosci Res* 2000; 59:47-62.
- Cuoghi B. Glial cells: basic components of clusters of supramedullary neurons in pufferfish. *J Neurocytol* 2001;30:503-13.
- Cuoghi B, Marini M. Ultrastructural and cytochemical features of the supramedullary neurons of the pufferfish *Diodon holacanthus* (L.) (Osteichthyes). *Tiss Cell* 2001;33:491-9.
- Cuoghi B, Mola L. Fish take their revenge: alternative models for modern neurobiology. *It J Zool* 2007;74:309-11.
- Cuoghi B, Blasoli L, Sabatini MA. ACTH occurrence in teleosts supramedullary neuron clusters: a neuron-glial common language? *Gen Comp Endocrinol* 2003;132:88-95.
- Cuoghi B, Marini M, Mola L. Histochemical and immunocytochemical localization of nitric oxide synthase in the supramedullary neurons of the pufferfish *Tetraodon fluviatilis*. *Brain Res* 2002;938:1-6.
- Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, et al. ATP mediates rapid microglial response to local brain injury *in vivo*. *Nature Neurosci* 2005;8:752-8.
- Delgado R, Carlin A, Airaghi L, Demitri MT, Meda L, Galimberti D, et al. Melanocortin peptides inhibit production of proinflammatory cytokines and nitric oxide by activated microglia. *J Leukoc Biol* 1998;63:740-5.
- Dickson DW, Mattiace LA, Kure K, Hutchins K, Lyman WD, Brosnan CF. Microglia in human disease, with an emphasis on acquired immune deficiency syndrome. *Lab Invest* 1991;64:135-56.
- Dowding AJ, Maggs A, Scholes J. Diversity amongst the microglia and regenerating fish CNS: immunohistochemical characterization using FL-1, an anti-macrophage monoclonal antibody. *Glia* 1991;4:345-64.
- Dowding AJ, Scholes J. Lymphocytes and macrophages outnumber oligodendroglia in normal fish spinal cord. *Proc Natl Acad Sci USA* 1993;90:10183-7.
- Ferrandino I, Pica A, Grimaldi MC. Immunohistochemical detection of ACTH and MSH cells in the hypophysis of the hermaphroditic teleost *Diplodus sargus*. *Eur J Histochem* 2000;44:397-406.
- Ghali RP, Herx LM, Maa A, Levine RL. Mononuclear cell proliferation and hyperplasia during Wallerian degeneration in the visual system of the goldfish in the presence or absence of regenerating optic axons. *Brain Res* 2000;854:178-88.
- Giulian D. Peptides from the regenerating central nervous system of goldfish stimulate glia. *Proc Natl Acad Sci USA* 1984;81:3567-71.
- Giulian D, Baker TJ, Shih LN, Lachman LB. Interleukin-1 of the central nervous system is produced by ameboid microglia. *J Exp Med* 1986;164:594-604.
- Giulian D, Young DG, Woodward J, Brown DC, Lachman LB. Interleukin-1 is an astroglial growth factor in the developing brain. *J Neurosci* 1988;8:709-14.
- Glezer I, Simard AR, Rivest S. Neuroprotective role of the innate immune system by microglia. *Neuroscience* 2007;147:867-83.
- Herbomel P, Thisse B, Thisse C. Ontogeny and behaviour of early macrophages in the zebrafish embryo. *Development* 1999;126: 3735-45.
- Herbomel P, Thisse B, Thisse C. Zebrafish early macrophages colonize



- cephalic mesenchyme and developing brain, retina and epidermis through a M-CSF receptor-dependent invasive process. *Dev Biol* 2001;238:274-88.
- Houalla T, Levine RL. The isolation and culture of microglia-like cells from the goldfish brain. *J Neurosci Met* 2003;131:121-31.
- Huising MO, Stet RJ, Savelkoul HF, Verburg-van Kemenade BM. The molecular evolution of the interleukin-1 family of cytokines; IL-18 in teleost fish. *Dev Comp Immunol* 2004;28:395-413.
