Microglia of teleosts: facing a challenge in neurobiology

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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.

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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-α 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

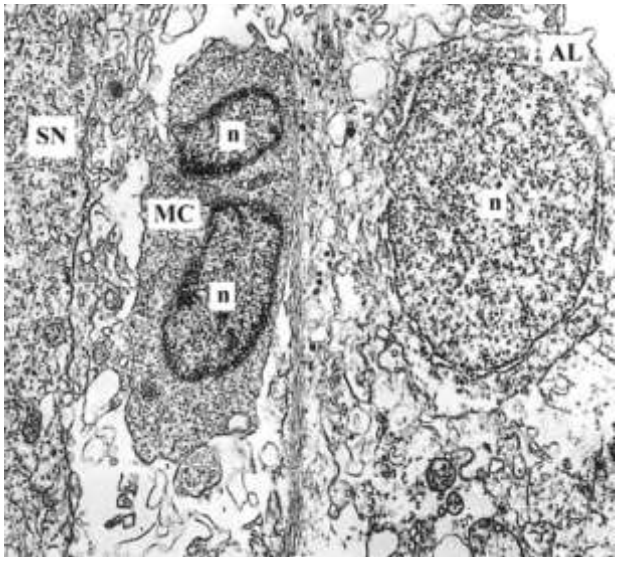

Figure 1. Microglial cell (MC) with polymorph nucleus (n) contacting a Supramedullary Neuron (SN) and located near to an astroglial-like cell (AL), with large oval-shaped nucleus (n). X 17.000.

(SN) cluster in the pufferfish *Diodon holacanthus* and *Tetraodon fluviatilis* (Figure 1) as small cells (maximum diameter 5-10 μm), 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 degenerated 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 Kuppfer'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 (α-MSH and β-End) in the elasmobranch brain (Chiba, 2001; Vallarino *et al.*, 1988; 1989) and in the lizard CNS (Vallarino, 1984).

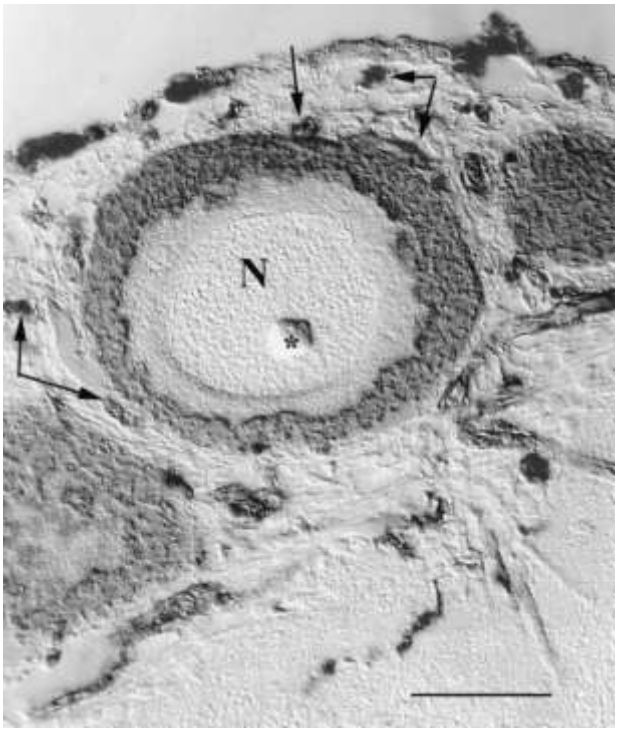

Figure 2. Transverse section of dorsal spinal cord with the cluster of *Tetraodon fluviatilis*. View of single SN with nucleus (N) and nucleolus (*). SN cytoplasm and surrounding glial cells (arrows) are immunopositive to anti-ACTH, BAS technique. Nomarski interference. Bar = 25 μm.

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 timescale 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-β1, an evolutionarily well-conserved growth factor. TGF-β1 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-β1 and laminin. In fact, macrophages and microglia are the two known sources of TGF-β1 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 regeneration, 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; Rieske *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-

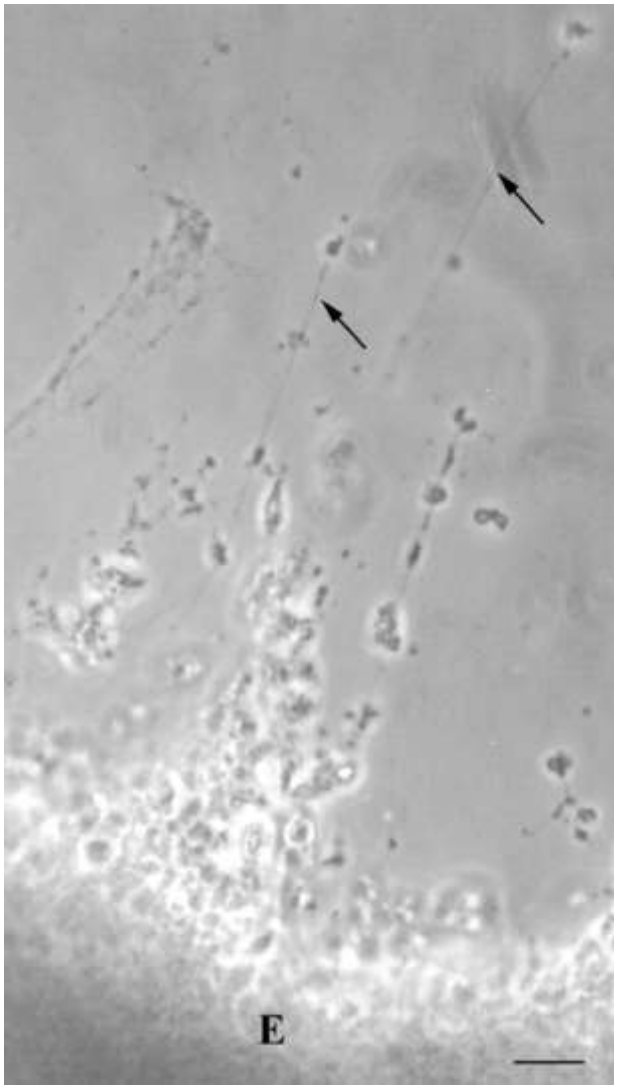

Figure 3. *Tetraodon fluviatilis* cluster explant and migrating cells. After 20 h of culture, actively migrating cells from the explant (E) on the substratum, following guide-lines (arrows). Bar = 10 μm.

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-1β 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 mensenchyme (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 μm 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.

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