- Ishikawa Y. Medakafish as a model system for vertebrate developmental genetics. *Bioessays* 2000;22:487-95.
- Jaillon O, Aury J, Brunet F, Petit JL, Stange-Thomann N, Muceli E, et al. Genome duplication in the teleost fish *Tetraodon nigroviridis* reveals the early vertebrate proto-karyotype. *Nature* 2004;431:946-57.
- Kasahara M, Naruse K, Sasaki S, Nakatani Y, Qu W, Ahsan B, et al. The medaka draft genome and insights into vertebrate genome evolution. *Nature* 2007;447:714-9.
- Kloss CUA, Kreutzberg GW, Raivich G. Proliferation of ramified microglia on an astrocyte monolayer: characterization of stimulatory and inhibitory cytokines. *J Neurosci Res* 1997;49:248-54.
- Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. *TINS* 1996;19:312-8.
- Levine RL, Evans MDC. The source of reactive cells during central Wallerian degeneration in the goldfish: a differential irradiation protocol. *Exper Neurol* 2002;173:136-44.
- Lewis KE, Eisen JS. From cells to circuits: development of the zebrafish spinal cord. *Prog Neurobiol* 2003;69:419-49.
- Lillo C, Velasco A, Jimeno D, Cid E, Aijón J, Lara JM. Non-neuronal cells involved in the degeneration and regeneration of the fish retina. *J Neurocytol* 2001;30:475-91.
- Ling EA, Wong WC. The origin and nature of ramified and amoeboid microglia: a historical review and current concepts. *Glia* 1993;7:9-18.
- Magazine HI, Liu Y, Bilfinger TV, Fricchione GL, Stefano GB. Morphine-induced conformational changes in human monocytes, granulocytes, and endothelial cells and in invertebrate immunocytes and microglia are mediated by nitric oxide. *J Immunol* 1996;156:4845-50.
- Mola L, Cuoghi B. The supramedullary neurons of fish: present status and goals for the future. *Brain Res Bull* 2004;64:195-204.
- Mola L, Sassi D, Cuoghi B. The supramedullary cells of the teleost *Coris julis* (L.): a noradrenergic neuronal system. *Eur J Histochem* 2002;46:329-32.
- Moore S, Thanos S. The concept of microglia in relation to central nervous system disease and regeneration. *Prog Neurobiol* 1996;48:441-60.
- Moorhouse L, Pederson K, Levine RL. Specific uptake of intracranial horseradish peroxidase (HRP) by microglial cell in goldfish. *Neurosci Lett* 1996:13-6.
- Murabe Y, Sano Y. Morphological studies on neuroglia. VI. Postnatal development of microglial cells. *Cell Tissue Res* 1982;225:469-85.
- Nakanishi M, Niidome T, Matsuda S, Akaike A, Kihara T, Sugimoto H. Microglia-derived interleukin-6 and leukaemia inhibitory factor promote astrocytic differentiation of neural stem/progenitor cells. *Eur J Neurosci* 2007;25:649-58.
- Nakajima K, Honda S, Tohyama Y, Imai Y, Kohsaka S, Kurihara T. Neurotrophin secretion from cultured microglia. *J Neurosci Res* 2001;65:322-31.
- Narasimhan K. Brain's guard cells show their agility. *Nature Neurosci* 2005;8:707.
- Olivereau M, Olivereau JM. Corticotropin-like immunoreactivity in the brain and pituitary of three teleost species (goldfish, trout and eel). *Cell Tissue Res* 1990;262:115-23.
- Pannese E. Neurocytology. Fine structure of neurons, nerve processes and neuroglial cells. GT Verlag, Stuttgart, 1994.
- Perry VH, Gordon S. Macrophages and microglia in the nervous system. *Trends Neurosci* 1998;11:273-7.
- Peruzzi E, Fontana G, Sonetti D. Presence and role of nitric oxide in the central nervous system of the freshwater snail *Planorbis corneus*: possible implication in neuron-microglia communication. *Brain Res* 2004;1005:9-20.
- Pinteaux E, Parker LC, Rothwell NJ, Luheshi GN. Expression on interleukin-1 receptors and their role in interleukin-1 actions in murine microglial cells. *J Neurochem* 2002;83:754-63.
- Rieske E, Graeber MB, Tetzlaff W, Czlonkowska A, Steit WJ, Kreutzberg GW. Microglia and microglia-derived brain macrophages in culture: generation from axotomized rat facial nuclei, identification and characterization in vitro. *Brain Res* 1989;492:1-14.
- Sarkar R, Meinberg EG, Stanley JC, Gordon D, Webb RC. Nitric oxide reversibly inhibits the migration of cultured vascular smooth muscle cells. *Circ Res* 1996;78:225-30.
- Scapigliati G, Buonocore F, Mazzini M. Biological activity of cytokines: an evolutionary perspective. *Curr Pharm Des* 2006;12:3071-81.
- Sonetti D, Peruzzi E. Neuron-microglia communication in the CNS of the freshwater snail *Planorbis corneus*. *Acta Biol Hung* 2004;55:273-85.
- Sonetti D, Ottaviani E, Bianchi F, Rodriguez M, Stefano ML, Sharrer B, et al. Microglia in invertebrate ganglia. *Proc Natl Acad Sci USA* 1994;91:9180-4.
- Springer AD, Wilson BR. Light microscopic study of degenerating cobalt-filled optic axons in goldfish: role of microglia and radial glia in debris removal. *J Comp Neurol* 1989;282:119-32.
- Vallarino M. Immunohistochemical localization of  $\alpha$ -melanocyte-stimulating hormone in the brain of the lizard, *Lacerta muralis*. *Cell Tissue Res* 1984;237:521-4.
- Vallarino M, Delbende C, Jegou S, Vaudry H. Alpha-melanocyte-stimulating hormone (a-MSH) in the brain of the cartilaginous fish. Immunohistochemical localization and biochemical characterization. *Peptides* 1988;9:899-907.
- Vallarino M, Tranchand Bunel DT, Delbende C, Ottonello I, Vaudry H. Distribution of the pro-opiomelanocortin-derived peptides, alpha-melanocyte-stimulating hormone (a-MSH), adrenocorticotrophic hormone (ACTH), and beta-endorphin in the brain of the dogfish *Scyliorhinus canicula*: An immunocytochemical study. *J Exp Zool* 1989;Suppl. 2:112-21.
- Velasco JA, Caminos E, Vecino E, Lara JM, Aijón J. Microglia in normal and regenerating visual pathways of the tench (*Tinca tinca* L., 1758; Teleost): a study with tomato lectin. *Brain Res* 1995;705:315-24.
- Velasco JA, Jimeno D, Lillo C, Caminos E, Lara JM, Aijón J. Enzyme histochemical identification of microglial cells in the retina of a fish (*Tinca tinca*). *Neurosci Lett* 1999;263:101-4.
- Vernadakis A. Glia-neuron intercommunications and synaptic plasticity. *Prog Neurobiol* 1996;49:185-214.
- Zupanc GKH. Up-regulation of somatostatin after lesions in the cerebellum of the teleost fish *Apteronotus leptorhynchus*. *Neurosci Lett* 1999;268:135-8.
- Zupanc GKH, Zupanc MM. New neurons for the injured brain: mechanisms of neuronal regeneration in adult teleost fish. *Regen Med* 2006;1:207-16.
- Zupanc GKH, Clint SC, Takimoto N, Hughes ATL, Wellbrock UM, Meissner D. Spatio-temporal distribution of microglial/macrophages during regeneration in the cerebellum of adult teleost fish, *Apteronotus leptorhynchus*: a quantitative analysis. *Brain Behav Evol* 2003;62:31-42.

